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GENETICS AND BREEDING

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ORIGIN, BIOLOGICAL PARTICULARITIES AND THE SPREADOF THE KARAKUL RACE SHEEP

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Abstract

The aim of the scientific research was to highlight the origin, biological particularities and spread in the world of Karakul race sheep for better knowledge and prediction of the impact of using this race as an breeding ameliorating raceand improve the morpho-productive qualities of local races. The research was conducted based on the synthesis analysis of a number of specialized bibliographic sources, published in different periods of spread and research of the Karakul sheep race in the world. The results of the research showed that the opinions of different researchers on the origin and period of formation of the Karakul sheep race, so far, are divergent. Some researchers believe that the Karakul sheep race has ancient origins (1300 years to en) and formed in the regions of ancient Mesopotamia, others mention that it has medieval origins and was brought to Central Asia by Arabs in the VIII century, and the third group of researchers report that the true Karakul race originated in the modern period (XVI-XVIII centuries) and was formed in Central Asia by the selection of local pseudo-Karakul sheep in the direction of improving the furskin qualities of newborn lambs. The main biological particularities of Karakul sheep is that the hairy sheath of newborn lambs is wound in loops of different types (wave, bob, ridge, etc.) and shapes (tubular, rib, flattened), with various varieties of curling (jacket , costal, flat, Kaukasian, moire), consisting of elastic, silky and glossy fibers, with a wide range of colors (black, greyish, gray, brown, pink, white), shades (dark, medium, light) and coloration (blue, gold, silver, pearl, bronze, platinum, diamond, amber, steel). Due to these specific biological particularities and the demands of the excessively large market for Karakul furskins in the late 19th - early 20th century, this race was spread all over the world, being exploited either for scientific research and pure race breeding, or at the crossbreeding with some local races to improve the furskin qualities of newborn lambs and the production of commercial furskins. The Karakul sheep race has low skills in milk and meat production (low body mass), being extremely sensitive to helminth infections in conditions of high air humidity, rain and wet pastures. Sheep of this race are widespread in warm arid countries, regions and areas with low humidity, large plains, semi-deserts, poor vegetation, where they are maintained throughout the year in natural grazing conditions, without capital investment, with minimal costs. The knowledge of these biological and environmental particularities, allows the application of cautious and reasonable strategies of selection and spread of this race as an ameliorating race to the development and improvement of the morpho-productive qualities of some local races.

Key words*: biological particularities, Karakul race, origin, spread.*

INTRODUCTION

According to research conducted on the basis of FAO data (www.fao.org/docrep/012/a 1250r. Pdf, 2016) more than 1129 sheep breeds are raised in the world (Buzu, 2016). In different geographical areas of the world were created by humans, raised and spread those races of sheep that, meeting the requirements of society, corresponded more adequately to local traditions and pedo-climatic conditions.

The Karakul sheep race was created in Asia, in arid and extensive pedo-climatic conditions, which allowed the maintenance of sheep grazing throughout the year, with minimal costs. The spread and improvement of the race occurred under the influence of the growing demand of the world market for the furskin of the newborn lamb, slaughtered 1-5 days after birth. According to its value, it is considered a luxury fur. Over the centuries, the selection of Karakul sheep in the main breeding areas has been unilateral, oriented towards improving the qualities of the furskin.

Thus, in the countries of Central Asia were created several intrarasial and elite types of Karakul sheep of different colors, shades and coloration, such as Karakul of Buhara, Karakalpak, Surhandaria, Karakum, Kâzâlkum, Kazakhstan, etc. (Алимбаев, 2011; Гигинейшвили, 1976; Дъячков, 1960, 1980; Дюсегалиев, 2010а, 2010б; Кошевой, 1975;

Стояновская, 1964; Юдин, 1943; Юсупбаев, 2011; www.agriculture.uz/ru.php?/research/ detail / 133-140, 2016).

In South-West Africa (Namibia), several intrarasial types of Karakul sheep of different colors with flattened and moile loops have been created (Мостерт, 1975; Филлингер, 1975а, 1975б; Шефер, 1975).

In Ukraine (Askania Nova) a prolific type of sheep was created - the Karakul of Askania (Перегон, 1972).

In Romania, at the Research and Production Station for Raising Sheep Popăuţi, Botoşani District, a local type of Karakul de Botoşani sheep was created, with different varieties of colors and shades, with increased skills of milk production (Барта et al., 1977; Pascal et al., 2010; Pascal, 2011).

In the Republic of Moldova, sheep breeding for milk-wool-furskins is one of the oldest and most traditional branches of the livestock sector(Ильев, 1965a, 1965b).

Sheep ensure the food security of the rural population with dairy products (cheese) and meat, and the processing industry - with raw materials (furskins, furs, hides, wool). They efficiently use natural pastures and plant debris after harvesting crops. For these reasons, oviculture is an accessible and indispensable branch for the local population and of major importance for the national economy.

According to historical traditions, in the northern and central areas of the country, the natives raised the Ţuşca sheep race, with mixed production skills for milk-wool-furskins, but the qualities of the furskins obtained from newborn lambs were inferior. In order to improve their qualities, the local Ţuşca race began to be absorbed by crossbreeding by the breeding race Karakul, imported from Central Asia. Imports of Karakul sheep into Bessarabia were made periodically, from 1884 until the beginning of World War II (Buzu, 2016).

During the post-World War II period (1947-1979), imports of Karakul sheep from Central Asia were made permanently, and the local Ţuşca sheep race was practically replaced by the Karakul race through mass absorption crosses (Богданович, 1957; Богданович et al., 1979; Богданович et al., 1983; Богданович et al.,1984; Ильев, 1957a, 1957b, 1966a, 1966b, 1969, 1976, 1984; Ильев & Богданович, 1966; Ильев et al., 1981). However, improving the number of sheep for the furskins was difficult. The share of first-class furskins in the Republic did not exceed 12.0-15.0% (Бузу et al., 1992). As a result of these crosses, it was observed that the level of milk production and body mass (meat production) in sheep began to decrease (Buzu, 1995; Богданович et al., 1979), which did not correspond to the traditions of sheep farming as milk and meat producers.

It should be noted that, until 1976, breeding crosses had a mass companion character, because the standard purpose of the required animal models was not elaborated, in the selection were not taken into account the genetic parameters of the selected characters and morpho-productive biological particularities of the initial races of sheep. As a result of these crosses, a population of mixed-breed sheep (Ţ x K) of different generations was created in the Republic, which began to be unofficially called the moldavian Karakul (Богданович, 1957; Ильев, 1957a). The productivity of these sheep continues to be low, even on breeding farms. This situation persisted because there were no objective methods for assessing furskin characteristics and sheep selection in local conditions.

To date, some of the most important characteristics have not been taken into account in determining the general breeding value (class) of Karakul sheep, according to the Instructions Guidelines in force (Buzu et al., 1996), some of the most important morpho-productive characters of selection are not taken into account, such as the production of milk and meat (body mass).

In fact, the main flaw of these Instructions is that the production of furskins is considered the only basic character, expressed by the class of lamb, and body mass and milk production are not taken into account in determining the class of the animal. So, between the values of the main morpho-productive characters and the breeding value of the animal there is an obvious rupture, which requires integration in a unique complex of the phenotypic, genotypic and economic values of the animal.

Research on the economic value of selection characters (Buzu & Spătaru, 2015) has shown that given that the selling price of furskins on

domestic and foreign markets remained at the level of 30-40 years ago, and the price of food products (meat, cheese) has skyrocketed during this period, 5-10 times, raising Karakul sheep without increased milk and meat skills in the Republic of Moldova becomes unprofitable. Therefore, the elaboration of an objective and efficient methodological framework for the complex selection of Karakul sheep according to the furskin qualities of the lambs, the body mass and the milk production of the sheep, considering the biological particularities of the animals, presents a particularly important and current problem.

In this context, the knowledge and highlighting of the biological particularities of the Karakul sheep race, as well as its genealogical links (origins) with ancestors of different intrarasial types from different areas or geographical zones of the world, allows the application of careful and reasonable selection and distribution strategies of this race as an ameliorating race to the development and improvement of the morpho-productive qualities of some local races.

MATERIALS AND METHODS

The research was conducted based on the synthesis analysis of a number of specialized bibliographic sources, published in different periods of spread and research of the Karakul sheep race in the world. Among the oldest and most important bibliographic sources studied by us were the publications of famous Austrian researchers (Adametz, 1911, 1927) and Tsarist Russia (Демянко, 1912; Карпов, 1912; Иванов, 1914; Юнг, 1914a, 1914b), from which we gathered some important information on the origin, biological particularities and spread of the Karakul sheep race in the early stages.

Important information on the origin of the Karakul sheep race has been found in the original publications of German researchers in the interwar period (Hornitsehek, 1939) and after the Second World War (Hundt, 1954; Trauer, 1963; Franke, 1973).

Other bibliographic sources, no less important, were the scientific reports of representatives of academia from different countries at international symposia for the Karakul sheep race, from which we became acquainted with the situation regarding the breeding and improvement of Karakul sheep in Romania (Барта et al., 1975), Germany (Вуссов, 1975), Austria (Турек, 1975) and other countries.

Much of the research information in this paper has been gathered from the scientific materials of some outstanding German researchers (Мостерт, 1975; Нел Джеймс, 1975; Филлингер, 1975a, 1975b) from the Karakul Race Research Station in Neidam, Namibia, whose works have been generously translated from English into Russian, edited and published by the renowned university professor from ВИЖ (Всесоюзный институт животноводста, Дубровицы, СССР) Гигинейшвили (1975), in the collection of scientific papers of the profile «Каракулеводство за рубежом».

Numerous valuable information on the origin, biological particularities, breeding and amelioration of Karakul sheep, as well as their use in crossbreeding with some local sheep races, has been gathered from the numerous scientific papers of the renowned researchers of the Institute of Scientific Research in Animal Husbandry for the steppe districts „Аскания-Нова", СССР (Иванов, 1964a, 1964b; Перегон, 1972) and the Union Institute for Scientific Research for Karakulture in Samarkand (Васин, 1936, 1971; Юдин, 1943; Дъячков, 1950, 1952, 1960, 1973, 1980; Кошевой, 1975; Стояновская, 1964; Одинцова, 1958).

Scientific information on the spread, the growth, breeding and use of Karakul sheep at the crossbreeding with the local Ţurcana race was taken from the bibliographic sources of some famous researchers in Romania (Nica, 1937, 1940; Ştefănescu, 1961; Ştefănescu et al., 1973; Pop et al., 1976; Pascal, 2007, 2011; Pascal et al., 2010).

Scientific informative data on the spread and biological particularities of the Karakul sheep race in the Republic of Moldova, as well as the results obtained at their crossbreeding with local sheep from the Ţuşca race, were collected from valuable scientific papers by the remarkable university professor Iliev Tudor (1992), known in the Soviet period as Ильев (1957, 1965, 1966, 1969, 1976, 1981, 1984). Much of the necessary scientific information

was taken from the works of the former director of the Moldavian Subsidiary for Scientific Research in Karakulture, Богданович (1957, 1979, 1983, 1984), as well as from some of our later works (Бузу et al., C.A., 1992; Buzu, 1995, 1996, 2001, 2015, 2016, 2017, 2018).
The materials of all the relevant scientific information were processed and systematized, according to the scientific synthesis methodology.

RESULTS AND DISCUSSIONS

The origin. From the point of view of zoological systematics, Karakul sheep belong to the class *Mammals*, order *Ongulate*, suborder *Artiodactyle* (*Paricopitate*), group *Ruminantia* (*Ruminant*), family *Cavicornia,* subfamily *Ovidee*, genus *Ovis*, species *Ovis aries*, subspecies *Ovis arkar* (Iliev, 1992; Pop et al., 1976; Ștefănescu et al., 1973). According to Борисенко (1967), most researchers, based on the analysis of fossils found by american professor Dyurst in the excavations of the town of Anau (suburb of Ashgabat) and their similarities with sheep of contemporary races, believe that long-tailed and fat sheep races come from wild forms of the subspecies *Ovis Vignei Arkar*, existing 5000-3500 years before our era. Given that the Karakul race is part of this group of races, we can conclude that *Ovis arkar* is the wild ancestor of this race.
Regarding the time (ancient or contemporary) and the place of formation of the Karakul race, as well as the process of occurrence of loops (result of mutations, interracial crosses, selection, etc.) in the scientific community, so far, there are divergent views.
A number of researchers (Trauer, 1963; Franke, 1973; Лангле, 1964), believe that the Karakul sheep race has ancient origins and comes from sheep with coarse wool and fat tails, whose lambs had furs with rolled loop. This loop was often embossed on various stone carved objects, bas-reliefs, ceramic tiles from the regions of Mesopotamia, as well as described by the great writers, travelers and geographers of antiquity. From the lambs with such curls were obtained the furskins known as „Merlușca". Furskins of this specification were used by the ancient Persians to make specific hats and were often brought as a gift to noble masters.
The American researcher, Юнг (1914a, 1914b), who visited the Emirate of Bukhara in the late 19th century and imported Karakul sheep to North America, believes that the Karakul sheep race originated in Turkestan in the 16th-17th centuries from the crossing of local Danadar sheep (black sheep, small , with long shiny wool, small head, straight nasal profile, with small ears straight in parts, thin legs and long tail similar to that of the dog), existing until recently in this region, with Afghan white Kurdiuk sheep with fine wool. The author is of the opinion that the European races Ţuşca, Reşetilovca, Sokoliska and Malâci also come from the Donadar race.
University professor Ильев (1969, p.8) in his work "Sheep breeding in Moldova" suggested the idea that *"Moldavian sheep Ţuşca has a common origin with the Karakul race, having as hereditary basis the skills of an old race of sheep for furskins that once spread on our country, southern Ukraine, Crimea, Uzbekistan, coming from Central Asia*"
According to the research of Hornitschek (1939), sheep with coarse wool and fat tail, whose lambs have loops of different shapes, are found in Syria, Iran, Palestine, Ethiopia, Somalia. But the most accurate forms of looping have been noted in Iraqi sheep, hinting that the Karakul race originated in that country.
According to us, the author might be right, considering that in Iraq there is a fairly large city called Kirkuk, similar to the name of one of the most valuable varieties of Kirpuk furskins, according to the official ГОСТ no. 8748-70 (1970). We believe that the name of this kind of furskin comes from the respective locality in Iraq.
Onur et al. (2013) report that in the province of Aydin in Turkey, sheep of the Cine Capari breed are currently race, which according to their external forms are very similar to the contemporary Karakul race. It is possible that the origin of this local races was related to the varieties of the Karakul race from neighboring countries (Syria, Iraq, Iran).
Adametz (1927) of the Agricultural University of Vienna, believes that the mutation of the fat tail in sheep first occurred in 2000 years until enin Syria, Palestine, Mesopotamia. Later, in

mutated-tailed sheep in these regions, the mutation of the furskin curls appeared from 1500-1600 years ago. Aspects depicting the loincloths were found on the ancient bas-reliefs of northern Syria 1300 years ago with the appearance of the king of the Hivites (Syrian tribe), whose hat and coat collar were made of looped furskins, similar to those of Karakul. The author notes that the remains of sheep for the furskins were found near Baghdad, but they are of mediocre quality. According to him, Turkestan (Bukhara, Hiva, etc.) was conquered by the Arabs in 751, populating it with their nomadic tribes from Syria and Mesopotamia, who brought with them cattle and Karakul sheep.

Academician Ivanov (1964б) supports Adametz's theory that Karakul sheep were brought to Bukhara in the 8th century by Arab invaders, hence the name "araby" sheep.

Демянко (1912) considers that the homeland of Karakul sheep is Southwest Asia, especially Bukhara, Hiva, Persia and Afghanistan.

Карпов has a similar opinion (1912, p. 3), who remarked that "*the only place in the world to create this "black rose" of sheep for furskins is the Emirate of Bukhara, and the main world trade auction for this fur is the fair in Nijnii Novgorod*".

Therefore, most researchers draw conclusions that the Karakul sheep race has ancient or medieval origins and was formed in Asia Minor or Central Asia.

At the same time, some specialists in the field (Гигинейшвили, 1976; Дъячков, 1973; Одинцова, 1958) consider that the race of sheep brought by the Arabs in the 8th century was not the Karakul race itself. The process of transforming local primitive races into high-performing races is quite sustainable and does not always end with the creation of specialized races, therefore, many ancient fat-tailed races have not reached the perfect level of furskin curling qualities. The creation of domestic animal races in the past is related to the emergence of capitalism, the growth of cities, the development of industry, the expansion of sales markets, the increase of the assortment requirements, quality and quantity of fur productions.

In this context, Одинцова (1958), Егоров (1971), Дъячков (1980) and others consider that the Karakul sheep race, specialized in the production of furskins, was created in the XVII-XVIII centuries in the Karakul district of the Emirate of Bukhara. The authors argue for the creation of this race, in large part, by developing trade relations with the furskins in Tsarist Russia on the markets of Astrakhan, from where, through the Volga River, they were transported to Nizjny Novgorod.From this international fur fair, Karakul and „Merluşca" furskins were spread by skippers throughout Europe and North America, where they were used to make hats, collars and jackets with fur on the outside. Therefore, in the early stages of the fur trade, the furskins of lambs and sheep of the Karakul race were known as "Astrahani-Karakul" (Bresson, 1940).

Biological particularities. The Karakul sheep race has a number of biological particularities, one of which is paramount and refers to the unique, very beautiful fur of the newborn lamb, slaughtered 1-5 days after birth. According to its value, the Karakul furskin is considered a luxury fur, located in the same line with the noblest natural furs (rat, mink, fox). This is explained by the superior and aesthetic qualities of the curl, the excellent silkyness of the hair coating, the perfect thermal properties, as well as the durable resistance to exploitation of furskins garments.

Unlike other racess, Karakul sheep have a very rich morpho-productive polymorphism. This polymorphism refers, first of all, to the properties of the hair coat (Adametz, 1911, 1927; Васин, 1936; Васин et al., 1971; Гигинейшвили, 1976; Buzu, 2016; Pascal, 2007).

Karakul lamb furskins with loops of different types (wave, bob, ridges, rings, etc.) and shapes (tubular, rib, flattened), with various varieties of curling (jacket, rib, flat, kaukasian, moored) have a wide range of colors (black, greyish, gray, brown, pink, white), shades (dark, medium, light) and coloration (blue, gold, silver, pearl, bronze, platinum, diamond, amber, steel, etc.). The fibers of the hair coat have various properties of gloss, silkiness, elasticity, etc. The specific properties of the hair coat, together with the skin properties, together form the furskin qualities of Karakul lambs (Дъячков & Письменная, 1952;

Иванов, 1964a; Кошевой, 1975; НелДжеймс, 1975; Шеффер, 1977; Buzu, 2016).

According to the characteristics of the biological particularities of the sheep of the Karakul race, after Васин (1971), they have less developed sweat glands compared to other races, because the abundant secretion of sweat is accompanied by considerable consumption of water from the body, which the Karakul sheep rarely receives. The same consumption of water is for frequent breathing, when animals use it to cool the body. Therefore, the respiration rate in Karakul sheep is lower compared to other sheep races.

According to the data of Алексеева (1953), the blood of Karakul sheep has a lower erythrocyte content, which indicates the more rational assimilation of oxygen compared to other races.

According to Васин (1936, 1971) and Дъячков (1950, 1973, 1980), Karakul sheep are quite small and late. The body weight of ewes is 40-45 kg, of rams 50-60 kg. This body mass of sheep is reached late, at the age of 6-7 years. The gestation period of Karakul sheep is relatively long - on average 151 days, while in the Romanov race - 148 days, in the English meat races 145-147 days. As a result, the body weight of the lamb at birth is relatively high and constitutes 11-12% of the mother's mass, while in English races for meat 7-9%. These particularities were formed in the process of evolution as properties of adaptability of the organism to the conditions of the desert and semi-desert environment with conditions of arid heat and drought. When calving takes place in unstable climatic conditions, under the open sky, without shelters, well-developed lambs survive more easily. Born quite chewed, the Karakul lamb grows rapidly and at the end of the first month of life reaches a body weight of 8-10 kg. Subsequently, the growth rate decreases. The ability of Karakul lambs to grow rapidly in the first months after birth is, again, a feature of adaptability, in which the lamb rushes to increase its weight in favorable conditions of maternal nutrition in ephemeral pastures, until the unfavorable conditions of terrible drought. The external appearance of the Karakul sheep expresses its general underdevelopment. In addition to his short stature, the Karakul sheep have narrow chest and back, bevelled saddles and croup, constitutional dryness and poorly expressed meat skills.

The prolificacy of the Karakul race is not high (105-110%), because under natural conditions, twin lambs usually perish due to poor development. Hence, the birth rate is low and is 70-90% (www.fao.org/docrep/010/ah806e/AH806E13.htm//Top/OfPage, 2016). The fat tail is an obvious adaptability of the Karakul sheep, expressed by the ability of fat to accumulate in the kurdiuk which allows it to provide reserves of energy food and water for periods of famine and drought.

Ильев (1969, p. 40) mentioned that "*Karakul sheep, compared to Ţuşca, give much less milk and, as a rule, end lactation much earlier than Ţuşca*". The fact that the milk production of Karakul sheep is low and lactation is shorter (100-130 days), is confirmed by several authors, publishing data on the amount of milk of ewes per lactation, from 25-40 kg in Bukhara (Дъячков,1980), 43.3-49.3 kg in Romania (Nica, 1937, 1940; Ștefănescu, 1961), 50 kg in Germany (Hundt, 1954), 40-45 kg in Afghanistan (www.fao.org/docrep/010/ah806e/AH806E13.htm, 2015), 40-50 kg in Iran (www.fao.org/docrep/010/ah806e/AH806E13.htm, 2015), up to 55.0 kg in the Republic of Moldova (Ильев, 1966b).

Karakul sheep are very mobile and active, have robust legs, light skeleton, which allows them to travel long distances during the day and night in search of vegetation for food, efficiently using dry and very rare vegetation from the pastures of deserts and semi-deserts. as a rule, it cannot be used by other species of domestic animals (Васин et al., 1971).

At the same time, Karakul sheep are extremely sensitive, compared to other sheep races, to the conditions of rain and wet pastures, infected with helminths. In some northern countries, helminthic invasions have become an impenetrable barrier to raising Karakul sheep in new conditions (Ильев, 1966b). According to this author, after the importation of Karakul sheep in 1913 from Bukhara to Todireşti, Bender, Bessarabia and, in 1933, in Cucuruzeni, Orhei, Moldova, where they were bred in purerace, they perished for two years due to strongyloidosis and dictyocaulosis, respectively, 17 and 60% of the imported herd.

Therefore, the knowledge of the biological particularities of the sheep race, expected for breeding, reproduction and genetic amelioration, is extremely necessary both for relectors, zoo-veterinary specialists and for breeders (owners) of these animals.

Spread of the Karakul race. Due to the biological particularities of Karakul sheep (unique in the world) and the ever-increasing demand for their lamb furskins, animals of this race were exported in the late 19th and early 20th centuries, either from Turkestan or other Russian governments, in Bosnia, 1894 (Турек, 1975), Germany, 1903 (Буссов, 1967) and 1913 (Иванов, 1914), USA Texas for the property of Yung, 1908 (Иванов, 1914), Romania, 1910 (Барта et al., 1977), Austria, 1907 (Турек, 1975), Namibia, 1907 (Филлингер, 1975a). From Europe, Karakul sheep have been exported to Africa, America (USA), Argentina, Canada and other parts of the world.

The expansion of fur markets in Europe has led to an increase in demand for the quantity and quality of Karakul furskins, leading to an increase in their price value. The unpretentiousness of Karakul sheep in maintenance, the relatively low cost of exploitation, the relatively convenient prices and the ever-increasing trade in furskins, have produced a real boom in the development of this race of sheep in the Central Asian Emirates, where Karakul sheep herds have increased sharply. At the end of the 19th century and the beginning of the 20th century, the growth rate of Karakul sheep herds accelerated, due to their high demand in Europe for the reproduction of breeding material. Thus, during this period, the Karakul sheep race becomes specialized in the production of a widely consumed productively, such as the furskin of the lamb in the first days after birth (Дъячков, 1980).

The spread of Karakul race sheep in Europe, and from here in many countries around the world, is due to the advancement and propagation, by Prof. University of Harikov Zaikevici A.E., since 1880, of the idea of genetic ameliorationof local sheep with coarse wool for the furskins, by crossing with the Karakul race from Bukhara (Иванов, 1914). The effect of improving the furskin qualities of lambs obtained from these crosses was confirmed by several sheep breeders at that time (Buzu, 2001).

To achieve this idea, starting with 1882, the Ministry of Property of the State of Tsarist Russia, together with the governmental zemstvels of Poltava, Tavria and Bessarabia and with some interested private investors, carried out a series of expeditions for the purchase of Karakul sheep from Turkestan (Юнг, 1914a).

According to the account of Карпов (1912, p. 4], due to the excessive rise in prices for Karakul furskins, increased interest in this race worldwide. Thus, "*the West, which not long ago knew this race more only from rare photographs and zoos, in the last time initiates whole expeditions for the acquisition of these precious animals, studies them in the homeland, implements their breeding in new territories, establishes Karakul sheep nurseries, even in Africa and America*".

According to the communication of Иванов (1914), the first import into Bessarabia of Karakul sheep was made in 1884 from the Transkaspia region with the support of the Zemstva of the Bessarabian government and the Russian Ministry of State Property. The second import was made from Bukhara in 1888 by the Agricultural Society of Bessarabia through the Agricultural Society of Poltava. Between 1889 and 1898, with the contribution of General Krupenschii (former governor of Bessarabia), several expeditions were made to import Karakul sheep from Bukhara. Later (1902-1913), representatives from Bessarabia participated in four import expeditions of Karakul sheep from Bukhara, organized by the Agrarian Society of Poltava Government. By the 1910s, the largest buyers of imported Karakul sheep became brothers - Sinadino boyars (of Greek ethnicity), who established in the village of Oniţcani, Orhei District, a farm of 700 heads of purerace Karakul sheep. According to the same author, in the Hotin District, Karakul sheep aroused a special interest. During this period, the Karakul sheep farms of General Krupenschii M.G. were established here by import in the village of Lomacineţ - 295 chap, of the Kaufman brothers in the village of Corjeuţi - 300 chap and Feteşti

village - 200 chap, of the owner Tevanov in Târnovo village - 278 chap.
In the Bender District, Zemstva set up an Agricultural Chamber of 110 purerace Karakul sheep, which were used in a directed way to improve the Țușca sheep in the area. In smaller quantities, Karakul sheep were purchased by several owners from Chisinau, Akerman, Soroca, Balti, Orhei and others (Buzu, 2001).
As we can see from this information, in Bessarabia at that time a real "boom" started to amelioration the Țușca aboriginal sheep by crossing with the imported Karakul sheep, which contributed to the improvement of the furskins qualities, without taking into account the impact of this crossing on the other important morpho-productive characters, such as: milk production and body mass (meat production).
According to Pecuta (1938), from the Karakul flock of the "Onitcani-Synadino" farm, Bessarabia, in the 1930s, sheep were exported to the south of France, Bulgaria, Algeria and Portugal, which acclimatized. relatively well.
At present, Karakul sheep are mainly distributed in Asia, mainly in Central Asia, as well as in Africa. In the countries of these regions and continents are the largest effectives of Karakul sheep in the world (Table 1).

Table 1. Karakul sheep effective in the main countries of distribution (after Buzu, 2017)

No. ord	Name of the country	Estimated effective, thousand head
1.	Uzbekistan	11,825.1
2.	Kazakhstan	6,079.1
3.	Turkmenistan	12,600.0
4.	Tajikistan	1,006.2
5.	Afganistan	5,256.4
6.	Namibia	2,871.4
7.	Republic of South Africa	1,250.0
8.	România	706.7
9.	Republic of Moldova	350.0

By generalizing the historiography of the spread and growth of Karakul sheep in the world, based on some estimates previously made by us (Buzu, 2017), we can conclude that they have taken root in sustainable growth „in those regions, parts and continents of the world with countries poorly developed (from Africa, Asia), with large rural human populations, living in arid areas of vast plains, semi-deserts with poor vegetation, where sheep are kept year-round in natural conditions without capital investment, with minimal costs".
For these human populations, sheep are an indispensable source of existence and survival in the difficult conditions of nature.
Due to special biological features (superior furskin qualities of newborn lambs), Karakul sheep have aroused curious interest around the world, spreading in small effectives on continents other than Asia and Africa.
Thus, in Western European countries (Austria, Germany, France etc.), as well as in South America (Argentina) and North America (USA), Karakul sheep have been imported for the purposes of scientific research and cross-breeding experiments with some races of local sheep. In some European countries (Austria, Germany), Karakul sheep have started to be bred (albeit in small effectives) for the purposes of purerace breeding and export marketing (South Africa, Namibia) of young.
Due to low meat and milk skills, as well as highly preserved specific heredity, the use of the Karakul race at the crossbreeding with local sheep races has had a negative impact on these morpho-productive traits. For example, in the Republic of Moldova, mass crossbreeding of local ewes (Ț x K) with Asian Karakul rams has led to some improvement in the furskin qualities of lambs and, at the same time, to a decrease in sheep's resistance to environmental weather, a significant decrease in viability, body mass and milk production (Buzu, 2018).
Due to the very specific biological particularities, the sustainable spread of the Karakul sheep race, unique in the world, was successful only in some regions and areas, which according to the pedo-climatic specificity corresponded more adequately to the physiological requirements of the animal organism and maintenance and exploitation conditions theirs.

CONCLUSIONS

The views of various researchers on the origin and formation of the Karakul sheep race have so far differed. Some researchers (Trauer, 1963; Franke, 1973; Лангле, 1964; Hornitsehek, 1939), consider that the Karakul sheep race has ancient origins (1300 years) and

formed them in the regions of the former Mesopotamians, others (Adametz, 1927) mention that it has medieval origins and was brought to Central Asia by the Arabs in the VIII century, and the third group of researchers (Юнг, 1914; Одинцова, 1958; Дъячков, 1980; Егоров et al., 1971) relates that the true Karakul race originated in the modern period (XVI-XVIII centuries) and was formed in Central Asia (Ashgabat, Bukhara regions) by selecting local pseudo-Karakul sheep in the direction of improving to perfection the furskin qualities of newborn lambs.

The main biological particularities of Karakul sheep is that the hairy shell of newborn lambs is wound in loops of different types (wave, bob, ridge, etc.) and shapes (tubular, rib, flattened), with various varieties of curling. (jacket, bag, flat, kaukasian, moire), consisting of elastic, silky and glossy fibers, with a wide range of colors (black, greyish, gray, brown, pink, white), shades (closed, medium, light) and coloration (blue, gold, silver, geml, pearl, bronze, platinum, diamond, amber, steel, etc). Due to this biological particularityes and the excessively high market requirements for the furskins of newborn lambs, the Karakul sheep race has been spread all over the world, being exploited for the purposes of scientific research and purerace breeding, or by crossing with some local races for amelioration furskin qualities of newborn lambs and the production of commercial furskins.

Karakul sheep have low skills in milk and meat production (low body mass), being extremely susceptible to helminth infections in conditions of high air humidity, rain and wet pastures.

Karakul sheep are widespread in warm arid countries, regions and areas with low humidity, wide plains, semi-deserts, poor vegetation, where they are maintained all year round in natural grazing conditions without capital investment, with minimal costs.

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THE MORPHO-PRODUCTIVE PARTICULARITIES OF QUEENS *APIS MELLIFERA CARPATICA* INSEMINATED INSTRUMENTALLY

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Abstract

The aim of the present research was to reveal, in special experiments, the morpho-productive advantages and disadvantages of instrumentally inseminated queens, compared to their contemporaries naturally paired in nuptial flight.Two scientific experiments were carried out at the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova (ASM), on two similar batches of bee colonies with instrumentally inseminated and naturally mated queens, of Apis mellifera Carpatica race, maintained in similar conditions feeding and maintenance. The results of the research showed that the instrumental insemination of queens is an innovative and efficient technological method for increasing the productivity of bee families, increasing production volumes and raising the economic efficiency of the beekeeping branch. Instrumentally inseminated queens have a higher prolificacy, compared to their contemporaries naturally mated in the nuptial flight, with 164 eggs/24 hours, or 10.0% (t_d = 6.1; P <0.001). Working bees from the families of instrumentally inseminated queens had a higher development of external morphometric indices, compared to their contemporaries from the families of queens naturally mated in nuptial flight, such as humus at: horn length - 0.19 mm, or 2.9% (t_d = 6.71; P<0.001), the ulnar index of the anterior right wing - by 4.3 absolute units, or by 10.2% (t_d = 5.00; P <0.001) and the share of bees with positive discoidal dislocation - by 11.0 absolute units, or by 15.4% (td = 3.10; P <0.01).The bee families of the instrumentally inseminated queens had a significantly higher development of the level of morpho-productive characters, compared to their contemporaries of the queens naturally mated in the nuptial flight, as follows: colony power - by 0.17 kg, or by 6.5% (td = 6.07; P<0.001), disease resistance - by 3.4 absolute units, or by 4.1% (td = 3.15; P <0.01), viability of the seedling - by 4.0 absolute units, or by 4.7% (td = 3.70; P <0.001) and honey production - by 8.39 kg, or by 19.9% (td = 5.31; P<0.001). Bee families of queen-daughters obtained from instrumentally inseminated mother-queens possess a higher genetic potential for productivity than their contemporaries of queen-daughters obtained from mother-queens naturally mated in nuptial flight, as follows: colony power- by 0.12 kg, or by 4.2% (td = 2.26; P<0.05), disease resistance - by 1.8 absolute units, or by 2.0% (td = 2.50; P<0.05), viability of the brood - by 1.9 absolute units, or by 2.1% (td = 2.84; P<0.01) and in honey production - by 5.30 kg, or with 12.6% (td = 3.01; P<0.01).In the following winter, bee families of queen-daughters obtained from instrumentally inseminated mother-queens had a higher wintering capacity (winter hardiness) than their contemporaries of queen-daughters obtained from mother-queens naturally paired in nuptial flight, by 4.0 absolute units, or by 5.0% (td = 2.25; P<0.05).

Key words*: Apis mellifera Carpatica, instrumental insemination, natural mating, queens.*

INTRODUCTION

In the Republic of Moldova is approved for breeding the local race of bees *Apis mellifera Carpatica*, which has a number of valuable morpho-productive biological properties, such as: good adaptation to specific pedo-climatic environmental conditions, increased resistance to wintering and disease, mildness, the increased prolificacy of queens and high skills of accumulating bee production in the nest. The clandestine crossbreeding of this local race leads to negative consequences, such as genetic segregation of the race and the result of crossbreeds with undesirable traits (aggressive, not adapted to local conditions) and low productivity (Siceanu et al., 2002).

For these reasons, according to the National Program for Genetic Conservation and Breeding of the Local Bee Population in the Republic of Moldova, their selection is recommended to be carried out by the method of purerace breeding (Cebotari, 2006).

In animal husbandry, the system of breeding purerace animals provides for the application of a series of methods of selection, over several generations, of individuals required by the performance of one or more characters, as well as of directed mating (nominal or in the group) in order to obtain a predicted (desired)

descendants, with certain specific morpho-productive properties.

In beekeeping, the application of these methods and selection procedures is quite difficult. The main obstacle in the progress of genetic breeding of *Apis mellifera* bee populations is the queen's biological reproductive particularities, characterized by her mating with drones in flight, in open air, in the so-called "nuptial flight" or "mating flight", at a considerable distance from the apiary - in special places for gathering drones. The queen, in this flight, has the instinct of polyandry, expressed by mating with several males, thus filling the spermatic container (spermoteca) with the mixed sperm of several drones of unknown origin. These biological features make it quite difficult to manage the process of mating queens with drones.

In this regard, beekeepers, breeders, for centuries have constantly sought methods to control the mating of queens with drones in order to manage selection, to obtain predictable descendants in the new generation with improved heredity (genetic potential for increased productivity).

Currently, a number of methods are known in beekeeping that allow beekeepers-breeders to carry out the somewhat paired pairing of queens. Among the most common of these are: intensive growth of drones in selected paternal bee colonies and prevention (non-admission) of their growth in all other colonies in the apiary (creation of a "drone barrier"); the double and total change of the queens in the apiary during two consecutive years; isolation of breeders during flight time (evening mating of queens with drones from selected families); isolation of drones in space (organization of breeding points in certain areas of isolated territories);instrumental insemination of queens (Билаш & Кривцов, 1991).

The latter method is the most accurate way to control the pairing of queens with drones of known origin and the most effective method of applying individual zootechnics selection in practice. The instrumental insemination of queens guarantees the knowledge (one hundred percent) of the genealogical origin of the offspring, ensures the possibility of obtaining descendants with a certain predicted heredity and a genetic potential of increased productivity, which makes it possible to increase the selection effect in the new generation.

At the same time, the opinions of beekeepers and researchers in this field, about the influence of instrumental insemination on the quality of queens and the productivity of bee colonies, continue to differ in three groups.

However, most authors, representing the first group (Roberts, 1946; Руттнер, 1975; Woyke, 1976; Wilde, 1987; Бородачев et al., 1987; Билаш & Кривцов, 1991; Boigenzahn et al., 1993; Szalai, 1995; Cermak, 2004; Cobey, 2007; Cebotari, 2013), considers that the instrumental insemination of queens has a beneficial effect on the growth and development of the brood, on the honey productivity of bee colonies, as well as on the longevity of queens.

According to the reports of the researchers of the second group (Cobey, 1998; Gerula, 1999; Konopaska, 1987; Laidlaw, 1992; Nelson, Laidlaw, 1988; Pritsch & Bienefeld, 2002; Vesely, 1984; Карасев, 2011), instrumentally inseminated queens, in practice, do not differ in their qualities from contemporaries naturally paired in the "nuptial flight", therefore, they do not cause any differences in the development of morphic-productive features of bee colonies.

In the opinion of other authors (Harbo & Szabo, 1984; Мукимов, 2002), instrumentally inseminated queens possess some phenotypic deficiencies such as: smaller brood, shorter lifespan, bees accept them more difficult, efficiency Instrumental insemination is low because the technical insemination procedure does not approach the physiological act of natural mating, as a result, their families possess a lower honey productivity. These deficiencies overshadow, to some extent, the advantages of the progressive method of reproduction (instrumental insemination) in beekeeping.

In this sense, the purpose of the present research was to reveal, in special experiments, the morpho-productive advantages and disadvantages of instrumentally inseminated queens, compared to their contemporaries naturally paired in the "nuptial flight".

MATERIALS AND METHODS

To solve the proposed goal, two scientific experiments were organized and conducted at the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova (ASM), on two similar groups of bee colonies with instrumentally inseminated and naturally paired queens of *Apis mellifera Carpatica,* maintained in similar conditions of feeding and care. The apiary was placed permanently (stationary) at the edge of a forest of broadleaf trees. The main sources of nectar and pollen in the apiary area were white acacia, lime and polyfloral vegetation.

The first experiment was conducted on two groups of bee families supplemented by young queens of the same age and with similar genetic background. The bee colonies in batch I (with a effective of 25 families) served as witnesses and were completed with queens naturally paired in the "nuptial flight" in the uninsulated space around the apiary. The bee colonies from group II - experimental (with a effective of 30 families) were completed with instrumentally inseminated queens.

Instrumental insemination of queens was performed in laboratory conditions according to the method of Руттнер Ф., 1975, using the special machine *Latshaw Insemination Instrument* (US production). The seminal material (sperm) freshly taken from the drones from the selected paternal families was inoculated into the respective genital organ of the queen in one go in an amount of 8 mm^3.

The second experiment was conducted on two batchs of bee families completed with queen-daughters of queen-mothers in the first experiment. The first batch consisted of 24 bee families completed with queen-daughters of queen-mothers naturally paired in the "nuptial flight" in the uninsulated space around the apiary. Experimental batch II consisted of 17 bee families completed with queens-daughters of queens-mothers inseminated instrumentally from experimental batch II of the first experiment. In this experiment, the queen-daughters of bee families from both batchs were naturally mated in the "nuptial flight" in the uninsulated space around the apiary.

Throughout the beekeeping season, in the bee families from both batchs involved in both the first and the second experiment, the development levels of the main morpho-productive characters were researched and evaluated, such as: queen prolificacy, morpho-metric characters of bees - the length of the fallopian tube, the ulnar index and the discoidal dislocation, as well as the morpho-productive characteristics of bee families: colony strength, disease resistance, viability of the brood, honey production and winter hardiness.

The development levels of the nominated morpho-productive characters of queens, bee colonies, as well as the external ones of working bees, were evaluated, according to the methodology developed by us (Cebotari & Buzu, 2010) for the Zootechnical Norm regarding the value of bee families, breeding and certification of beekeeping breeding material, approved by Government Decision of the Republic of Moldova no. 306 of 28.04.2011.

The data obtained in the research were statistically processed using the computer software "STATISTICA-12", and the significance of the results was assessed, according to the certainty criteria of Student's error-free probability theory, by the methods of variational biometric statistics according to Плохинский (1989).

RESULTS AND DISCUSSIONS

The research results showed that the instrumental insemination of *Apis mellifera Carpatica* queens has a positive impact, first of all, on the development of the main morpho-productive biological characters of the first generation bee families, therefore, of the queen's own bee families. Subsequently, the positive effect is reflected on the level of development of the morpho-productive characters of the bee colonies from the second generation, therefore, of the bee families of the queen-daughters.

In the first experiment, performed on two batchs of bee families with naturally paired queens and instrumentally inseminated, it was found that the prolificacy of the queens, as well as most of the morpho-productive characters of bee families with instrumentally inseminated queens had a significant growth evolution, compared to those of families with queens naturally paired in the nuptial flight of the uninsulated space around the apiary (Table 1).

Table 1. Level of development of morpho-productive traits of bee colonies supplemented with naturally mated and instrumentally inseminated queens

Character name	Batch 1, colonies with naturally mating queens (N = 25)	Batch 2, colonies with instrumentally inseminated queens (N = 30)	The difference, batch 2 - batch 1		t_d
			absolutely	%	
Prolificity, eggs / 24 hours	1648 ± 20	1812 ± 18	+164	10.0	6.10^{***}
Horn length, mm	6.45 ± 0.02	6.64 ± 0.02	+0.19	2.9	6.71^{***}
Cubital index,%	42.0 ± 0.5	46.3 ± 0.7	+4.3	10.2	5.0^{***}
Discoid displacement +,%	71.5 ± 2.9	82.5 ± 2.1	+11.0	15.4	3.1^{**}
Colony power, kg	2.62 ± 0.02	2.79 ± 0.02	+0.17	6.5	6.07^{***}
Disease resistance,%	83.5 ± 0.9	86.9 ± 0.6	+3.4	4.1	3.15^{**}
Brood viability,%	85.1 ± 0.9	89.1 ± 0.6	+4.0	4.7	3.70^{***}
Honey production, kg	42.19 ± 1.11	50.58 ± 1.13	+8.39	19.9	5.31^{***}
Winter hardiness,%	87.9 ± 1.1	90.3 ± 1.0	+2.4	2.7	1.61

Remark:$^{+}$ - positive discoidal dislocation; ** - P< 0.01; *** - P< 0.001.

It was found that instrumental insemination has a positive influence, first of all, on the queen's prolificacy. Thus, the instrumentally inseminated queens from experimental batch 2 exceeded by prolificacy, their contemporaries naturally mated from batch I (control), with 164 eggs/24 hours or by 10.0% (t_d = 6.10; P<0.001).

According to us, the increased prolificacy of the queens in batch 2 is explained by the fact that instrumental insemination ensures a fuller filling of the queen's spermotec with semen and sperm needed to fertilize the eggs. The saturated degree of filling of the spermotec with the necessary sperm exerts a hormonal stimulation on the activity of the queen's ovarian glands, causing the activation of ovogenesis and increasing the prolificacy of queens.

On the contrary, the natural mating of queens in the nuptial flight does not always ensure a sufficient saturation of the spermotec with male semen. Therefore, some queens perform a few nuptial flights in the hope of filling the spermotec with the necessary semen, and others, remain with the semiplegic spermotec and usually have a lower prolificacy.

Due to the fact that working bees from the families of instrumentally inseminated queens come from the paternal line from drones from selected families, with well-developed morpho-productive characters, they (bees) inherited from their parents a higher level of development of morpho-metric characters outside.

Thus, the worker bees from the families of experimental batch 2 of the instrumentally inseminated queens significantly exceeded the length of the horn, their contemporaries from the families of batch I (control) of the naturally paired queens, by 0.19 mm or 2.9% (t_d = 6.71; P <0.001). This means that worker bees from batch 2 families have a higher capacity to capture nectar from the flower corolla tube of different honey plants, especially those with the deeper corolla flower tube.

The ulnar index of the anterior right wing of worker bees from the experimental batch 2 families of instrumentally inseminated queens was significantly higher compared to their contemporaries from the families of batch I (control) of naturally paired queens, with 4.3 absolute units, or 10, 2% (t_d = 5.0; P <0.001), which more adequately corresponds to the race standard.

The share of bees with positive discoid dislocation of the radial and ulnar ribs of the anterior right wing in the group 2 bee families with instrumentally inseminated queens was higher compared to their contemporaries in the group 1 families with queens naturally mated in nuptial flight, with 11.0 absolute units, or by 15.4% (t_d = 3.1; P<0.01), which also more adequately corresponds to the race standard according to this character.

Due to the greater prolificacy of instrumentally inseminated queens, their bee colonies had a more abundant development of perennial brood, which contributed to the increase in the amount of bees in the nest. Thus, the power of bee families in batch 2 with instrumentally inseminated queens was significantly higher, compared to their contemporaries in batch 1

with queens naturally mated in the nuptial flight, by 0.17 kg or 6.5% (t_d = 6,07; P<0.001). More eloquently, the advantages of bee families of instrumentally inseminated queens, compared to those of queens naturally paired in nuptial flight, are reflected in the histogram (Figure 1).

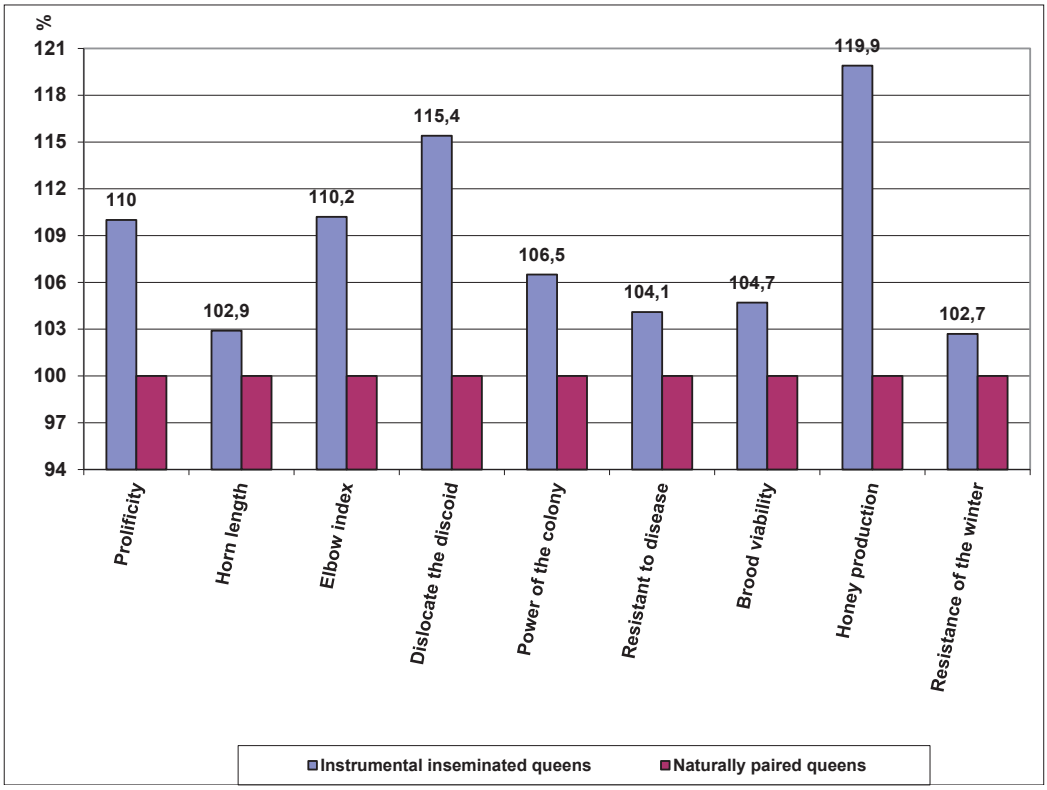

Figure 1. The value of the morpho-productive characters of the bee families with instrumentally inseminated and naturally paired queens

The histogram shows that the bee families in batch 2 with instrumentally inseminated queens essentially exceed, to a different extent, their contemporaries in batch 1 with queens naturally mated in the nuptial flight, according to the value of all the characters investigated.

Due to the insemination of queens with drone semen from selected families, a generation of vigorous and disease-resistant working families was obtained, which in turn ensured an increased viability of the brood.

Thus, bee families in batch 2 with instrumentally inseminated queens had a higher resistance to disease (hygienic instinct) compared to bee colonies in batch 1 with queens naturally mated in nuptial flight, with 3.4 absolute units, or with 4.1% (t_d = 3.15; P<0.01). At the same time, the viability of the brood of bee families from batch 2 with instrumentally inseminated queens was significantly higher, compared to that of the brood of bee families from batch 1 with queens naturally mated in the nuptial flight, with 4.0 absolute units, or with 4.7% (t_d = 3.7; P<0.001).

Overall, given that the prolificacy of instrumentally inseminated queens was significantly higher, as well as the morpho-productive characters of the bee families of these queens had a more advanced development, they ultimately ensured a clear increase in capacity. accumulation in the nest of bee production.

Thus, the honey production accumulated in the nest increased from 42.19 ± 1.11 kg in the bee families from batch 1 with queens naturally

mated in the nuptial flight, to 50.58 ± 1.13 kg in the bee families from batch 2 with instrumentally inseminated queens. The increase was quite significant, constituting 8.39 kg, or 19.9% (t_d = 5.31; P<0.001). This increase had the highest certainty threshold, according to the theory of probability of error-free predictions after Student (Плохинский, 1989).

It is important to mention that the bee families from batch 2 with instrumentally inseminated queens entered the winter strong and vigorous. In the spring of the following beekeeping year, it was found that bee families in batch 2 with instrumentally inseminated queens had a higher tendency to winter resistance compared to their contemporaries in batch 1 with queens naturally mated in the nuptial flight.

In the second experiment, conducted on two batchs of bee families completed with queen-daughters of queen-mothers in the first experiment, it was found that the natural mating of queens in nuptial flight causes a slight genetic segregation of the degree of development of morpho-productive characters of the second generation bee colonies. This is confirmed by the fact that the difference between the level of development of the morpho-productive characters of the bee families with queen-daughters from batch 1 and those with queen-daughters from batch 2 decreased.

At the same time, research has shown that the families of queen-daughters of instrumentally inseminated mother-queens differed in the positive direction from the contemporaries of daughter-daughters naturally paired in nuptial flight according to the biological features of the main morpho-productive characters (Table 2).

Table 2. The value of the morpho-productive characters of the bee families with queen-daughters obtained from naturally paired mother-queens and instrumentally inseminated

Character name	Batch 1, the queen-daughters of the mother-queens naturally paired (N = 24)	Batch 2, the queen-daughters of the instrumental-inseminated mother-queens (N = 17)	The difference, batch 2 - batch 1		t_d
			absolutely	%	
Prolificity, eggs / 24 hours	1782 ± 12	1815 ± 23	+33	1.9	1.27
Horn length, mm	6.57 ± 0.01	6.61 ± 0.01	+0.04	0.6	2.86**
Cubital index,%	45.7 ± 0.6	46.9 ± 1.1	+1.2	2.6	0.96
Discoid displacement +,%	77.0 ± 3.6	83.0 ± 2.1	+6.0	7.8	1.43
Colony power, kg	2.89 ± 0.02	3.01 ± 0.05	+0.12	4.2	2.26**
Disease resistance,%	88.5 ± 0.4	90.3 ± 0.6	+1.8	2.0	2.50*
Brood viability,%	90.1 ± 0.3	92.0 ± 0.6	+1.9	2.1	2.84**
Honey production, kg	41.92 ± 1.06	47.22 ± 1.41	+5.30	12.6	3.01**
Winter hardiness,%	80.7 ± 1.1	84.7 ± 1.4	+4.0	5.0	2.25*

Remark:+ - positive discoidal dislocation; * - P< 0.05; ** - P< 0.01.

From these particularities, the difference is more pronounced according to the characteristics of the horn length, the viability of the brood, the resistance to diseases, the strength of the colony and, most importantly, the production of honey.

At the same time, analyzing the prolificacy of the queens in the comparative experimental batchs, we found that the queens of the bee families in the experimental batch 2, which are the daughters of the instrumentally inseminated queens, had only a higher tendency of prolificacy compared to the contemporary queens in batch 1, because the difference between the value of this character of the comparative queens was not significant (P>0.1).

Such trends were also observed at the level of development of some external characters, such as the ulnar index and the positive discoidal dislocation of the nerves of the radial cell and the ulnar one of the anterior right wing.

Thus, the ulnar index in working bees of families in experimental batch 2 had a tendency to exceed, compared to contemporaries in batch 1, by 1.2 absolute units, or by 2.6%, the difference being insignificant (P>0.1).

Such a trend was also found in the positive discoidal dislocation of the nerves of the radial and ulnar cells of the anterior right wing, which

is slightly higher in bees of families in batch 2, compared to contemporaries in batch 1, with 6.0 absolute units, or 7.8%, but this difference is not significant (P>0.1).

At the same time, the working bees of the families from batch 2, whose mothers were the daughters of instrumentally inseminated queens, substantially exceeded their contemporaries from batch 1, whose mothers were the daughters of queens naturally mated in the nuptial flight, by the length of the horn - by 0.04 mm, or 0.6% (t_d = 2.86; P<0.01). Given that the variability of this external character of bees is very narrow, this difference is quite significant with the certainty of the second threshold of the probability theory of error-free predictions after Student.

Significant differences in favor of bee families in batch 2 were found after other morpho-productive characters, quite important, such as disease resistance (hygienic instinct) and viability of the brood, which shows directly or indirectly towards a higher resistance of these families against specific diseases.

Thus, the bee families of daughter queens obtained from instrumentally inseminated mother queens outnumbered their contemporaries, whose mothers were naturally mated in the nuptial flight, by disease resistance - by 1.8 absolute units, or by 2.0% (P<0.05) and according to the viability of the brood - by 1.9 absolute units, or by 2.1% (P<0.01).

More eloquently, the advantages of bee families with queen-daughters obtained from instrumentally inseminated mother-queens, compared to the families of queen-daughters obtained from naturally-paired queen-mothers, are reflected in the histogram (Figure 2).

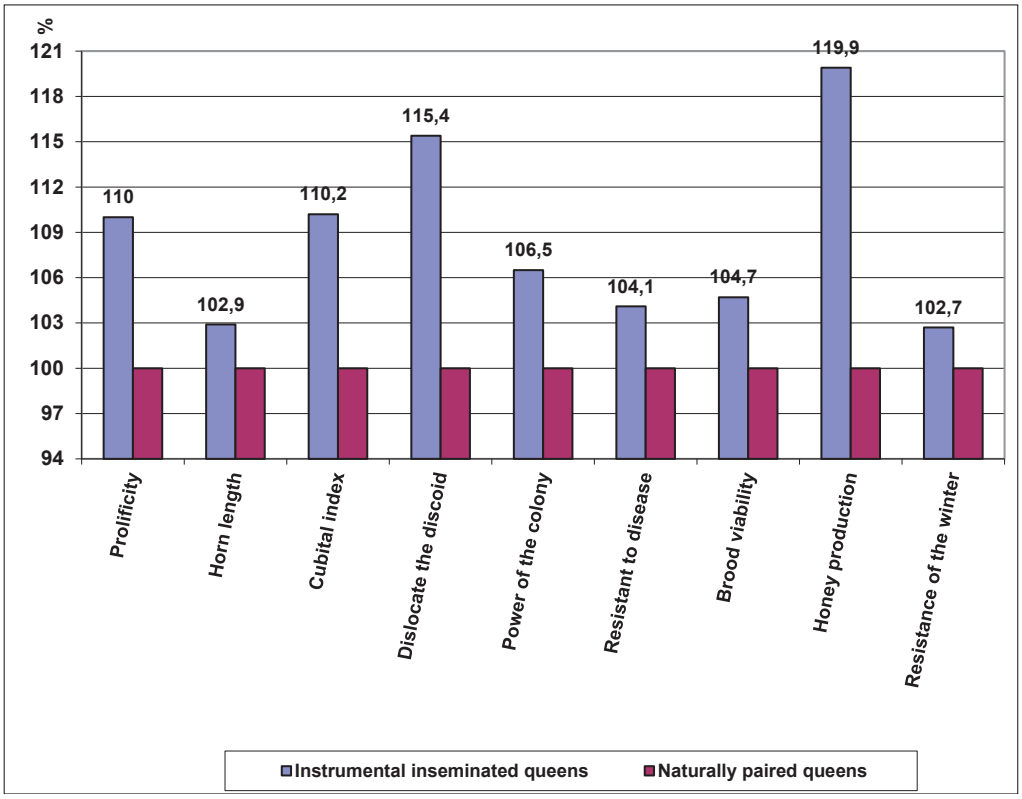

Figure 2. The value of the morpho-productive characters of the bee families with queen-daughters obtained from naturally paired queen-mothers and instrumentally inseminated ones

From the histogram it can be seen that the bee families with queen-daughters of the queen-mothers inseminated instrumentally, exceed to a different extent, according to the level of

development of most of the morpho-productive characters researched, the bee families with queen-daughters of queen-mothers naturally paired in the nuptial flight.

Thus, the bee families in experimental batch 2, whose queens came from instrumentally inseminated mother queens, significantly outnumbered their contemporaries from mother pairs naturally mated in nuptial flight and after winter hardiness by 4.0 absolute units, or with 5.0% (t_d = 2.25; P<0.05), as well as after the strength of the colonies - by 0.12 kg, or by 4.2% (t_d = 2.26; P<0.05).

The most substantial advantage of bee families in batch 2, with queen-daughters of instrumentally inseminated mothers, is that they considerably outnumber their contemporaries in the control batch by the production of honey accumulated in the nest - by 5.3 kg, or by 12.6% (t_d = 3.01; P<0.01). This difference is highly significant with the certainty of the second threshold of the probability theory of error-free predictions after Student (Плохинский, 1989).

The substantial overtaking of bee families with queens-daughters of queens-mothers inseminated instrumentally after honey production and winter hardiness, compared to those in the control batch, is due to the fact that the selection process in the bee population of the experimental apiary was directed to improve, first and foremost, these traits, as well as the strength of the colony, disease resistance and viability of the brood. As can be seen, these characters acquired in the selection process a more consolidated heredity, which is constantly passed down through inheritance from mothers to descendants.

The obtained data confirm the scientific conclusions of some researchers (Билаш & Кривцов, 1991; Бородачев et al., 1987), which states, that daughters of instrumentally inseminated mother queens, under all equal conditions, have a higher genetic potential for productivity than their contemporaries obtained from mother queens naturally paired in nuptial flight.

Therefore, generalizing the results of our research, we can conclude that the bee families of instrumentally inseminated mother queens, as well as of daughter queens obtained from instrumentally inseminated mother queens, certainly possess a higher level of character development morpho-productive, compared to the contemporaries of queen-mothers and queen-daughters naturally paired in nuptial flight.

This was achieved due to the directed mating of the parental partners and the formation in the descendants of the working bees, as well as in the queen-daughters of a claimed inheritance with genetic potential of increasedproductivity. In all other equal conditions of maintenance and supply of nectar-polleniferous food, instrumentally inseminated mother-queens, as well as their daughter-queens, generate increased productivity and a higher economic effect.

In this sense, the advantages of instrumentally inseminated queens, as well as of daughter-queens obtained from them, are quite obvious and with high certainty. These advantages must not be ignored both by scientific researchers in the theoretical research-innovation activity and by beekeepers in the practical selection activity for the genetic improvement of bee colony populations, increasing productivity and production volumes, increasing the economic efficiency of the beekeeping branch.

CONCLUSIONS

1. Instrumental insemination of queens is an innovative technological method effective for increasing the productivity of bee families, increasing production volumes and raising the economic efficiency of the beekeeping branch.
2. Instrumentally inseminated queens have a higher prolificacy, compared to their contemporaries naturally paired in the nuptial flight, with 164 eggs / 24 hours, or 10.0% (td = 6.1; P<0.001).
3. Working bees from the families of instrumentally inseminated queens had a higher development of external morpho-metric indices compared to their contemporaries from the families of queens naturally mated in nuptial flight, as humid to: horn length - by 0.19 mm, or by 2.9% (t_d = 6.71; P <0.001), the ulnar index of the anterior right wing - by 4.3 absolute units, or by 10.2% (t_d = 5.00; P<0.001) and the weight bees with positive discoid dislocation - by 11.0 absolute units, or by 15.4% (t_d = 3.10; P<0.01).

4. The bee families of the instrumentally inseminated queens had a significantly higher development of the level of morpho-productive characters, compared to their contemporaries of the queens naturally mated in the nuptial flight, as hummed to: power of the colony - by 0.17 kg, or with6.5% (t_d = 6.07; P<0.001), disease resistance - by 3.4 absolute units, or by 4.1% (t_d = 3.15; P<0.01), viability of the brood -by 4.0 absolute units, or by 4.7% (t_d = 3.70; P<0.001) and honey production - by 8.39 kg, or by 19.9% (t_d = 5.31; P<0.001).

5. Bee families of daughter- queens obtained from instrumentally inseminated mother queens have a higher genetic potential for productivity than their contemporaries of daughter- queens obtained from mother- queens naturally mated in nuptial flight, as follows: colony strength - 0.12 kg, or 4.2% (t_d = 2.26; P <0.05), disease resistance - 1.8 absolute units, or 2.0% (t_d = 2,50; P <0.05), viability of the brood - by 1.9 absolute units, or by 2.1% (t_d = 2.84; P<0.01) and in honey production - by 5.30 kg, or 12.6% (t_d = 3.01; P<0.01).

6. In the following winter, bee families of daughter- queens obtained from instrumentally inseminated mother- queens had a higher wintering capacity (winter hardiness) than their contemporaries of daughter- queens obtained from mother- queens naturally mated innuptial flight, with 4.0 absolute units, or 5.0% (t_d = 2.25; P<0.05).

7. The use for implantation (substitution of old queens) of instrumentally inseminated queens, as well as of daughter- queens obtained from them, ensures a selection effect in the genetic amelioration of the population of bee colonies in the apiary.

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HIGHLY EFFECTIVE METHOD OF DNA EXTRACTION FROM BLOOD: A FIRST STEP FOR ANALYSIS OF GENETIC DIVERSITY OF INDIGENOUS CATTLE BREEDS

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Abstract

In molecular genetics analysis, isolation and quantification of DNA represents a step extremely important. DNA isolation methods are based on purity, integrity and the amount of DNA obtained. The degree of DNA purity is one of the most important factors in the reproducibility of the PCR method. DNA is considered pure if the ratio of the two spectrophotometric readings, A260/A280, shows values between 1.7 - 2.0. Values lower than 1.7 indicate impurities with proteins, and a ratio higher than 2.0 indicates impurities with other contaminants. The aim of this research was to evaluate the effectiveness of the automatic method of DNA extraction from a number of 24 blood samples collected from Pinzgauer cattle breed, for the analysis of genetic diversity. The amounts of DNA extracted from the 24 blood samples ranged from 13-61.9 ng/µl and the absorbance ratio A260/A280 showed values between 1.61-2.13. The results obtained demonstrated the effectiveness of the automatic method of DNA extraction, and thus, isolated and quantified genetic material can be used further in the next stages of genomic analysis of this breed.

Key words*: cows, DNA quantification, genomic analysis, Pinzgau.*

INTRODUCTION

The problem of preserving the genetic resources, respectively the indigenous cattle breeds, with cultural-historical importance, endangered, represents a topic of major importance.

The Pinzgau breed has a major importance in the livestock sector, being adapted for growth and exploitation in areas with altitudes between 400-1600 m, rich in rainfall and fertile natural meadows, being resistant to severe environmental conditions (Feldhamer, 2007).

In the last 15 years, about 300 breeds of approx. 6,000 belonging to different species of farm animals have been identified by the Food and Agriculture Organization of the United Nations (FAO) as endangered. Among cattle breeds, the most endangered are the podolian breeds from different parts of Europe. Podolian cattle belong to a group of very old European breeds, having ancestors in Bos primigenius (Gentry, 2011).

Piera Di Lorenzo et al. (2018) studied the genetic diversity of Podolian breeds and aimed to reconstruct their origin. A number of 18 podolian breeds (Piemontese, Bianca di Val Padana, Romagnola, Mucco Pisano, Calvana, Chianina, Maremmana, Marchigiana, Italian Podolian, Ukrainian Gray, Romanian Gray, Hungarian Gray, Slavonian Syrmian Pod., Istrian) have been studied phylogenetically.

To interpret the results, nine other cattle breeds were compared (Valdostana, Gray Alpine, Italian Brown, Italian Red Pied, Cabannina, Reggiana, Agerolese, Cinisara, Modicana).

The global analysis clearly highlighted some peculiarities of some genes in the mtDNA group. The analysis of the main components indicated a genetic proximity between five breeds (Chianina, Marchigiana, Maremmana, Podolica Italiana and Romagnola).

A suggestive hypothesis shows the ancestral double contribution to the current genealogical background of podolian breeds (Piera Di Lorenzo et al., 2018).

Another type of genetic research focused on the study genetic markers associated with the characteristics of milk/meat production, especially in the case of endangered cattle

breeds, which is useful for assessing the conservation value of genetic resources of animal origin as well as for determining the degree of uniformity of breed (Lewin, 2004).
Many other molecular markers associated with the place of formation and domestication of the breed were analyzed over time by numerous researchers in the field.
The issue of preserving local breeds is important for ensuring the food security of the Romanian population, given the recent climate changes, not only of rising temperatures, but temperature fluctuations and extreme phenomena that could bring new challenges in the future.
A first step in studying the genetic diversity of these breeds is the extraction of DNA from biological samples.
During the process of DNA denaturation, the nitrogenous bases located inside molecule manifests absorption capacity of ultraviolet radiation at $\lambda = 260$ nm, if we compare to the situation when they were arranged inside the double helix (Thomas, 1994).
The hyper chromic effect affects the absorbance ratio of working solutions at wavelengths of 260 and 280 nm (A260/A280), which must be between 1.8 and 2, though some authors allow values between 1.7 and 2.
Lower values suggest higher absorbance and, indirectly, higher protein concentrations in the working samples, which absorb ultraviolet light at 280 nm; higher ratio values indicate RNA contamination.
This paper aims to validate the optimal method of extraction and quantification of total DNA from blood samples collected from the Pinzgau cattle breed, a first step in the analysis of genetic diversity.

MATERIALS AND METHODS

The quantification of the genetic diversity of Pinzgau cattle breed was initiated by the stage of collecting a number of 24 biological samples, respectively blood. Blood samples were collected by jugular vein puncture, using Vacutainer tubes with EDTA to prevent clotting, with a capacity of 2 ml and 18 G collection needles.
DNA extraction from blood samples was performed by the automated method with Maxwell equipment 16. This equipment can process up to 16 samples in 40 minutes, and the extracted DNA can be used in a variety of applications, including PCR and agarose gel electrophoresis.
The amount of total DNA was quantified using a Nanodrop spectrophotometer and the optical density was measured at the absorption rate A260 nm and A280 nm, subsequently making the ratio between the two absorption rates.
Nanodrop is a spectrophotometer, with a measuring spectrum between 220-750 nm, which measures samples with a volume of 1µl, with a very high accuracy and reproducibility. It uses high-performance technology that involves tensioning the surfaces on which the measurement is made to retain the sample.
The results were statistically interpreted using a series of basic indicators, such as the arithmetic mean, standard deviation, standard error and confidence limit of the mean, coefficient of variation.

RESULTS AND DISCUSSIONS

Most biological substances have a characteristic absorption rate in the field of ultraviolet (UV) radiation (Higgins, 1988).
Thus, the absorption rate of 260 nm corresponds to nucleic acids, 280 nm to proteins and 230 nm to various contaminants.
The interpretation of the quantification results is based on the fact that the DNA is considered sufficiently pure, if the ratio of the two readings, respectively A260/A280, has values included in range 1.7-2.0.
Values lower than 1.7 indicate impurities with proteins, and those higher than 2.0 indicate impurities with other contaminants.
Beer Lambert's law shows that there is a linear relationship between the concentration of a compound and its absorbance at a certain wavelength. The calculation of the DNA concentration is based on this fact, but assessments are also made on the purity of the DNA in relation to the proteins (Tamura, 2013).
Regarding the age of the individuals from whom the blood samples were taken, there is a series of information that shows that the age range for the 24 females was between 12-90 months, with an average of 52 months.

As can be seen in figure 1, a number of 11 females are 5-10 years old, 8 are age limits between 2-5 years and 5 are in the range of 1-2 years (Table 1 and Figure 1).

Table 1. Identification data of Pinzgau individuals from which biological material was collected for the purpose of genetic analysis

No.	Cattle breed	Age (months)
1.	Pinzgau	16
2.	Pinzgau	83
3.	Pinzgau	72
4.	Pinzgau	65
5.	Pinzgau	60
6.	Pinzgau	33
7.	Pinzgau	58
8.	Pinzgau	64
9.	Pinzgau	23
10.	Pinzgau	34
11.	Pinzgau	33
12.	Pinzgau	90
14.	Pinzgau	86
15.	Pinzgau	84
16.	Pinzgau	81
17.	Pinzgau	66
18.	Pinzgau	66
19.	Pinzgau	50
20.	Pinzgau	63
21.	Pinzgau	12
22.	Pinzgau	20
23.	Pinzgau	18
24.	Pinzgau	18

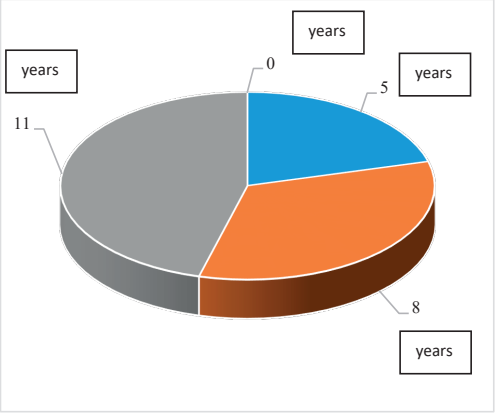

Figure 1. Numerical distribution by age of Pinzgau cattle breed

Following the quantification of DNA samples extracted from the 24 females, the concentrations varied in the range of 4.1-61.9 ng/µl, the average concentrations being 27.7 ng/µl (Table 2) and the ratio of the two absorptions, respectively A260/A280, presented values in the range 0.68-2.13, with an average of 1.68.

Table 2. Results of spectrophotometric quantification of total DNA extracted from blood samples from the Pinzgau cattle breed

ID Sample	Abs260	Abs280	Abs230	260/280	260/230	Concentration DNA (ng/µl)
P_01	0.352	0.218	0.375	1.61	0.94	17.6
P_02	0.447	0.289	0.480	1.55	0.93	22.3
P_03	1.24	0.842	1.212	1.47	1.02	61.9
P_04	0.369	0.230	0.339	1.60	1.09	18.4
P_05	0.486	0.305	0.430	1.59	1.13	24.3
P_06	0.844	0.500	0.661	1.69	1.28	42.2
P_07	0.371	0.218	0.382	1.70	0.97	18.5
P_08	0.385	0.227	0.373	1.70	1.03	19.2
P_09	0.589	0.369	0.627	1.60	0.94	29.4
P_10	0.796	0.506	1.024	1.57	0.78	39.7
P_11	0.404	0.266	0.409	1.52	0.99	20.2
P_12	0.603	0.377	0.800	1.60	0.75	30.1
P_13	0.821	0.488	0.559	1.68	1.47	41.0
P_14	0.922	0.526	0.579	1.75	1.59	46.0
P_15	0.959	0.570	0.666	1.68	1.44	47.9
P_16	0.547	0.315	0.486	1.74	1.13	27.3
P_17	0.347	0.168	0.311	2.07	1.12	17.3
P_18	0.355	0.171	0.301	2.08	1.18	17.7
P_19	0.262	0.147	0.241	1.78	1.09	13.0
P_20	0.084	0.123	0.280	0.68	0.30	4.10
P_21	0.306	0.169	0.400	1.81	0.76	15.2
P_22	0.398	0.187	0.226	2.13	1.76	19.9
P_23	0.627	0.299	0.557	2.10	1.13	31.3
P_24	0.806	0.468	0.826	1.72	0.98	40.3

Figure 2 shows the DNA concentration values that were in the range of 4.1-61.9 ng/µl and an average of 27.7 ng/µl.

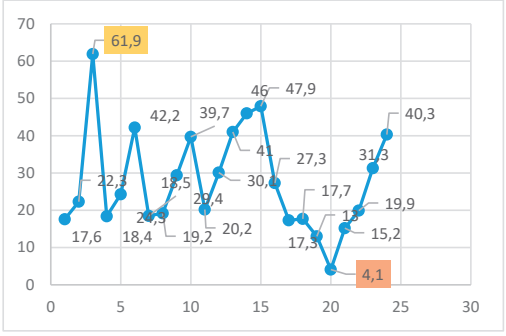

Figure 2. DNA concentration values (ng/µl)

A comparison between the minimum and maximum values, respectively the average value of the data obtained on the concentration of quantified DNA can be seen in Figure 3.

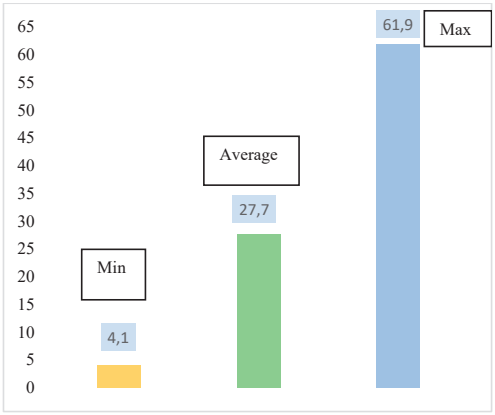

Figure 3. Comparison between the mean value of the DNA concentration, in relation to the minimum / maximum values (ng/µl)

The results of the quantification analysis demonstrated the effectiveness of the DNA extraction method (Figure 3).
Another important aspect in molecular genetics analyzes concerns the purity of the extracted DNA which is evaluated on the basis of the A260 / A280 absorbance ratio. The values obtained are usually classified into 3 ranges (<1.7; 1.7-2.0; > 2.0).
The percentage distribution of the 24 samples, depending on the value of the absorbent ratio A260 / A280 is represented in Figure 4.

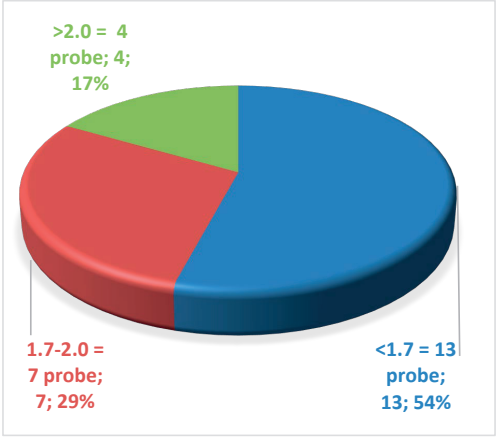

Figure 4. Percentage distribution of samples according to the value of the absorbent ratio A260/A280

Out of the total of 24 samples, 13 presented a value lower than 1.7, a number of 7 samples had values in the range 1.7-2.0 and 4 samples had values higher than 2.0.

A statistical expression of the values obtained for the DNA concentration can be consulted in Table 3, which results in a value of the confidence limit of 5.75 and represents the confidence interval that demonstrates that the calculated parameters are in the desired range.

Table 3. Statistical calculation of values associated with DNA concentration

Statistical parameters	DNA concentration (ng/µl)
Minimum value	4.1
Maximum value	61.9
Average	27.7
Standard deviation	13.61
Standard error	5.55
Confidence limit	5.75

In this case, a homogeneity of the data can be observed, not recording aberrant values compared to the average results of DNA sample concentrations which is 27.7 (ng/µl). The values obtained from the spectrophotometric analysis are found around the average.

CONCLUSIONS

Molecular analysis techniques have evolved considerably over time, allowing the investigation of genome diversity by sequencing it, in different species of zootechnical interest. Thus, the phylogeny of many animal species has been studied by researchers since 1980 (Wellmann, 2019; Wheeler, 2011).
Research in the past has been based mainly on the study of genetic markers associated with characters in individuals of different species of interest, as well as research on microsatellites due to the very high polymorphism and the large amount of genetic information.
The results of the investigations regarding the analysis of the total DNA of the Romanian Pinzgau cattle breed, led to the formulation of the conclusion that the spectrophotometric quantification of the totally isolated DNA validated quantitatively and qualitatively the stage of isolation and purification of nucleic acids, making possible the transition to the next stages of analysis.
Thus, the use and testing of new molecular marker research techniques will help clarify issues regarding the genetic diversity of species of interest.

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GENETIC DIVERSITY OF PINZGAU CATTLE BREED: A SYSTEMATIC REVIEW

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Abstract

The present work aims to review the most important aspects regard to genomic characterization of Pinzgau cattle breed. In Romania, this cattle breed is part of the country´s genetic and cultural heritage and faces the impact of bottleneck and the lack of diversity due to a significant decline in population. Natural populations´ genetic structure is influenced by a limited gene flow that occurs when the geographic distances between them increase. In our country, the Food and Agriculture Organization of the United Nations (FAO) maintained the status of ' endangered-maintained ' cattle breed in 2000 considered to be threatened with extinction. This paper wants to highlight the fact that the conservation of this breed is a national priority and also, reviews the most relevant information in the literature on the genetic diversity of this cattle breed.

Key words: *cattle breeds, genetic diversity, Pinzgau cattle.*

INTRODUCTION

The Pinzgau breed is named after its origin area, near Salzburg, Austria, and is a mountain cattle breed.

The breed originated from local mountain breeds in the 19th century and evolved in three directions: traction, milk and meat. In Romania, the Red Pinzgau breed has been established since the second half of the nineteenth century, after the absorption of crosses made between the local cattle breeds Grey Steppe, Mocanita and Pizgau of Austria. Also, "Cow of Dorna" or "Black Pinzgau" was created by the crossing of local cattle with specific mountain breeds: Pinzgauer, Mölltal, Zillertal etc. (Popa et al., 2012).

Over the last few decades, the biodiversity of cattle has decreased dramatically. The genetic degradation observed was mainly due to the specialization of livestock production in favor of cosmopolitan and high quality breeds, so, the control of the genetic diversity of cattle has therefore become an important concern in the management of livestock breeding programmes (Kukučková et al., 2017).

The Pinzgau breed, currently, meets in more than 25 countries around the world. Special color mottled red-brown spots on the side of the body and white line became the character of the breed.

In Romania, the Pinzgau breed is meets in three areas: the NW of Moldova, the SW of Transylvania and the W of Transylvania-Apuseni. Transylvanian Pinzgau breed has a strong constitution, lively temperament, docile disposition, average precocity, high endurance, good adaptation ability, resistance to disease and environment (Kadlecik et al., 2004).

Dorna cow is less than 1-2 cm tall than Red Pinzgauer, the rectangular body is more pronounced, the bones and muscles are better developed and the background is black (Fisteag, 1958).

According to FAO reports, the Pinzgau breed is threatened with extinction, entering the category of endangered cattle. FAO studies often draw attention to the numerical decline of different species, the classification of different breeds, taking into account the number of individuals for each breed, the ratio of females to males, and their inclusion in active

conservation or maintenance programs by companies or research institutions, as one of the following categories: extinct, critical, endangered, critical-maintained, endangered-maintained, not at risk (Scherf, 2000).
Due to its rusticity, resistance to a particular hilly and mountainous climate, with a remarkable successful longevity and survival, the expression of its genetic characteristics distinct from other breeds, Pinzgau must be considered a component of national genetic resources.
The main objective of this study was to provide a thorough insight into the genomic characterisation of the Pinzgau cattle breed (especially the Romanian Pinzgau breed) through the use of high-performance molecular information.

MATERIALS AND METHODS

In order to reach the objectives of this study, 13 bibliographic sources from the specialized literature were consulted. The main issues addressed refer to the morphological and productive evaluation of the Pinzgau breed, especially in our country, as well as information regarding the genetic diversity of this breed, which is currently in danger of extinction.
The research methods used in this study were the observation, analysis and graphical interpretation of data from the specialized literature regarding numerical evolution, morpho-productive characteristics and genetic analysis of the Pinzgau cattle breed.

RESULTS AND DISCUSSIONS

1. The morphological and productive characteristics of the Pinzgau cattle breed

The Pinzgau breed was first developed between 1690 and 1740 by the crossing of local red bulls with the Bern type of Switzerland, after 1740, the resulting animals were used for breeding in the true breed and is located in the mountain areas of Romania at an altitude of over 1000 m. This breed is originates in Austria, Salzburg, Tyrol, alpine and subalpine areas (Maciuc, 2006).
In 1820, Pinzgau breed were exported to countries such as Romania, Yugoslavia, the Czech Republic and Slovakia. Throughout South Africa, Canada, the USA and Australia, even under the harshest weather conditions, Pinzgau thrived (Kadlecik, 2004).
An extensive study on the morphological and production characteristics of Pinzgau cattle, from the Apuseni Mountains area and from the Hațeg and Petroșani Depression was carried out between 1956-1962 by Dincă et al., whose synthesis is presented below. The average body weight of the cows was 382.3 kg, the cows in the area of the valleys had 392 kg body weight, and those in the mountain area and in the premontane area 371 kg.
Figure 1 shows the weight of the Pinzgau (females) breed, for 2014, in comparison with the cows from the breeds: Brown, Romanian Black Spotted, Romanian Spotted and other beef cattle breeds, undefined.

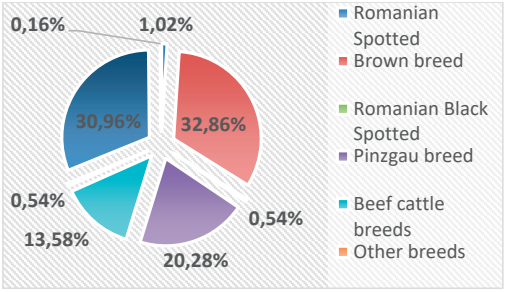

Figure 1. The share of Pinzgau cattle breed from the total of cows in Romania, registered in the herd book (2014)

In Romania, as mentioned by FAO-1993, the Pinzgauer cattle included 1092 females registered in the national herd book. However, the recorded population trend was considered to be decreasing. According to Figure 1, in 2014, the Pinzgau breed represented 20.28% of the total number of cattle from the bovine species, in the territory of our country.
For 2015, the situation regarding the weight of the Pinzgau breed from the total number of cattle in Romania is presented in Figure 2.

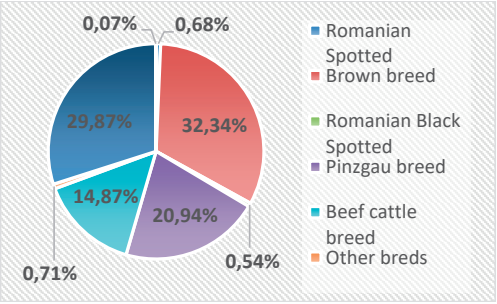

Figure 2. The share of Pinzgau cattle breed from the total f cows in Romania, registered in the herd book (2015)

According to Figure 2, in 2015, the Pinzgau breed represented 20.94% of the total number of cattle from the bovine species, in the territory of our country, a higher percentage by 0.66 compared to the previous year.

Regarding the exterior, the conformation and the constitution, in general, the cattle of this breed present a pleasant, attractive and harmonious exterior, having a characteristic conformation to the breeds with intermediate production skills (Georgescu et al., 1998).

The body development is relatively variable, depending on the area of spread and the local geoclimatic conditions (Table 1).

Within the breed there is a variety known as Dorna Cow or Black Pinzgau. It is a special type of breed, widespread in the area of the same name, especially around the localities of Vatra Dornei, Câmpulung Moldovenesc and Gura Humorului.

The researchers conducted by Acatincăi in 2004, show that there are morphological differences between the cattle of the Pinzgau breed, found in the northwest of Moldova, the Apuseni Mountains or the Dorna Depression.

The phenotypic performances in the direction of milk production are different, with limits between 1900-3500 kg and 3.62- 4% fat. As for the herds from the official production control, they have a very sinuous evolution (Table 2).

Table 1. The main morphological characteristics of the Pinzgau breed

Characteristic (cm)	NW Moldovei (cattle)	Apuseni Mountains (cattle)	Cows	Bulls	Dorna Cow
Waist	126.3	129.4	128.6	134	125
Length of the trunk	151.2	155.0	153.1	163.1	151
Thoracic depth	67.4	67.9	67.5	74.3	64
Thoracic perimeter	178.8	179.3	179.1	199.7	176
Body weight	469.2	482.8	471.1	662.9	444

**Acatincăi, 2004*

Table 2. Number of lactation, average milk production and fat content

Pinzgau cattle breed			
Lactation	I	IV	VII
Average milk production (kg)	2849.79	3313.89	3300
Fat content of milk (%)	3.77	3.75	3.73

*Official Control of Production, during 2014-2015

Regarding the evolution of milk production according to the lactation rank, it has a linear character.

Imported animals of the Pinzgau breed have produced a lower yield of milk under the conditions of processing in Romania than in Austria. The protein, fat and lactose content of milk was also lower (Gîlcă & Gîlcă, 2012).

2. Genomic uniqueness of the Pinzgau cattle breed

The genome of many breeds of cattle was studied by researchers and the research results have been published over time in numerous articles. The research was centred on the analysis of genetic markers correlated with the characteristics of the productions, in the case of cattle breeds threatened with extinction (as is the case with the Pinzgau breed), this was useful for the understanding of the importance of the survival of genetic capital relevant to animal origin, the degree of uniformity of the breed and, corroborated by several other important molecular markers, their place of development and domestication. The genetic structure of natural populations is determined

by the minimal gene flow that happens as the spatial differences between them increase. Genetic segregation of the breeds decreased the variation at the molecular stage, which can be controlled by growing homozygosity (Felius et al., 2014).

Investigating Pinzgauer populations in Austria, Bavaria, Germany, Erhardt (1996) discovered a new K-casein variant (K-CN G) with a frequency of 0.003 by isoelectric focusing in polyacrylamide gels and by alkaline polyacrylamide gel electrophoresis. K-CN G was not present in milk samples of Limpurger, another endangered breed.

A. Caroli et al. analized in the original Pinzgauer cattle, milk protein genetic variation and casein haplotype structure. A total of 485 dairy specimens from Original Pinzgauer from Austria (n = 275) and Germany (n = 210) were isoelectrofocused to evaluate the genetic variation influencing the protein amino acid charge in dairy proteins αS1-casein, β-casein, π-casein, α-lactalbumin and β-lactoglobulin. A rather elevated genetic variation influencing the amino acid charge of dairy proteins characterizes the Original Pinzgauer breed, with a total of 15 alleles, 12 of which were discovered at a frequency > 0.05. With 4 alleles identified, the most polymorphic protein was β-casein. CSN1S1*B, CSN2*A2, CSN1S2*A, CSN3*A, LGB*A, and LAA*B were the predominant allles. A comparatively high frequency of CSN1S2*B (0.202 in the entire information set)

was discovered, primarily occurring within the haplotype C-A2-B-A (in the order CSN1S1-CSN2-CSN1S2-CSN3), which appears to be unique to the original Pinzgauer, potentially due to the survival of an ancient haplotype or *Bos indicus* introgression.

A specific white spotting phenotype, termed finching or line-backed spotting, is known for all Pinzgauer cattle and occurs occasionally in Tux-Zillertaler cattle, two Austrian breeds. The so-called Pinzgauer spotting is inherited as an autosomal incompletely dominant trait. Based on 777k SNP data, a genome-wide association study using 27 white spotted and 16 solid-colored Tux-Zillertaler cattle revealed a strong signal at the Kit locus on chromosome 6. Haplotype analyzes described the Kit coding region's critical interval of 122 kb downstream. Whole-genome sequencing of a Pinzgauer cattle and comparison with 338 control genomes disclosed a complicated structural version composed of a deletion of 9.4-kb and a reversed duplication of 1.5 kb fused from chromosome 4 to a 310-kb duplicated section. A diagnostic PCR for this structural variant (Kitpinz) was created for the simple genotyping of carriers and confirmed the presence of the variant allele in all Pinzgauer and most white spotted Tux-Zillertaler cattle. The introgression of the Kitpinz variant confirms admixture and the reported historical relationship with Austrian Tux-Zillertaler of these short-headed breeds and suggests a mutation event that occurs before breed formation (Kuttel et. al., 2019).

Ivan Pavlík et. al. (2014) researched genetic variation in the Pinzgau breed in Austria and Slovakia. A total of 12,442 individuals were used in the sample reflecting the reference population and have been studied four sub-populations. The mean inbreeding coefficient (five generations taken into account) was 0.0186, 0.0242, 0.0151 and 0.0126 for Austrian dairy products (AD), Austrian beef (AB), Slovak dairy products (SD) and Slovak beef (SB) respectively. The effective size of the population varied from 122.5 (AD) to 809.4 (SB).

Genetic evaluation represents an important tool in breeding and cattle selection, Romanian Pinzgau being a part of active breeds adapted to local conditions with local origin. Currently, in Romania, Pinzgauer cattle breed is included in a genetic program of conservation.

CONCLUSIONS

Pinzgau cattle breed represents a valuable genetic reserve for livestock of Romania. This is more strategically necessary than ever to maintain as much livestock variety as possible and to ensure a prompt and timely response to the needs of future generations.

The Romanian Pinzgau must be regarded as a part of national genetic wealth, owing to its rusticity, tolerance to the unique hilly and mountainous climate, with a remarkable endurance, an indication of its genetic distinction from other breeds.

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CONTRIBUTIONS TO STUDIES REGARDING THE MORPHOLOGICAL AND REPRODUCTION CHARACTERS OF SHAGYA ARABIANS HORSE BREED

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Abstract

The paper presents the results of a study accomplished in the Rădăuți stud farm, which aimed to analyze some morphological and reproductive aspects of Shagya Arabian horses. The selected population was represented by broodmares from 2000y generation, introduced in the reproductive herd until now. The average value of height was 159.188 ± 0.593 cm, the average of heart girth was 177.188 ± 1.013 cm, and the cannon perimeter was 18,594 ± 0,184 cm for the studied horses. Regarding the reproduction aspects, it was revealed that the studied parameters had the following average values: 4.7 ± 0.3 years for age of introduction to reproduction, 4.5 ± 0.2 years for the age at first foaling, 338.7 ± 0.9 days for the gestation length, 149 ± 18.3 days for the service-period parameter, 503 ± 19.9 days for the foaling-interval parameter, and 14.6 ± 1.2 years for the reproductive longevity of broodmares. The conclusion was that all data obtained fall within the normal limits specified by the literature.

Key words: *broodmares, morphological, Rădăuți, reproductive, Shagya.*

INTRODUCTION

The main purpose of a stud farm is to obtain high value descendants, which have to perpetuate and improve the characteristics of reproduction population.

The selection process of horses, involves high attention for the exterior traits of reproducing heads, aspect highlighted also in the ranking activity of horses, which tracks promoting only the individuals that gather a specific score for maintaining in the reproduction herd (Doliș et al., 2017).

Basically, the entire practice of a stud farm depends on the reproductive activity of this type of unit. Highly ranged performances regarding the reproduction are correlated with many aspects from the general management applied in the stud farm, like the professional and educational degree of the staff, but especially the feeding, care and shelter system of horses exploited for reproduction (Gordon, 2004).

It is a fact well known that compared to other species, the efficiency of reproduction activity of horses is lower. Thus, in normal condition, the fecundity percent registered in their case is 65%, and the natality percent seldom exceeds 50%, but in stud farms, where rearing conditions are optimized, the first parameter can be more than 90%, and the second one 80-85% (Dumitrescu, 1986; Georgescu et al., 1990; Gîlcă & Doliș, 2006; Mărginean et al., 2005; Velea et al., 1980).

Reduced values of these reproduction indices are the result of so-called physiological sterility, in which case the main culprit is precisely the man.

The study aimed to create an image as close as possible of the quality of reproduction horses and reproductive activity of this unit, based on analysis of data existing in the stud farm registers. Likewise, we considered it an opportunity to contribute to the development of breeding activity of Shagya Arabian horses.

MATERIALS AND METHODS

The biological material was represented by 16 broodmares from Shagya Arabian horse breed, from the 2000 generation, which were

promoted in the National Stud Farm, based on the results obtained at qualification tests, and also at ranking activity in 2003.
The mares were recorded as having a high reproductive activity (up to 17 years), which was able to offer enough data to accomplish a complex study, and apposite conclusions.
Regarding the morphological aspects, there were studied the 3 main body dimensions, which are followed in the ranking activities, like: height, heart girth and cannon perimeter. Based on this data, there were calculated also three body indices to reflect as clearly as possible the exterior traits of the analyzed horses (the massiveness index, which represents the ratio between the heart girth and the height; the bone index – the ratio between the cannon perimeter and the height; the dactyl-thorax index – the ratio between the cannon girth and the thoracic perimeter).
The necessary data for analyzing the 16 broodmares were taken from the stud farm's registers; based on this extract there were calculated some reproductive indexes, like: the age at introducing to reproduction (the difference between data at first foaling and mare's birth), the age at first foaling (the difference between the date of first foaling and mare's birth), gestation length (the difference between date of foaling and date of prolific mount), service-period parameter (the difference between foaling date and the prolific mount after foaling), the foaling-interval (the difference between two successive foaling or the sum between SP and next gestation length).

RESULTS AND DISCUSSIONS

Data obtained after consulting the registers were centralized and also statistical interpreted in Table 1. The results showed that the average value of height was 159.188 ± 0.593 cm, the minimum value was 156 cm and the maximum 164 cm, revealing that this character is very homogenous for the whole studied population (V% = 1.49%).

Table 1. Data regarding several body dimensions and indexes on studied broodmares

Broodmare's identification name	Height (cm)	Heart girth (cm)	Cannon perimeter (cm)	Dactyl-thorax index (%)	Bone index (%)	Massiveness index (%)
0	1	2	3	4	5	**6**
425-EL-SBAA XII-35	161	180	19	10.56	11.8	111.8
426-EL-SBAA XII-37	159	176	19	10.8	11.95	110.69
428-SHAGYA LXII-3	163	180	17.5	9.72	10.74	110.43
429-SHAGYA LXII-6	164	179	17.5	9.78	10.67	109.15
430-SIGLAVY-BAGDADY XV-58	157	172	18	10.47	11.46	109.55
431-DAHOMAN XXXIX-51	156	183	19	10.38	12.18	117.31
432-EL-SBAA XII-42	158	174	19	10.92	12.03	110.13
433-HADBAN XXXV-17	159	182	19	10.44	11.95	114.47
434-SHAGYA LXII-7	161	184	19.5	10.6	12.11	114.29
435-SHAGYA LXII-8	162	177	18	10.17	11.11	109.26
436-EL-SBAA XII-38	158	180	20	11.11	12.66	113.92
437-EL-SBAA XII-36	158	173	18	10.4	11.39	109.49
438-KOHEILAN XXXIX-15	156	174	19	10.92	12.18	111.54
439-DAHOMAN XXXIX-53	158	176	18	10.23	11.39	111.39
440-HADBAN XXXV-18	158	171	18	10.53	11.39	108.23
441-SIGLAVY-BAGDADY XV-61	159	174	19	10.92	11.95	109.43
$\overline{X}$	159.188	177.188	18.594	10.496	11.685	111.317
var	5.629	16.429	0.541	0.156	0.301	6.184
s	2.373	4.053	0.735	0.395	0.549	2.487
$\pm s_{\overline{x}}$	0.593	1.013	0.184	0.099	0.137	0.622
V%	1.490	2.288	3.954	3.764	4.698	2.234
MIN	156	171	17.5	9.722	10.671	108.228
MAX	**164**	**184**	**20**	**11.111**	**12.658**	117.308

The heart girth had limits of 171-184 cm, and an average value of 177.188 ± 1.013 cm showing that the studied population behaved as homogenous group (V%=2.288%). The cannon circumference had an average value of 18.594 ± 0.184 cm (the absolute values were 17.5 and 20 cm) so this character is also highly homogenous.

Regarding the dactyl-thorax index the average value was 10.496 ± 0.099% (from 9.72-11.11%), the bone index was 11.685 ± 0.137% (the extreme values oscillated between 10.67-12.66%), and the massiveness index had an average value of 111.317 ± 0.622% (the limits were ranged between (108.23-117.31%).

These values were similar to those exposed in the literature, and they reflect the image of horses with fine and fine-robust conformation, with a medium body development, and smooth and strong bones, which fits the Shagya Arabian horse breed in the category of horse riding, and also light carriage breeds (Dolis et al., 2017; Georgescu, 1990; Gilca & Dolis, 2006; Marginean et al., 2005).

The data statistical processed showed that the parameter introduction to first mount had an average value of 1714.8 ± 104.1 days (4.7 ± 0.3 years), and the absolute limits ranged between 1242 and 3041 days (3.4-8.3 years); this result indicated a high variability of the character (V% = 24.3%). This percent was influenced by one of the broodmares (Shagya LXII-3) which recorded the first mount at the age of more than 8 years, while most of them recorded much earlier (at 4-5 years). Dispensing this mare, the average value decreases to 1626.4 ± 56.9 days (4.5 ± 0.2 years), which leads to the variability of 14%.

Another observation is that 81.25% of the studied broodmares were introduced to the first mount in 2000 and 2005, respectively 43.75% (7 heads) and 37.50 % (6 heads) - at the age of 4-5 years, the rest were included in the season of 2003 (12.50%/2 heads), at the age of approximately 4 years, respectively in the season of 2008 (6.25%/1 head), at an abnormal age, of more 8 years.

The data obtained are in the limits provided by the literature, which specify that young horses can be first introduced to breeding when they have achieved at least 75% of adult development, respectively the age of 2½-3 years in heavy breeds, 3-3½ years for intermediate breeds and 3½-4 years for light breeds (Dumitrescu, 1986; Tănase & Nacu 2005; Ujică, 1981; Ujică, 1988; Velea et al., 1980).

Unlike the age of introduction to reproduction, which depends on the management of the stud farm, the age at first foaling is correlated to the physiological status of the genital organs of females, the hormonal and neuronal functioning of the mare, and the results of fecundity which is also very important.

For the studied population, 43.75% of the mares foaled after the first time breeding, respectively in the first year of the breeding season, another 43.75% in the second year, while 12.5% of them ever did (El-Sbaa XII-36 and Koheilan XXXIX-15). If we exclude these two mares, the age at the first foaling, for the whole studied population, was on average 2186.6 ± 164.0 days (or 6.0 ± 0.4 years) – the absolute limits ranged from 1755 to 4200 days or 4.8 and 11.5 years (Figure 1).

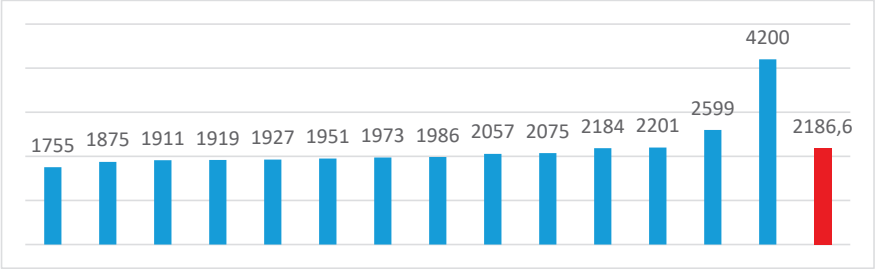

Figure 1. The absolute and average values of age at first foaling (days)

The variability for this character was high (28.1%) and, as in the case of age at first mating, was due to the mare Shagya LXII-3, which, as seen, recorded the first mating only after the age of 8. Eliminating this mare from the calculation, the average reaches 2031.8 ± 58.2 days (5.6 ± 0.2 years), and the variability decreases to 10.3%, which makes these values normal.

If there are taken into study only the mares which foaled from the first year of breeding use, the age at first foaling is reduced, on

average, to 1963.1 ± 21.4 days (5.4 ± 0.1 years), which is a desirable value in any stud farm. In this case, the variability is small (2.9%), and the group is homogeneous in terms of this character.

From the statistical processing of the data of gestation length, it is observed that on the whole population and taking into account all gestation lengths are completed with foaling; the average duration of gestation was 338.7 ± 0.9 days, the limits of absolute values were 313 and respectively 366 days (Figure 2).

The average length of the first gestation was 332.8 ± 5.2 days, calculated for 13 mares, as the gestation of the Dahoman mare XXXIX-51 ended with late abortion, at 273 days, and was excluded. The limits were between 320 and 344 days, so the group studied being homogeneous from this point of view (V% = 2.4%).

In the case of the second gestation, for 11 mares, the average was 341.1 ± 3 days, with limits between 323 and 357 days. Also, in the case of this second gestation there was a case of abortion, which occurred on the 291st day and was not taken into account (Shagya LXII-3). The average of the third gestation, calculated for 12 mares, was 341.1 ± 2.7 days, with limits between 325 and 357 days.

The duration of the fourth gestation the average value was 343.1 ± 2.4 days, which was calculated for 11 mares. The absolute values ranged between 330 and 358 days, the group being homogeneous from this point of view (V%=2.3). The mare El-Sbaa XII-35 during the fourth gestation was aborted at 267 days, so it was excluded from the calculation.

The fifth gestation had an average duration of 338.3 ± 1.2 days, with limits between 333 and 344 days. Of the 12 mares in this case, one (Hadban XXXV-17) had an abortion at 255 days, not being considered.

The sixth and seventh gestations with an average of 329.5 days, were available for just 2 broodmares (328 and 344 days).

The 13th, 14th, and 15th gestations were recorded in the case of a single mare (El-Sbaa XII-38) and lasted between 313 and 352 days.

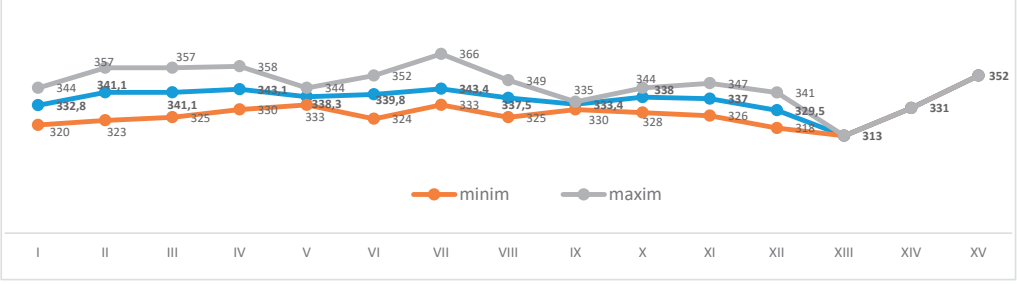

Figure 2. The dynamics of gestation length (days)

In the literature, the gestation length of mares is, on average, 11 months, with variations between 307 and 412 days.

The data on the service period were centralized and statistically processed (Figure 3).

From these data, it is observed that, in general, counting all foaling, respectively fertile amounts, SP in the studied population had an average value of 149 ± 18.3 days, the absolute values oscillating in very wide limits, between 6 and 783, which also determined a very high variability of the character, between 84 and 139.2%.

The fourth gestation was completed with late abortion (Dahoman XXXIX-51, El-Sbaa XII-35, Hadban XXXV-17, Shagya LXII-3) but they were assimilated as normal gestations.

The lowest value of the average length of SP was recorded after the eighth foaling, calculated for 6 mares, respectively 77.5 ± 38.4 days. The absolute minimum of service-period parameter, recorded in this study, was 6 days after foaling.

The highest mean value of SP length was recorded after the sixth foaling, respectively 235 ± 90.4 days. The absolute maximum was recorded after the fourth foaling, 783 days (Shagya LXII-7). In this case, after the fourth foaling, the highest variability of the character was registered, respectively 139.2%.

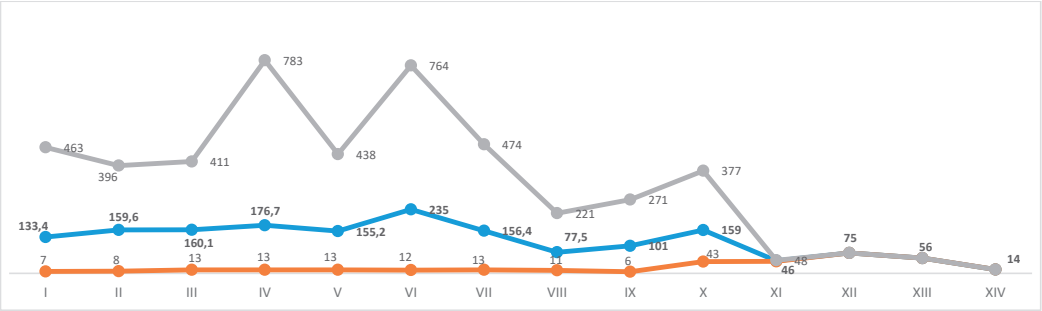

Figure 3. The dynamics of the service-period length (days)

Counting all the intervals between foaling (89), at the level of the entire population the FI had an average value of 503 ± 19.9 days and absolute values that ranged between 326 and 1125 days (Figure 4).

The absolute minimum for this character was registered in the population studied in the case of the first FI, respectively the one registered between the first and the second foaling (326 days).

The absolute maximum in this study was recorded in the case of the fourth FI (1125 days).

The variability of this character in the population was generally high (18.1-46.4%).

The calculations did not take into account the 4 abortions, mentioned above, from the mares Dahoman XXXIX-51, El-Sbaa XII-35, Hadban XXXV-17 and Shagya LXII-3.

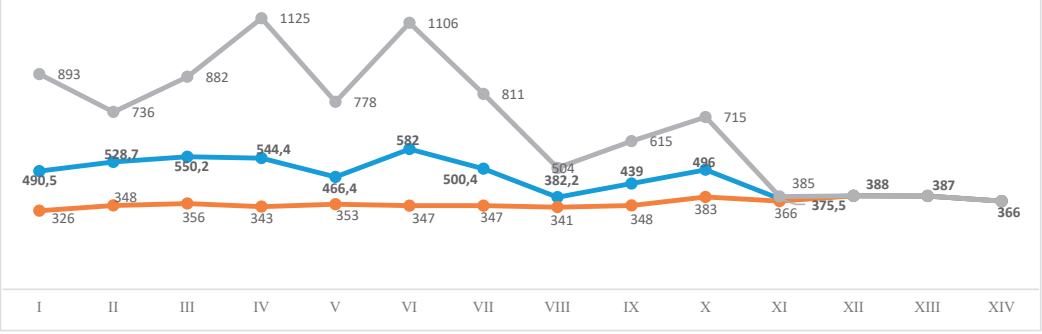

Figure 4. The dynamics of foaling-interval length (days)

The reproductive longevity of the studied broodmares was estimated based on the age they had at the last record in the reproduction registers, respectively breeding or foaling (Figure 5).

Thus, it was observed that for 75% of mares the last breeding event recorded in the records was a insemination, namely a non-fertile one, after which the mare was excluded from the breeding nucleus, on the occasion of the first classification. For 25% of mares the last recorded breeding event was foaling. Of the 16 mares of the 2000y generation, taken into the study, three are still active in the stud farm. These are: Shagya XII-7 (last mounted on 05.05.2019 - non-pregnant); Siglavy Bagday XV-58 (last foaling on 29.01.2020); El Sbaa XII-38 (last foaling 10.05.2020).

Statistical data processing shows that the reproductive longevity of the mares studied was on average 5335.6 ± 429.2 days (14.6 ± 1.2 years), with limits of 1995 and 7339 days (5.5 and 20,1 years).

The variability of this character in the studied population was high, of 32%.

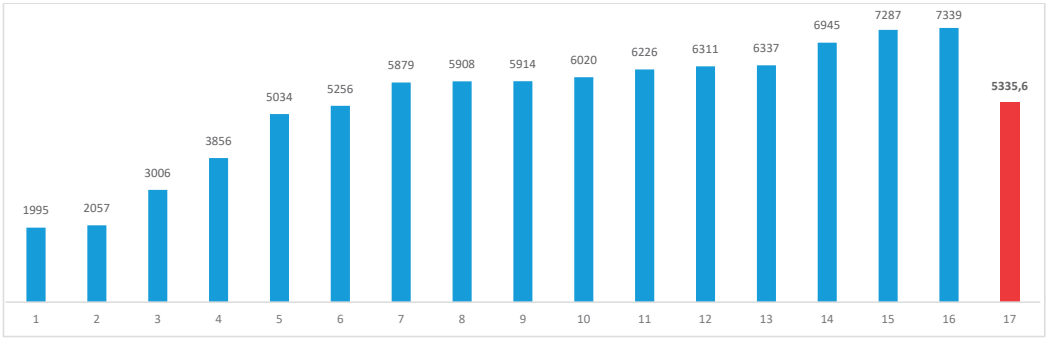

Figure 5. The reproductive longevity of broodmares (days)

CONCLUSIONS

Following the study on the breeding activity carried out on the 16 mares of the Shagya Arab breed from the 2000y generation, promoted in the herd of the Rădăuţi Stud Farm, the following conclusions were drawn:

- the mare's height had an average value of 159.188 ± 0.593 cm;
- the heart girth had an average value of 177,188 ± 1,013 cm;
- the cannon perimeter had an average value of 18.594 ± 0.184 cm;
- the average age of introduction to reproduction of mares was 1626.4 ± 56.9 days, respectively 4.5 ± 0.2 years;
- the average age of mares at the first foaling was 2186.6 ± 164.0 days, respectively 6.0 ± 0.4 years;
- the average gestation length of broodmares was 338.7 ± 0.9 days, the limits of the absolute values registered to be 313, respectively 366 days;
- the service period had an average value of 149 ± 18.3 days, the absolute values oscillating in the limits of 6 and 783 days;
- the foaling-interval was on average 503 ± 19.9 days, the absolute values recorded ranged between 326 and 1125 days;
- the reproductive longevity was on average 5335.6 ± 29.2 days (14.6 ± 1.2 years), with limits of 1995 and 7339 days (5.5 and 20.1 years).

Given the conclusions drawn during this study it is important to maintain, respectively promote in the breeding herd of the stud farm only the best specimens, able to bring genetic progress. Also, it is very important to offer the best conditions of housing, food and horse care for the broodmares to be healthy and strong.

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STUDY ON PRODUCTIVITY OF COWS OF HOLSTEIN BREED IN THE DYNAMICS OF LACTATION AND CORRELATION BETWEEN THE MAIN ECONOMICALLY USEFUL FEATURES

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Abstract

The article presents the results of studies of milk productivity of Holstein cows in the dynamics of lactation, correlations between the level of milk yield, fat content, amount of milk fat, and live weight. The research was carried out in the herd of the breeding farm of Society of limited liability "Doksancom", on Holstein cows in the dynamics of lactation, as well as first-calf heifers of various origins. For each subsequent lactation, the milk yield of cows in the herd of Society of limited liability "Doksancom" increases. It was established that milk productivity for the second lactation was by 1468 kg of milk, for the third - 1215 kg of milk, for the fourth-sixth lactation - 1199 kg of milk more than for the first lactation, the difference is significant at P<0.001, P<0.001 and P<0.01 respectively. Comparative analysis of the milk yield of first-calf heifers raised in various farms showed that first-calf cows raised in the herd of Society of limited liability "Doksancom" had the highest productivity - 9518 kg of milk, which is by 925 kg more than first-calf cows purchased from the breeding farm of Joint-Stock Company "Aydyn", the difference is significant (P<0.05) and per 1200 kg - first-calf cows imported from Holland (P <0.01). The relationship between milk yield and the percentage of fat in milk of cows was in a negative correlation from weak -0.154 (IV-VI lactation) to moderate -0.409 - -0.449, II and III lactation, respectively.

Key words*: correlation, first-calf cow, heifer, live weight, milk yield.*

INTRODUCTION

In modern conditions, one of the priorities is to increase the productivity of cattle, and not the increase of their number. Industrial production of milk requires a certain concentration of animals on farms, its narrow specialization, a high level of mechanization and automation of animal services. At the same time, a great attention is paid not only to production technology, but also to the quality of the livestock used for production (Gorelik et al., 2014). In the dairy cattle breeding of the developed countries of the world, the leading place is occupied by the highly productive Holstein breed.

The determining factor in the qualitative transformation of cattle breeding in the Republic of Moldova is the further development of livestock breeding, the presence of highly productive dairy cattle, adapted to modern technologies, climatic and feed conditions of the republic, as well as improving the food supply and the creation of advanced technologies for keeping animals.

As it is known, the milk productivity of cows is an important economically useful sign, one of the main indicators of animal husbandry. As a biological feature, it fluctuates depending on a number of factors, such as: season of the year, lactation in a row, membership in genealogical lines, generation and types. When breeding cows of dairy breeds and especially Holstein breed, it is taken into account the complex of economically useful traits, it is paid attention to the productivity and quality of milk - milk fat and protein milk.

Thus, under optimal feeding and keeping conditions, milk yield of Holstein cows in the breeding herd of Society of limited liability "Doksancom" amounted to 8980 kg of milk (first lactation), 10082 kg (second lactation) and 10530 kg (third lactation) milk per lactation (Foksha & Konstandoglo, 2019) .

The realization of the genetic potential for milk production and amounted to higher for the third

lactation and amounted to 108.3%, which is on average by 7.1% more than at the animals of the first two lactations. Milk productivity of cows is the result of the interaction of a complex of physiological processes of the body, which are controlled by many gene systems and determine the hereditary status of the breed. The study of the relationship between economic and useful traits has a great importance for breeding and pedigree work, as these dependencies can be used in the selection of animals of the desired types in the process of creation (Nicoro et al., 1968).

The practical value of correlations between signs is that they allow selection for a smaller number of signs with a positive relationship between them. At the same time, the rates of genetic improvement of herds are significantly accelerated (Belyaev, 1966).

The relationship between features is by the correlation coefficient, while the correlation is observed between both quantitative and qualitative signs (Stenkin & Mulyanov, 2014; Abrompolsky & Abylkasymov, 2005; Gaidukova & Tyutyunikov, 2013). In dairy cattle breeding, the most important is the identification of the nature and magnitude of correlation between the level of milk yield and the mass fraction of fat in milk. The correlation between milk yield and the mass fraction of fat and protein in milk is usually negative (Ruzsky, 1982; Osipenko et al., 1985; Yeghiazaryan & Braginets, 2010; Smith & Omoas, 1984; Sonderegger, 1986; Vleck, 1985; Abrompolsky & Abylkasymov, 2005). Therefore, in each individual case, it is necessary to determine the form, direction and degree of correlation (Dautbaev, 1995; Egiazaryan & Braginets, 2010; Stenkin & Mulyanov, 2014).

The data of many scientists confirm the positive relationship between milk productivity and live weight, and the fact that bigger animals have greater milk productivity (Kutrovsky, 2006, 2007; Brilling, 1985; Ratheises, 1972). According to many scientists and practitioners of livestock science, the relationship between milk yield, qualitative indicators of milk with age is positive (Shmeleva & Basonov, 2014; Vilver, 2015).

As it is known, the milk production of cows during lactation is subject to significant fluctuations. After calving, the daily milk yields of cows increase, reaching a maximum at 2-3 months of lactation, then gradually decrease (Katmakov, 2004), this process is graphically reflected by the lactation curve. The nature of the lactation curve depends on the maximum daily milk yield, the subsequent degree of its decrease and the duration of lactation. High-yielding cows within each breed are characterized by a large increase in productivity in the second or third months of lactation and its slow decline thereafter. It is known that the increase of milk productivity per lactation depends (among other conditions) from the maximum milk yield and on till the degree of its preservation during lactation. At the same maximum milk yield, this increase per lactation will be greater than the more constant lactation curve (Ernst et al., 1992; Kostomakhin, 2007).

The aim of our research was to study the milk productivity of Holstein cows in the dynamics of lactation, the correlation between the main economically useful traits, and also to compare the milk productivity of first-calf cows raised in different farms.

MATERIALS AND METHODS

The research was carried out in 2019-2020 in the herd of the breeding farm (of Society of limited liability) - of SLL "Doksancom" on Holstein cows (n = 259), including: 112 heads - first lactation; 90 heads - second lactation; 36 heads - third lactation; 21 heads - fourth - sixth lactation. Of the 112 first-calf cows, 67 were raised in the herd of SLL "Doksancom", 20 heads were purchased from the breeding farm of (Joint-Stock Company) J-SC "Aydyn", 25 goals - imported from Holland.

The main data on the milk production of animals were taken from forms of zootechnical and pedigree accounting. All the analyzed number of cows was kept in optimal conditions of feeding and keeping in accordance with the basic zootechnical and hygiene requirements.

Were used zootechnical research methods with biometric processing of materials by the method of variation statistics according to Plokhinsky (1978) and Mercurieva (1983): arithmetic mean (X), arithmetic mean error (Sx), coefficient of variability (Cv), correlation coefficient (r) and correlation coefficient error (m).

Lactation curves of cows were constructed, and was calculated the milk coefficient (MC - of milk produced per 100 kg of live weight), proposed by Startsev (1965) using the formula: MC = MY / LW, where MC is the milk coefficient, kg; M Y- milk yield for 305 days of lactation, kg; LW - live weight, kg. The relationship between milk productivity indicators and milk quality, live weight was determined by calculating the correlation coefficient using Microsoft Excel, the reliability of the indicators was determined by Student.

RESULTS AND DISCUSSIONS

The results of studying the nature of milk production of cows of the breeding farm of Society limited liability "Doksancom" in the dynamics of lactation are shown in Table 1.

Table 1. Dynamics of milk production of cows of SLL "Doksancom" (X ± Sx)

No	Indicators	Lactation			On average fourth - sixth lactation
		first	second	third	
1.	The number of cows, head	112	90	36	21
2.	Live weight, kg	578±2.1	642±4.5	678±3.4	687±6.4
3.	Milk, kg	9085±183.5	10553±231***	10300±313***	10284±400**
4.	Fat content, %	3.88±0.01	3.92±0.01	3.89±0.016	3.91±0.014
5.	Amount of fat, kg	352±7.0	412±8.7	402±11.4	402±15.5
6.	MC*, kg	1558±33.7	1629±35.4	1502±44	1498±61

Note: **P>0.01; ***P>0,001; MC* - of milk produced per 100 kg of live weight

It was established that primiparous cows in first lactation had milk production at 88.2% compared to mature cows (standard 70-75%), which averaged 9085 kg of milk fat of 3.88%. With increasing number of lactations it also increased the production of milk per lactation, the amount of fat. Thus the milk production on second lactation was by 1468 kg more milk and constituted on average 10553 kg of milk, on third lactation - by 1215 kg, on fourth - sixth lactations - by 1199 kg, the difference being significant, with P<0.001, P<0.001 and P<0.01 respectively. The amount of overall fat spotted to the second lactation by 60 kg, with the third lactation and more - by 50 kg, the difference being significant, with P<0.001-P<0.01 correspondingly.

Live weight corresponded to and was higher than the breed standard at the end of the first, second, third and older lactations. First heifers had a live weight higher than the standard by 28 kg (550 kg), second lactation - by 42 kg (600 kg), third lactation - by 28 kg (650 kg), and third lactation and more - by 37 kg (Figure 1).

For a more complete characterization of milk productivity and the efficiency of using animals, it was calculated the milk production coefficient, which can be used to establish the highlighting of the dairy type of cattle.

The highest milk production coefficient per 100 kg of live weight, have the cows of the second lactation - 1629 kg of milk. The milk production coefficient of first-calf heifers was 1558 kg, according to the third - 1502 kg. The relatively high indices of the milk production coefficient can be explained by the fact that cows have relatively high milk productivity for a number of analyzed lactations, which indicates the highlighting of the milk type.

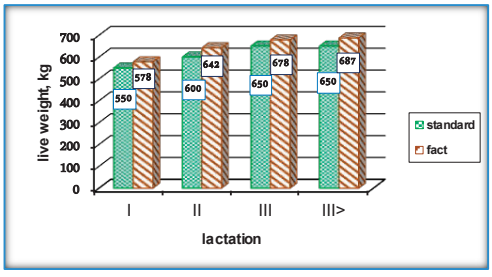

Figure 1. Live weight of cows in lactation dynamics, SLL "Doksancom"

The change in milk yield at highly productive cows of herd of the SLL “Doksancom” is shown in the following figures of lactation curves (Figures 2-5).

As it can be seen from the figures, at cows no. 7320 and no. 7582 lactation curve increases by the 2nd month lactation and within 2-3 months of lactation stabilizes, by the fourth month it rises to the peak of lactation. Then it gradually decreases until the end of lactation by an

average of 9.6% with fluctuations from 4.5 to 18.1% (no. 7320) and by 10.6% with fluctuations from 6.6 to 19.8% (no. 7582).

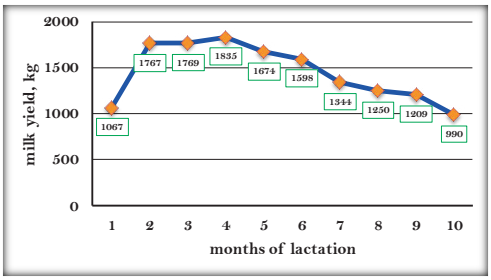

Figure 2. Lactation curve of cow no.7320, fourth lactation, milk yield 14513 kg of milk

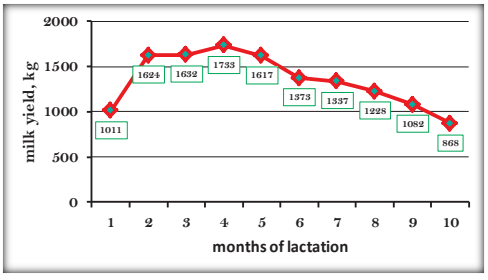

Figure 3. Lactation curve of cow no. 7582, of the third lactation, milk yield 13,422 kg of milk

At cow no. 1667 from the first to 4 months of lactation, there is a gradual increase in the average monthly milk yield, the peak of the lactation curve falls on 4 months of lactation, and then over the next months the curve gradually and smoothly decreases until the end of lactation. Lactation curve of cow no. 2967 (Figure 4) is somewhat different from the others in that the average monthly milk yield for the second month of lactation is slightly less (by 38 kg) of the first month of lactation.

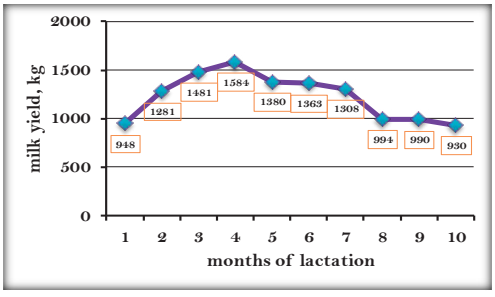

Figure 4. Lactation curve of cow no. 1667, fourth lactation, milk yield 12556 kg of milk

The peak of lactation occurs in the third month, followed by a gradual decrease until the end of lactation. It should be noted that the results of our studies are consistent with the data (Nekrasov et al., 2011), which also fixed the maximum values of the average monthly milk yield for 2-3-4 months lactation and the conclusion (Devyatov, 1983; Aldrich, 1987) that the lactation curve, regardless of productivity, has a certain optimal form with balanced feeding.

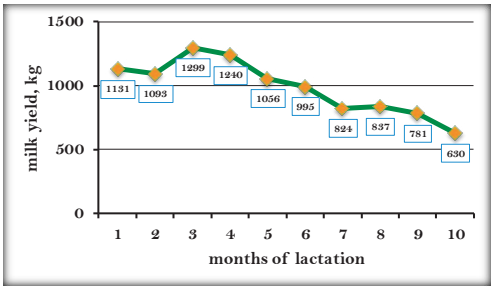

Figure 5. Lactation curve of cow no. 2967, of the second lactation, milk yield 9886 kg of milk

Thus, the lactation curve at all analyzed Holstein cows of the herd of SLL "Doksancom" changes with a certain regularity and has a leveled character, characterized by a high stable type, which is characteristic to animals with a strong constitution and high milk production.

The milk productivity of cows, as it is known, depends on a large number of factors, one of which is compliance with the technology of breeding and exploitation of animals in various farms and countries. In this regard, we carried out a comparative analysis of the milk yield of first-calf heifers raised in the herd of SLL "Doksancom", purchased from the breeding farm of J-SC. "Aydyn" and imported from Holland, tab. 2.

Analysis of Table 2 showed that the highest productivity was observed at first-calf cows raised in the herd of SLL "Doksancom" - 9518 kg of milk, which is by 925 kg more than heifers purchased from the breeding farm of J-SC "Aydyn", the difference is significant ($P<0.05$) and for 1200 kg - heifers imported from the Netherlands ($P<0.01$).

The amount of milk fat per lactation of first-calf heifers of all groups was high, but the

superiority should be given to first-calf cows raised in SLL "Doksancom", which exceeded by this feature first-calf cows from J-SC "Aydyn" by 39 kg, from Holland - 53 kg.

In terms of live weight, all first-calf heifers assessed averaged 578 kg, which exceeds the breed standard at the end of the first lactation by 28 kg (standard 550 kg).

Table 2. Milk productivity of first-calf cows, depending on origin (X ± Sx)

No	Indicators	Society of limited liability "Doksancom"	Joint-Stock Company "Aydyn"	From the Netherlands	On average
1.	The number of cows, head	67	20	25	112
2.	Live weight, kg	581±2.9	577±3.6	571±4.0	578±2.1
3.	Milk, kg	9518±275.3	8593±243.2	8318±220.3	9085±183.5
4.	Fat content, %	3.91±0.01	3.86±0.01	3.83±0.01	3.88±0.01
5.	Amount of fat, kg	371±10.4	332±9.4	318±8.1	352±7.0

Of great importance in breeding work with dairy cattle has the correlation between economically useful traits. So, for example, the variability of the mass content and the amount of fat in milk, as well as the live weight, depend on the variability of the milk yield of cows for lactation.

The results of studying the correlation between productivity indicators in the herd of SLL "Doksancom" are given in Table. 3.

Table 3. Correlation between productivity indicators and live weight, r ± m

No	Correlated trait	I lactation	II lactation	III lactation	IV-VI lactations
1.	milk yield (X) - fat, % (Y)	-0.156±0.09	-0.409±0.1	-0.449±0.15	-0.154±0.05
2.	milk yield (X) - fat, kg (Y)	+0.993±0.0	+0.989±0.01	+0.986±0.02	0.995±0.02
3.	milk yield (X) - live weight (Y)	+0.189±0.08	+0.103±0.11	+0.271±0.16	-0.083±0.23

The relationship between milk yield and the percentage of fat in milk of cows was in a negative correlation from weak -0.154 (IV-VI lactation) to moderate -0.409 - -0.449 (II - III lactation), respectively. As it can be seen, one-way selection for the level of milk yield led to an increase in the negative relationship between these features. The presence of a negative relationship between the level of milk yield and fat content in milk makes it difficult to conduct successful selection and indicates the need for simultaneous selection for milk yield and fat content in milk.

It should be noted a high correlation between the amount of milk and the amount of milk fat, which for the first lactation is +0.993, second lactation - +0.989, third lactation - +0.986 and fourth-sixth lactation - +0.995. A comparative analysis of the results of the relationship between milk yield and the amount of milk fat established a high reliable positive relationship between the second and the first ($P<0.001$), between the third and first lactations ($P<0.05$).

Between the milk yield of the live weight of cows, a weak positive correlation was revealed for the first (+0.189), second (+0.103) and third (+0.271) lactations, a weak negative (-0.083) - for the fourth-sixth lactations. Low correlation coefficients between milk yield for all lactations and live weight indicate non-linear relationships between them, which characterizes the uniformity of the herd in terms of live weight.

CONCLUSIONS

1. With increasing number of lactations it also increased the production of milk per lactation, the amount of fat. Thus the milk production on lactation II was by 1468 kg more milk and constituted on average 10553 kg of milk, on lactation III - by 1215 kg, on lactations IV-VI - by 1199 kg, the difference being significant, with $P<0.001$, $P<0.001$ and $P<0.01$, respectively.
2. The high indicators of the milk production ratio are explained by the fact that cows have relatively high milk productivity for a number of lactations, which indicates the highlight of

the dairy type of the analyzed population of Holstein cattle.

3. A comparative analysis of the milk yield of first-calf heifers showed that first-calf cows raised in the herd of Society of limited liability "Doksancom" had the highest productivity - 9518 kg of milk, which is by 925 kg more than that of first-calf cows purchased from the breeding farm of Joint-Stock Company "Aydyn", the difference is significant ($P<0.05$) and for 1200 kg - first-calf cows imported from Holland ($P<0.01$).The lactation curve of Holstein cows of the herd of Society of limited liability "Doksancom" changes with a certain regularity and has a flattened character.

4. Low correlation coefficients between milk yield for all lactations and live weight indicate a non-linear nature of the relationships between them, which characterizes the uniformity of the herd of Society of limited liability "Doksancom" in terms of live weight.

5. In order to increase the productivity of the population when selecting animals for their own reproduction in JSC "Aydyn" and SLL "Doksancom" it should be taken into account the stability of the lactation curves and milk production coefficients.

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USE OF ENTROPIC AND INFORMATION ANALYSIS OF LIVING WEIGHT OF DAIRY COWS FOR PRODUCTIVITY

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Abstract

In the article, due to the use of entropy-informational analysis, the statistical parameters of the biological system, the degree of its entropy of absolute and relative organization, which is represented by the live weight of dairy cows, have been studied. It has been established that biological systems, regardless of the breed factor, become more ordered at the age of nine and fifteen months, as well as at their birth. It has also been proven that cows of the Ukrainian black-and-white dairy breed have lower values of unconditional entropy, that is, entropy tends to zero, and the system becomes more orderly. Thus, animals with high levels of ordering of systems in terms of live weight, respectively, will have a high degree of ordering of systems represented by the main indicators of milk productivity. This can serve as a kind of marker when predicting it.

Key words*: body weight, dairy cattle, entropy, entropy and information analysis (EIA).*

INTRODUCTION

The processes of system development in define direction can be modeled by the exploring information transfer mechanism. Whereas it gives opportunity to clarify the mechanisms of system progress inclusive of its amplification, orderliness and organization degree increase (Gill, 2010; García-Garibay, 2010; Merkyryeva, 1989).

Entropy in general is the degree of uncertainty, any system disorderliness, it is some degree of chaos, disorder expression. Entropy in the context of selection is a capability to show variability in time under the influence of probable factors (Shennon, 1963).

Compared to immunogenotypic analysis that allow to estimate only by the alleles of blood types and protein polymorphism types, EIA takes into account also heterozygosity or homozygosity by the basic features of selection. It gives an opportunity for deeper evaluation of the population variability that is useful while predicting of productivity – selection planning (Gill, 2010; Halushko, 2009; Karateeva, 2013; Pidpala et al., 2018).

In recent years, many statistical approaches have been proposed for detecting gene-gene (G x G) interactions, including numerous methods based on information theory, inspired by the concept of entropy. They are considered to be especially powerful and, because of their non-linearity, they are better able to capture non-linear relationships between genetic variants and / or variables. In addition, the presented estimates based on entropy are fundamentally different in their design and even in the basic definition of interactions (Ferrario & König, 2018).

Kwon et al. (2014) used IGENT, iNTeraction method based on GEnome information theory. IGENT is an efficient algorithm for detecting genome-wide gene-gene interactions (GGI) and gene-environment interactions (GEI). And it was found that entropy-based gene interaction (GGI) analysis reveals much of the unexplained inheritance of complex traits.

Malten & König (2020) modified an efficient method for the simultaneous detection of the main effects and effects of gene interaction which is the entropy-based IGENT method. This modification is based on conditional mutual information, subject to the equilibrium of links. The modified estimate is investigated in complex modeling based on five models of genetic interaction using entropy analysis.

Stock-breeder's system concept has to include complete analysis of entropy qualities changes of biological systems of any complexity as they accommodate define number of information that indicative to particular population. The systems are bounded within these populations,

and for such systems the features entropy is growing or remaining the same (Gill, 2010; Kramarenko, 2005).

Bio-computational gene grouping facilitates genetic analysis, sequencing, and structural analysis. To calculate the Shannon's entropy of DNA sequences of genes in the extraction of cluster genes that control milk production in dairy cows, Dehghanzadeh et al. (2017) used the Kulback-Leibler (KL) divergence equation which is based on the similarities and differences of nucleotides and different orders of relative entropy. The research results showed that exons with the maximum entropy value are suitable for genotype analysis using molecular markers, and both coding and non-coding sequences have low or high complexity of system organization.

KL divergence can be used to cluster large gene sets of dairy cattle with other methods to group biologically significant gene sets (Dehghanzadeh et al., 2017).

The relationship between indicators of milk production and genes associated with it was also studied by Dehghanzadeh et al. (2020). Entropy is a measure of the uncertainty of a set of information. In his study (Dehghanzadeh et al., 2020), which is based on the relative entropy of genes and exons, the Kullback-Leibler divergence was calculated using the clustering of genes responsible for the milk production of cows. As a result of reseach on the study of metabolic pathways of genes based on gene annotations, it was found that the proposed clustering method gives correct, logical and quick results. At the same time, this method did not have the disadvantages associated with alignment, made it possible to take into account genes of actual length and content, and also did not require high memory for long sequences. Therefore, Dehghanzadeh et al. (2020) believe that the performance of their proposed method can be used with other competitive gene clustering techniques for grouping a biologically significant set of genes. Therefore, the proposed method can be considered as a method for predicting genes associated with performance indicators and genes with weak genomic annotations (Dehghanzadeh et al., 2020).

Using entropy information analysis, Ruiz-Marín et al. (2010) studied the etiology of complex diseases caused by a combination of genetic and environmental factors. Using symbolic dynamics and symbolic entropy as a measure of gene dependence Ruiz-Marín et al. (2010) developed a new, simple, consistent and powerful test for detecting the genetic association of biallelic / SNP markers. This test is based on entropy measures and avoids smoothed nonparametric estimates and is more efficient than Fisher's analysis, especially for a large number of markers (Ruiz-Marín et al., 2010).

Borowska et al. (2018) used information theory as an alternative statistical approach to identify regions of the genome and candidate genes associated with economically useful traits of livestock. The following sperm quality variables were analyzed: CASA sperm kinematics (total motility, mean path speed, straight-line speed, curvilinear speed, amplitude of lateral head displacement, lateral beat frequency, straightness, linearity), sperm membrane integrity (plasmolem, mitochondrial function), the content of ATP in sperm. Entropy and conditional entropy were estimated for each SNP. Conditional entropy quantifies the remaining uncertainty about the values of a variable, taking into account SNPs. The most informative SNPs for each variable were determined. The results of the study showed that important regions of the genome and candidate genes that determine the variable qualities of bovine semen are located on several chromosomes. Scientists have proved the reliability of the effect of SNPs on some variables in the quality of Holstein-Friesian bovine sperm using entropy analysis (Borowska et al., 2018).

Based on the assessment of the results of entropy-information analysis of the signs of milk productivity of Holstein cows, Pidpaloi et al. (2018), it was established the degree of organization and information content for Holstein cows of German and Ukrainian breeding of adjacent generations based on the characteristics of productivity, reproductive and adaptive ability of animals. The following traits were characterized by a high level of determinism: the content of fat and protein in milk in both Holstein cows of German and Ukrainian breeding (Pidpala et al., 2018).

Thus, the use of entropy analysis to study the state of a specific biological system will

provide objective data on the influence of various factors on the level of performance indicators of animals. This, in turn, will improve the accuracy of assessing animals and will make it possible to predict various options for selecting animals for their further use in selection and breeding work.

MATERIALS AND METHODS

The object of research was the full grown cows of three breeds what are specific for South region of Ukraine (n = 189): Red Steppe Breed (n = 88), Ukrainian Black-and-white Dairy Breed (n = 52), Ukrainian Red Dairy Breed (n = 49). These cows belong to two lead farms in Nikolayev region: Red Steppe and Ukrainian Black-and-white Dairy cows to GP «PR Stepnoy», and Ukrainian Red Dairy cows to PSPH «Kozyrskoe». Body weight of newborn cows and three-, six-, nine-, 12-, 15-, and 18-months cows was the subject to study. Entropy-information manipulation of data was made by the generally accepted procedure in S.S Kramarenko version (Kramarenko, 2005).

The measure of intrapopulation unconditional entropy of quantitative character was calculated using a formula:

$$H = -\sum_{i=1}^{k} \left(p_i \cdot \log_2 \cdot p_i \right)$$

where:

H - entropy of concrete statistical system;

pi - probability (or frequency) of characteristic variability by gradations of variation series;

k - quantity of probable system variants (characters).

Maximum possible theoretically determinate entropy for this system stage is calculated by the formula:

$$H_{\max} = \log_2 \cdot k$$

where:

Hmax - the degree of complexity or maximum system indeterminateness;

k - maximum number of system conditions of the characteristic.

The level of absolute system orderliness is calculated using a formula:

$$O = H_{\max} - H$$

The level of relative system orderliness is calculated using a formula:

$$R = 1 - H / H_{\max}$$

Entropy zero level demonstrates the highest orderliness. In determine systems level of relative entropy is high and reaches one. $R = 0$ in completely disorganize systems.

Two-factor analysis of variance was used for establishment of factors effect on the system organization.

RESULTS AND DISCUSSIONS

Ouma et al. (2007) used maximum entropy in search theory with an emphasis on heterogeneity among animals in terms of their live weight and its impact on adaptation to specific conditions of livestock management technology. The data obtained indicate the existence of heterogeneity in live weight and their adaptation to certain conditions. Thus, cattle with higher live weight showed better adaptation to industrial technologies than animals with lower live weight (Ouma et al., 2007).

Based on the entropy of Fukuda et al. (2013), an RBF neural network model was built to predict the weight of pigs based on the growth parameters of Landrace sows. The results showed that the RBF neural network modeling method using entropy analysis was an effective way to build a pig weight prediction model. Entropy removed the collinearity of the explanatory variables in linear regression analysis and allowed for predicting the live weight of pigs better than the linear regression model (Fukuda et al., 2013).

At the same time, there are no data on the study of live weight of cattle using entropy and its main indicators. This technique will improve the accuracy of assessing animals and will make it possible at an early age to predict various options for selecting animals for their further use in their selection. This was the goal of our research.

It should be noted that entropy level is variable within 1.597 ..... 3.228 bit by using body weight entropy-information analysis of cows at birth and in the age of three, six and nine months, and also in the age of 12, 15, and 18 months. It means that range of variability is rather wide (Table 1). Absolute entropy by the body weight of Red Steppe Breed cows within the prescribed periods comes up to 2.165 .... 3.228 bit, Ukrainian Red Dairy Breed 2.024 ..... 3.171 bit and Ukrainian Black-and-white

Dairy Breed - 1.597 ..... 3.179 bit. It shows system organization high level exactly at Ukrainian Black-and-white Dairy Breed representatives.

Table 1. EIA of body weight variability (kg) of cows in the Southern Ukraine

Cows age	*n*	Body mass entropy parameters of cows, bit			
		H ± SEH	*Hmax*	*O*	*R*
Newborn	88	2.165±0.076	3.322	1.157	0.348
3 months	88	3.228±0.039		0.094	0.028
6 months	88	2.241±0.114		1.081	0.325
9 months	88	2.222±0.111		1.110	0.331
12 months	88	3.108±0.057		0.214	0.065
15 months	88	2.733±0.086		0.589	0.117
18 months	88	3.138±0.054		0.184	0.055
Newborn	49	2.024±0.080	3.322	1.298	0.391
3 months	49	3,044±0.061		0.278	0.084
6 months	49	3.103±0.045		0.218	0.066
9 months	49	3.090±0.049		0.232	0.070
12 months	49	3.171±0.060		0.151	0.045
15 months	49	3.073±0.052		0.248	0.075
18 months	49	3.083±0.049		0.239	0.072
Newborn	52	1.597±0.096	3.322	1.725	0.519
3 months	52	3.084±0.071		0.237	0.071
6 months	52	3.179±0.063		0.143	0.043
9 months	52	2.814±0,010		0.508	0.153
12 months	52	2.848±0.064		0.474	0.143
15 months	52	2.991±0,066		0.330	0.099
18 months	52	2.966±0.072		0.356	0.101

Absolute and relative entropy in this group increases: *O* from 0.143 to 1.725 bit and *R* from 0.043 to 0.519 bit inclusive. Animals with high indexes of the system orderliness by the body weight respectively will have high level of the system orderliness by main characters of milk productivity. It can be kind of marker in milk productivity forecasting.

It has been noted that the tendency of orderliness degree increases at birth, and at the age of nine and fifteen months that is not depend on the breed, it is demonstrated by entropy low values of Red Steppe Breed (2.0165; 2.222; 2.273 bit), Ukrainian Red Dairy Breed (2.024; 3.090; 3.073 bit), Ukrainian Black-and-white Dairy Breed (1.597; 2.814; 2.991 bit). The system becomes more organize in the end of growing period.

As a result, presented biological systems by cows body weight generally by Antomonov classification (Antomonov, 1977) are found as the stochastic quasideterministic systems, as their relative orderliness is not more than 0.1, that points at their high separability during the stock breeding.

Performed analysis of variance established that age factor or body weight period formation to 61% (P≤0.01) have essential influence on the dynamics of orderliness degree display during the whole cows growing period. It is not depend on the breed only (η^{x2} = 1%), random factors have impact (38%) (Table 2).

Table 2. Factorial conditionality of the entropy level of cows body weight

Impact factors	***SS***	***Df***	***MS***	***F***	***p***	η^{x2}
A - breed	0.224649	2	0.112324	1.293	0.310	1.0
B - age	3.047053	6	0.507842	5.847	0.005	61.0
Random factors	1.042297	12	0.086858			38.0
Total variability	4.313999	20				

CONCLUSIONS

It is proved, that Biological systems that are presented by body weight of Red Steppe, Ukrainian Red Dairy and Ukrainian Black-and-white Dairy cows belong to stochastic quasideterministic systems.

It was find out that orderliness degree increases at birth, and at the age of nine and fifteen months. It is appropriate to use received results of different systems as accessory parameters in the selective stock breeding and in the milk productivity forecasting.

It was deduced statistically significant factors influence on body weight formation during development: body weight formation period (age) dominates (to 61%) in comparison with breed factor - 1.0%.

Thus, the obtained results make it possible to assert that the use of entropy-informational analysis in the selection of animals can be used as an additional indicator of their assessment for the main economically useful traits, in particular, their live weight. That will allow you to get a more accurate and complete assessment and predict their future live weight at an early stage of development.

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MEAT QUALITIES OF PIGS OF DIFFERENT GENOTYPES BY MELANOCORTIN RECEPTOR GENE 4 (MC4R) AND ITS CONNECTION WITH SOME BIOCHEMICAL INDICATORS OF BLOOD SERUM

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Abstract

Results of studies about fattening and meat qualities of Large White young pigs of different genotypes by melanocortin receptor gene 4 (MC4R) are presented, as well as level of correlations between these traits and some biochemical indicators of blood serum. It was established that biochemical parameters of blood serum and concentration of total lipoproteins (mg%) of young pigs correspond to physiological norm of clinically healthy animals, fattening and meat qualities of animals of different genotypes by melanocortin receptor gene 4 (MC4R) correspond to I and elite class. Considering intrabreed differentiation by genotype, difference between animals of II (MC4RAG) and I (MC4RAA) groups in average daily gain in live weight during the control fattening period is 91.9 g (td = 7.00, P<0.001), in length of chilled carcass is 0.8 cm (td = 0.78, P>0.05), in length of the bacon side of chilled half carcass is 1.2 cm (td = 0.48, P>0.05), in thickness of fatback at the level of 6-7 thoracic vertebrae is 1.8 mm (td = 2.22, P<0.01) and in age of reaching live weight of 100 kg is 9.9 days (td = 5.78, P<0.001).

Key words*: biochemical parameters of blood serum, correlation, fattening qualities, genotype, young pigs.*

INTRODUCTION

The practice and scientific researches of native and foreign scientists indicate that an effective method of determining the genetic potential by the reproductive traits of pigs in the main herd, as well as the fattening and meat qualities of their offspring, is the use of modern genetic methods DNA markers (Zhukorskii & Tsereniuk, 2015; Tsereniuk, 2014; Bazhov & Konlatskii, 1989; Khalak et al., 2020; Topikha et al., 2012; Lykhach et al., 2016; Noguera et al., 2014; Ryzhova & Kalashnikova, 2003; Zinovieva et al., 2013; Loban et al., 2011; Kim et al., 2006; Walsh et al., 1991; Dyman et al., 2001; Muñoz et al., 2011; Berezovskii & Khatko, 2005; Berezovskii, 1999; Vlizlo, 2012; Lakin, 1990).

Research work provided by different scientific institutions have established that the polygenic hereditary traits of pigs are determined by the complex interaction of animal genotype and environmental conditions. Consequently, in order to increase the economy of high-quality pork production, taking into account the optimization of fattening conditions and housing of animals of different gender and age groups in selecting and breeding work, it is necessary to use modern genetic methods (DNA markers) in selection and breeding work. It was proved in works of Konoval et al., 2008; Hetmantseva et al., 2012; Hladyr et al., 2009; Dyman et al., 2001; Yepishko, 2008; Zinovieva & Ernst, 2006; Korinnyi et al., 2005; Konoval et al., 2007; Loban, 2010; Khalak et al., 2020; Khalak et al., 2020.

The aim of the research is to study the fattening and meat qualities of Large White young pigs of different genotypes by melanocortin receptor gene 4 (MC4R), as well as to determine the level of correlations between these traits and some biochemical parameters of blood serum.

MATERIALS AND METHODS

The studies were carried out in the conditions of agricultural formations of Dnipropetrovsk region, among them there are the animal husbandry laboratory of State Institution - Institute of Grain Crops, National Academy of Sciences of Ukraine, the research centre for biosafety and environmental control of resources of the agro-industrial complex of Dnipro State Agrarian and Economic University and the laboratory of genetics of Institute of Pig Breeding and the agro-industrial institution NAAS of Ukraine.

Large White young pigs of Hungarian origin were the object of research.

DNA isolation from biomaterial samples (earmark) was performed with using ion exchange resin *Chelex-100* (Walsh et al., 1991), DNA typing with using the PLR-RFLP technique (Dyman et al., 2001) at the *MC4R* gene locus (Muñoz et al., 2011).

Evaluation of young pigs by fattening and meat qualities was carried out taking into account the following indicators: average daily gain in live weight for the period of control feeding, kg; age of achievement of live weight of 100 kg, days; thickness of fatback at the level of 6-7 thoracic vertebrae, mm; length of chilled carcass, cm; length of bacon side of chilled half carcass, cm (Berezovskii & Khatko, 2005).

Integrated assessment of the fattening and meat qualities of young pigs in the experimental groups was carried out with the use of complex index of fattening and meat qualities (B. Tyler index) (Iв):

$$Iв = 100 + (242 \times K) - (4.13 \times L) \qquad (1),$$

where: Iв - complex index of fattening and meat qualities (B. Tyler index), points; K - average daily gain in live weight, kg; L - thickness of fatback at the level of 6-7 thoracic vertebrae, mm; 242; 4.13 - constant coefficients (Berezovskii, 1999).

In the blood serum, the total protein content (g/l), the urea content (mmol/l) and the concentration of total lipoproteins (mg%) were determined (Vlizlo et al., 2012).

The pair correlation coefficient (r), its error (S_r), and the reliability criterion (t_r) were calculated using the formulas 2, 3, 4:

$$r = \frac{\sum xy - \frac{\sum x \cdot \sum y}{n}}{\sqrt{C_x \cdot C_y}} \qquad (2)$$

$$S_r = \sqrt{\frac{1 - r^2}{n - 2}} \qquad (3)$$

$$t_r = \frac{r}{S_r} \qquad (4)$$

The strength of correlations between the traits was determined using the Chaddock scale (Table 1) (Sidorova et al., 2003).

Table 1. Chaddock scale for of strength of correlation gradation

Correlation coefficient value	Correlation strength
0.1-0.3	Weak
0.3-0.5	Moderate
0.5-0.7	Noticeable
0.7-0.9	High (close)
0.9-0.99	Strong

Biometric processing of the obtained research results was carried out according to the method of Lakin (1990).

RESULTS AND DISCUSSIONS

Analysis of primary zootechnical registration and research results indicate that Large White young pigs from the controlled herd reach live weight of 100 kg in 171.4 ± 1.20 days (Cv = 3.73%), the average daily gain in live weight during the period of control fattening is 784.0 ± 11.34 g (Cv = 7.65%), thickness of fatback at the level of 6-7 thoracic vertebrae is 20.9 ± 0.365 mm (Cv = 9.22%), chilled carcass length is 96.5 ± 0.85 cm (Cv = 2.65%), length of the

bacon side of the chilled half carcass is 84.7 ± 1.91 cm (Cv = 6.78%). The complex index of fattening and meat qualities (B. Tyler index) (IV) varies from 131.45 to 169.93 points.

The coefficient of variability of fattening and meat qualities of Large White young pigs from the controlled herd varied from 2.37 to 9.23% (Table 2).

Table 2. Indicators of variability of fattening and meat qualities of Large White young pigs from a controlled herd

Indicators, units	Biometric indicators	
	$\sigma \pm S_\sigma$	$Cv \pm Sc_v$, %
Average daily gain in live weight during the control fattening period, kg	60.40±13.512	7.70±1.722
Age of achievement of live weight of 100 kg, days	5.11±1.143	2.98±0.667
Thickness of fatback at the level of 6-7 thoracic vertebrae, mm	1.93±0.431	9.23±2.064
Length of chilled carcass, cm	2.29±0.512	2.37±0.530
Length of the bacon side of chilled half carcass, cm	5.61±1.255	6.62±1.480

Laboratory studies have shown that the total protein content in the blood serum of young pigs in the experimental group is 82.00 ± 2.108 g/l (Cv = 7.71%), the urea content is 4.69 ± 0.208 mmol/l (Cv = 16.60%), the concentration of total lipoproteins is 611.36 ± 48.872 mg% (Cv = 33.66%).

The results of studies of blood serum biochemical parameters, fattening and meat qualities of young pigs of different interbreed differentiation by genotype are shown in Tables 3 and 4.

It was established that the biochemical parameters of blood serum of young pigs from I (MC4RAA) and II groups (MC4RAG) correspond to the physiological norm of clinically healthy animals (Bazhov & Konlatskii, 1989).

Table 3. Biochemical parameters of blood serum, fattening and meat qualities of young pigs of different intrabreed differentiation by genotype SNP c. 1426 G> A melanocortin receptor gene 4 (Mc4r), n = 5

Indicators, units	Biometric indicators	Genotype	
		$MC4R^{AA}$	$MC4R^{AG}$
		Group	
		I	II
Total protein content, g/l	$\bar{X} \pm S_{\bar{X}}$	83.25±2.528	85.00±1.527
	$\sigma \pm S_\sigma$	5.05±1.598	2.64±0.835
	$Cv \pm Sc_v$,%	6.08±1.924	3.11±0.984
Urea content, mmol/l	$\bar{X} \pm S_{\bar{X}}$	4.46±0.238	4.72±0.331
	$\sigma \pm S_\sigma$	0.58±0.183	0.87±0.275
	$Cv \pm Sc_v$,%	13.11±4.148	18.59±5.882
Concentration of total lipoproteins, mg%	$\bar{X} \pm S_{\bar{X}}$	528.00±38.782	581.33±31.399
	$\sigma \pm S_\sigma$	109,69±34.712	76.91±24.338
	$Cv \pm Sc_v$,%	20.78±6.575	13.23±4.186

The difference between animals from the indicated groups and genotypes in terms of the total protein content is 1.75 g/l (td = 0.59, P>0.05), in the urea content is 0.26 mmol/l (td = 0.65, P>0.05), in the concentration of total lipoproteins is 53.33 mg% (td = 1.06, P>0.05).

The analysis of results of control fattening showed that young pigs from the II group ($MC4R^{AG}$), in comparison with their peers from the I group ($MC4R^{AA}$), are characterized by higher indicators of the average daily gain in live weight during the control fattening period (by 91.9 g, td = 7.00, P<0.001), length of chilled carcass (by 0.8 cm; td = 0.78, P>0.05), length of the bacon side of the chilled half carcass (by 1.2 cm; td = 0.48, P>0.05), smaller indicators of thickness of fatback at the level of 6-7 thoracic vertebrae (by 1.8 mm, td = 2.22, P<0.01), and age of achievement of live weight of 100 kg (by 9.9 days, td = 5.78 , P<0.001).

Table 4. Fattening and meat qualities of young pigs of different genotypes by melanocortin receptor gene 4 (Mc4R), n = 10

Indicators, units	Biometric indicators	Genotype	
		$MC4R^{AA}$	$MC4R^{AG}$
		Group	
		I	II
Average daily gain in live weight during the control fattening period, kg	$\bar{X} \pm S_{\bar{x}}$	721.6±10.84	813.5±7.40
	σ±Sσ	34.15±7.639	23.42±5.239
	Cv±Scv, %	4.73±1.058	2.88±0.644
Age of achievement of live weight of 100 kg, days	$\bar{X} \pm S_{\bar{x}}$	178.0±1.16	168.1±1.271
	σ±Sσ	3.69±0.825	4.02±0.899
	Cv±Scv, %	2.07±0.463	2.39±0.534
Thickness of fatback at the level of 6-7 thoracic vertebrae, mm	$\bar{X} \pm S_{\bar{x}}$	22.3±0.57	20.5±0.58
	σ±Sσ	1.82±0.407	1.84±0.411
	Cv±Scv, %	8.19±1.832	8.97±2.00
Length of chilled carcass, cm	$\bar{X} \pm S_{\bar{x}}$	95.4±0.82	96.2±0.61
	σ±Sσ	2.60±0.581	1.93±0.431
	Cv±Scv, %	2.71±0.607	2.01±0.449
Length of the bacon side of chilled half carcass, cm	$\bar{X} \pm S_{\bar{x}}$	82.0±2.22	83.2±1.14
	σ±Sσ	7.03±1.572	3.61±0.807
	Cv±Scv, %	8.58±1.919	4.34±0.970
Complex index of fattening and meat qualities (B. Tyler index), points	$\bar{X} \pm S_{\bar{x}}$	141.77±2.018	157.22±3.029
	σ±Sσ	6.38±1.427	9.58±2.143
	Cv±Scv, %	4.50±1.006	6.09±1.362

The difference between animals from the II (MC4RAG) and I (MC4RAA) groups in terms of the complex index of fattening and meat qualities (B. Tyler index) is 15.45 points (td = 4.25, P<0.001).

The coefficient of variability of biochemical parameters of blood serum, fattening and meat quality of young pigs of different intrabreed differentiation by melanocortin receptor gene 4 (Mc4R) varies from 2.01 (the length of chilled carcasses of animals of the $MC4R^{AG}$ genotype) to 20.78% (the concentration of total lipoproteins in blood of The results of calculating the coefficients of pair correlation between the biochemical parameters of blood serum, fattening and meat qualities of Large White young pigs are shown in Table 5.

It was found that correlation coefficient between biochemical parameters of blood serum, fattening and meat qualities of Large White young pigs varied from -0.533 to +0.375.

Table 5. Coefficients of paired correlation between biochemical parameters of blood serum, fattening and meat qualities of Large White young pigs

Features		Biometric indicators		Correlation strength
x	*y*	r±Sr	tr	
Average daily gain in live weight during the control fattening period, kg	1	-0.209±0.2445	0.85	Weak
	2	0.277±0.2402	1.15	Weak
	3	0.155±0.2470	0.63	Weak
Age of achievement of live weight of 100 kg, days	1	0.107±0.2486	0.43	Weak
	2	0.024±0.2499	0.10	Weak
	3	0.074±0.2493	0.30	Weak
Thickness of fatback at the level of 6-7 thoracic vertebrae, mm	1	0.375±0.2318	1.62	Moderate
	2	0.007±0.2500	0.03	Weak
	3	0.132±0.2478	0.53	Weak
Length of chilled carcass, cm	1	-0.321±0.2368	1.36	Moderate
	2	-0.445±0.2239	1.99	Moderate
	3	0.023±0.2499	0.09	Weak
Length of the bacon side of chilled half carcass, cm	1	-0.533±0.2115	2.52	Noticeable
	2	0.019±0.2500	0.08	Weak
	3	-0.101±0.2487	0.41	Weak

Note: 1 - total protein content, г/л; 2 - urea content, mmol/l; 3 - concentration of total lipoproteins, mg%

A reliable relationship with a probability of $P<0.05$ was established between the length of bacon side of chilled half carcass and the total protein content in the blood serum ($r = -0.533$, $tr = 2.52$).

Thus, the experiment showed that the biochemical parameters of blood serum of young pigs correspond to the physiological norm of clinically healthy animals; the fattening and meat qualities of animals of different genotypes by melanocortin receptor gene 4 (MC4R) correspond to class I and elite class.

CONCLUSIONS

It was established that the biochemical parameters of the blood serum of young pigs correspond to the physiological norm of clinically healthy animals, the fattening and meat qualities of animals of different genotypes by melanocortin receptor gene 4 (MC4R) correspond to class I and elite class.

Young pigs of MC4RAG genotype significantly exceeded their peers of MC4RAA genotype Average daily gain in live weight during the control fattening period by 91.9 g, in chilled carcass length by 0.8 cm, in length of the bacon side of chilled half carcass by 1.2 cm, in thickness of fatback at the level of 6-7 thoracic vertebrae by 1.8 mm and in age of achievement of live weight of 100 kg by 9.9 days.

Correlation links between biochemical parameters of blood serum (total protein content, urea content, concentration of total lipoproteins), fattening and meat qualities of young pigs are unreliable by 93.3% and cannot be used in selection as markers of these traits.

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THE BIOCHEMICAL INDICATORS OF BLOOD SERUM AND THEIR RELATIONSHIP WITH FATTENING AND MEAT QUALITIES OF YOUNG SWINE OF DIFFERENT INBREED DIFFERENTIATION ACCORDING TO THE SAZER-FREDIN INDEX

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Abstract

The article presents the results of studies of protein metabolism and their relationship with fattening and meat qualities of young pigs of large white breeds of different interbreed differentiation according to the index of A. Sazer - H. Fredin. It was found that the biochemical parameters of the serum of young pigs of the experimental group correspond to the physiological norm of clinically healthy animals. Taking into account the interbreed differentiation of young pigs of the large white breed according to the index of A. Sazer - H. Fredin, it was found that the animals of group II outperformed peers of I on average daily live weight gain during control fattening, age of 100 kg live weight, fat thickness at 6-7 thoracic vertebrae, the length of the chilled carcass, the length of the bacon half of the chilled half-carcass on average by 4.03%. The pairwise correlation coefficient between the biochemical parameters of blood serum, fattening, and meat qualities of young pigs of large white breeds range from -0.533 to +0.528.

Key words: *correlation, economic efficiency, variability, young pigs.*

INTRODUCTION

Studies of domestic and foreign scientists show that an essential factor in intensifying the selection process in pig breeding is developing and implementing innovative methods for assessing animals' breeding value and the search for biological markers of early prediction of quantitative traits (Getya, 2009; Esfandyari et al., 2015; Khalak et al., 2020).

To assess the breeding value of pigs, the information on the productivity of parents, siblings, and semi-siblings, the animal's productivity, and their offspring's productivity are used (Tserenyuk, 2009; Khalak, 2015). The effectiveness of these methods is different, and therefore in world practice, use a comprehensive assessment of pigs using signs of productivity and biochemical parameters of serum. Thus, according to (Bazhov & Komlatsky, 1989), it was found that the selection for groups with different indicators of growth energy according to the long-headedness index at 2 months of age, the difference in live weight at the age of 7 months is 8.6-2.1 kg; according to the body mass index 4.6-13.9 kg; taking into account these indices 16.2-20.4 kg. The correlation coefficient between the index's value and the actual growth rate in the control cultivation is equal to 0.727-0.862, in the control fattening - 0.712-0.856. The authors note that the indices of exchange at 4 months of age are closely related to economic benefits. The correlation coefficients of protein metabolism indices with productive traits exceed 0.70, lipid - 0.50-0.88, protein-lipid -

0.64-0.95. The opposite pattern is established in the work of (Khalak & Gutyj, 2020). The authors' studies showed that the pairwise correlation coefficients between the concentration of total lipoproteins, serum aspartate aminotransferase (AsT), and alanine aminotransferase (AlT) activity, fattening and meat qualities of young white pigs are characterized as multidirectional and weakly (-0.157 ± 0.2059), average daily increase in live weight during the period of control fattening, kg × alkaline phosphatase activity, units/l.) - + 0.161 ± 0.2058 (age of reaching live weight 100 kg, days × concentration of total lipoproteins, mg %) selection and evaluation indices. The authors argue that the use of biochemical parameters of blood serum for early prediction of fattening and meat qualities of young pigs is ineffective.

Vashchenko's (2019) studies show that the evaluation of factory-type pigs "Bagachansky" on linear models using DNA marker data (g. 143 CTSL C>T) and without it, showed a high correlation ($r = 0.96 \pm 0.001$, $p \leq 0.001$), which can be explained by the low frequency of the allele g. 143 $CTSL^T$ in the population. The results of DNA typing of Myrhorod pig breed according to the MC4R gene should be used as a fixed factor in determining the breeding value by the BLUP method based on "age of 100 kg" and "fat thickness". The correlation between the estimates obtained from the models with and without the use of data on the genotype of pigs by the MC4R gene, based on age at 100 kg is 0.76 ± 0.109 ($p \leq 0.001$); based on "fat thickness" - 0.71 ± 0.119 ($p \leq 0.001$)

The work aims to investigate some indicators of protein metabolism and their connection with fattening and meat qualities of young pigs of the large white breed of different interbreed differentiation according to the index of A. Sazer - H. Fredin, as well as to calculate the level of correlations between traits and economic effectiveness of research results.

MATERIALS AND METHODS

The research was conducted in agricultural formations and processing enterprises of the Dnipro region, the research center of biosafety and ecological control of agro-industrial resources of Dnipro State Agrarian and Economic University and livestock laboratory of the Institute of Grain Crops of NAAS. The work was performed according to the research program of NAAS of Ukraine №30, "Innovative technologies of breeding, industrial and organic production of pig products" ("swine breeding").

The object of the study was young pigs of the large white breed of Hungarian selection. Conditions for feeding and keeping animals of the experimental groups were identical and complied with zootechnical standards.

Evaluation of animals of the specified production group and genotype on indicators of fattening and meat qualities was carried out taking into account the following indicators: average daily gain of live weight for the period of control fattening, g; the age of live weight 100 kg, days; fat thickness at the level of 6-7 thoracic vertebrae, mm; length of the chilled carcass, cm; length of bacon half of chilled half-carcass., cm (Berezovsky & Khatko, 2005).

The formula calculated the A. Sazer - H. Fredin index and the complex index of fattening and meat qualities (Tyler B. index):

$$I = \frac{1}{\sigma_g} \times \Delta G_1 - \frac{1}{\sigma_f} \times \Delta F_1, \quad (1)$$

where: I - index of A. Sazer - H. Fredin; ΔG1 - the growth rate in deviations from the average; ΔF_1 - fat thickness in deviations from the average; σ_g - the phenotypic standard deviation of growth rate; σf is the phenotypic standard deviation of the fat thickness (Kozlovsky et al., 1982);

$$I_в = 100 + (242 \times K) - (4{,}13 \times L) \quad (2)$$

where: $I_в$ - the complex index of fattening and meat qualities (Tyler B. index), points; K - average daily weight gain, kg; L - the fat thickness at the level of 6-7 thoracic vertebrae, mm; 242; 4,13 - the constant coefficients (Vashchenko, 2019).

The serum of 5-month-old animals was determined by the content of total protein (g/l), the urea content (mmol/l), and the creatinine concentration (mg%) (Vlizlo et al., 2012).

Economic efficiency of research results and biometric processing of the obtained data

(Lakin, 1990) was carried out according to generally accepted methods.

The Chaddock scale determined the strength of the correlations between the traits (Sidorova et al., 2003) (Table 1).

Table 1. Chaddock scale for gradation of correlation strength

The value of the correlation coefficient	The correlation strength
0.1-0.3	Weak
0.3-0.5	Moderate
0.5-0.7	Noticeable
0.7-0.9	High
0.9-0.99	Very high

RESULTS AND DISCUSSIONS

It was found that the biochemical parameters of the serum of young pigs of the experimental group correspond to the physiological norm of clinically healthy animals. Thus, the total protein content is 82.0 ± 2.10 g/l (Cv = 7.71%), urea content is 4.77 ± 0.30 mmol/l (Cv = 18.01%) and creatinine concentration is 90, 86 ± 4.193 μmol/l (Sv = 17.88%).

Analysis of the results of the study shows that the average daily increase in live weight of young pigs during the control period of fattening is 777.1 ± 11.11 g (Sv=7.29%), the age of 100 kg live weight - 172.1 ± 1.18 days (Cv = 3.52%), fat thickness at the level of 6-7 thoracic vertebrae - 20.9 ± 0.36 mm (Cv = 9.22%), length of the cooled carcass – 96.3 ± 0.43 cm 2.38%), the length of the bacon half of the chilled half-carcass - 83.3 ± 1.06 cm (Sv = 6.73%), the complex index of fattening and meat qualities (Tyler B. index) - 152.64 ± 2.115 points (Sv = 7.33%). The index of A. Sazer - H. Fredin ranges from -1.791 to +3.211 points.

The results of studies of biochemical parameters of blood serum of young pigs of the large white breed of different classes of distribution according to the index of A. Sazer - H. Fredin are shown in Table 2.

Table 2. Biochemical parameters of blood serum of young pigs of the large white breed of different classes of distribution according to the index of A. Sazer - H. Fredin, n = 5

The indicator, units of measurement	The biometric Indicators	Index of A. Sazer - H. Fredin	
		The index gradation	
		-1.791 - -0.329	+0.003 - +3.211
		Group	
		I	II
The content of total protein, g/l	$\overline{X} \pm S_{\overline{X}}$	83.0±1.61	80.0±6.11
	σ±Sσ	3.94±1.246	10.58±3.348
	$Cv \pm S_{Cv}$, %	4.74±1.500	13.22±4.183
The urea content, mmol/l	$\overline{X} \pm S_{\overline{X}}$	4.72±0.303	4.63±0.307
	σ±Sσ	0.80±0.253	0.81±0.256
	$Cv \pm S_{Cv}$, %	16.94±5.440	17.53±5.547
Creatinine concentration, μmol/l	$\overline{X} \pm S_{\overline{X}}$	80.6±5.88	96.0±4.95
	σ±Sσ	13.16±4.164	15.67±4.958
	$Cv \pm S_{Cv}$, %	16.32±5.164	16.32±5.164

It was found that the difference between the groups in total protein content is 3.0 g/l (td=0.47, P>0.05), the urea content - 0.09 mmol/l (td = 2.25, P<0.05), the creatinine concentration - 15.4 μmol/l (td = 2.20, P<0.05).

The results of the study of fattening and meat qualities of young pigs of different interbreed differentiation according to the index of A. Sazer - H. Fredin are given in table 3.

It was found that the young pigs of group II outperformed peers of I on average daily live weight gain for the period of control fattening by 28.2 g (td = 1.43, P>0.05), the age of reaching a live weight of 100 kg - by 5.1 days (td = 2.21, P<0.05), the thickness of the fat at the level of 6-7 thoracic vertebrae - 2.3 mm (td = 3.70, P<0.01), the length of the cooled carcass – 1.3 cm (td = 1.52, P>0.05), the length of the bacon half of the cooled half-carcass - 1.9 cm (td = 0.87, P>0.05).

Table 3. Fattening and meat qualities of young pigs of the large white breed of different classes of distribution according to the index of A. Sazer - H. Fredin

The indicator, units of measurement	The biometric Indicators	Index of A. Sazer - H. Fredin	
		The index gradation	
		-1.791 - -0.329	+0.003 - +3.211
		Group	
		I	II
The average daily gain of live weight during the period of control fattening, g	n	12	16
	$\overline{X} \pm S_{\overline{X}}$	759.8±14.96	788.0±12.82
	$\sigma \pm S_{\sigma}$	55,14±11.276	51,31±9,081
	$Cv \pm S_{Cv}$, %	7.25±1.482	6.51±1.152
The age of reaching live weight 100 kg, days	$\overline{X} \pm S_{\overline{X}}$	178.1±1.67	173.0±1.62
	$\sigma \pm S_{\sigma}$	5.20±1.063	6.50±1.150
	$Cv \pm S_{Cv}$, %	2.91±0.595	3.75±0.663
The thickness of the fat at the level of 6-7 thoracic vertebrae, mm	$\overline{X} \pm S_{\overline{X}}$	22.1±0.48	19.8±0.40
	$\sigma \pm S_{\sigma}$	1.69±0.345	1.61±0.284
	$Cv \pm S_{Cv}$, %	7.64±1,564	8.13±1.421
The length of the cooled carcass, cm	$\overline{X} \pm S_{\overline{X}}$	95.4±0.58	96.7±0.63
	$\sigma \pm S_{\sigma}$	2.02±0.413	2.54±0.449
	$Cv \pm S_{Cv}$, %	2.11±0.429	2.62±0.469
The length of the bacon half of the cooled carcass, cm	$\overline{X} \pm S_{\overline{X}}$	82.2±1.69	84.1±1.36
	$\sigma \pm S_{\sigma}$	5.86±1.198	5.45±0.964
	$Cv \pm S_{Cv}$, %	7.12±1.458	6.48±1.146
A comprehensive index of fattening and meat qualities (Tyler B. index) points	$\overline{X} \pm S_{\overline{X}}$	149.56±3.293	154.95±2.701
	$\sigma \pm S_{\sigma}$	11.41±2.333	10.80±1.911
	$Cv \pm S_{Cv}$, %	7.62±1.558	6.96±1.231

The difference between groups of animals in the complex index of fattening and meat qualities (Tyler B. index) is equal to 5.39 points (td = 1.26, P> 0.05).

The coefficient of variation of biochemical parameters of blood serum, fattening, and meat qualities of young pigs of the experimental groups range from 2.11 to 17.53%.

The calculation of the pairwise correlation coefficients between the biochemical parameters of blood serum, fattening, and meat qualities of young pigs of the large white breeds are shown in Table 4.

The pairwise correlation coefficient between the biochemical parameters of blood serum, fattening, and meat qualities of young pigs of the large white breeds range from -0.533 to +0.528.

A significant relationship was found between the following pairs of features: total protein content × length of bacon half of chilled half-carcass (0.533 ± 0.1727**), total protein content, g/l × index of A. Sazer - H. Fredin (-0.395 ± 0.1875), urea content × length of the chilled carcass (-0.445 ± 0.1828), creatinine concentration × length of the chilled carcass (0.528 ± 0.1734), creatinine concentration × length of bacon half of chilled half-carcass (0.519 ± 0.1745), creatinine concentration × index of A. Sazer - H. Fredin (0.497 ± 0.1771).

According to the results of calculating the economic efficiency of using young pigs of different distribution classes according to index A. Sazer - H. Fredin found that the maximum increase in additional products was obtained from group II animals, namely +1.38% (Table 5).

Table 4. The pairwise correlation coefficient between biochemical parameters of blood serum, fattening, and meat qualities of young pigs of large white breed

The signs		The biometric Indicators		The correlation strength
x	*y*	r±Sr	tr	
The content of total protein, g/l	1	-0.252±0.1975	1.28	Weak
	2	0.162±0.2014	0.80	Weak
	3	0.375±0,1892	1.98	Moderate
	4	-0.321±0.1933	1.66	Moderate
	5	-0.533±0.1727**	3.09	Moderate
	6	-0.309±0.1941	1.59	Moderate
	7	-0.395±0.1875*	2.11	Moderate
The urea content, mmol/l	1	0.101±0.2031	0.50	Weak
	2	0.331±0.1926	1.72	Moderate
	3	0.007±0.2041	0.03	Weak
	4	-0.445±0.1828*	2.43	Помірна
	5	0.019±0.2041	0.09	Weak
	6	-0.013±0.2041	0.06	Weak
	7	0.016±0.2041	0.08	Weak
The creatinine concentration, μmol/l	1	0.142±0.2021	0.70	Weak
	2	-0.015±0.2041	0.07	Weak
	3	-0.338±0.1921	1.76	Moderate
	4	0.528±0.1734**	3.05	Noticeable
	5	0.519±0.1745**	2.97	Noticeable
	6	0.140±0.2021	0.69	Weak
	7	0.497±0.1771**	2.81	Moderate

Note: 1 - Average daily gain of live weight during the period of control fattening; 2 - Age of reaching a live weight of 100 kg, days; 3 - The thickness of the fat at the level of 6-7 thoracic vertebrae, mm; 4 - Length of the chilled carcass, cm; 5 - Length of bacon half of chilled half-carcass, cm; 6 - Comprehensive index of fattening and meat qualities (Tyler B. index), points; 7 - Index of A. Sazer - H. Fredin, points, * - P<0.05; ** - P<0.01; *** - P<0.001

Table 5. The economic efficiency of research results

The group	Index gradations A. Sazer - H. Fredin	The average daily increase in live weight during the period of control fattening from 30 to 100 kg, g	The supplement of additional products,%	The cost of additional products, UAH/US dollars/animal.
General sample	-1.791 – +3.211	777.1±11.11	–	–
I	-1.791 – -0.329	759.8±19.96	-2.22	-579.65 / -20.96
II	+0.003 – +3.211	788.0±12.82	+1.38	+360.32 / +13.03

Note: * - the selling price of young pigs at the time of the research was UAH 44.8. or 1.62 US dollars for 1 kg of live weight.

The cost of additional products received from animals of these groups is +360.32 UAH/animal.

CONCLUSIONS

It was found that the biochemical parameters of the serum of young pigs of the experimental group correspond to the physiological norm of clinically healthy animals. The difference between the groups in terms of total protein content (g/l), urea content (mmol/l), and creatinine concentration (μmol/l) averages 7.18%.

Young pigs of the controlled herd at the age of reaching a live weight of 100 kg, fat thickness at the level of 6-7 thoracic vertebrae, and the chilled carcass's length, according to the current Instructions for grading pigs belong to class I and elite class.

Taking into account the interbreed differentiation of young pigs of the large white breed according to the index of A. Sazer - H. Fredin, it was found that the animals of group II outweighed peers I on average daily live weight gain during the control period of fattening by 3.62% (td = 1.43, P>0.05). The age of reaching a live weight of 100 kg - by

2.86% (td = 2.21, P<0.05), the thickness of the fat at the level of 6-7 thoracic vertebrae - by 10.10% (td = 3.70, P<0,01), the length of the cooled carcass - by 1,34% (td = 1.52, P>0.05), the length of the bacon half of the cooled half-carcass - by 2.25% (td = 0.87, P>0.05).

The coefficient of pair correlation between biochemical parameters of blood serum, fattening, and meat qualities of young pigs of large white breed vary from -0.533 to +0.528.

The use of young pigs of group II (index A. Sazer - H. Fredin ranges from +0.003 to +3.211 points) provides additional products at the level of +1.38%, and its cost is 360.32 UAH/animal.

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LONGEVITY AND THE MAIN REASONS FOR COW RETIREMENT

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Abstract

The influence of the genotype for the Holstein breed on the longevity and life-long productivity of black-and-white cows was studied, and the intensity and reasons for the retirement of animals from the herd were analyzed. For this purpose, in the conditions of the breeding farm of the CJSC "Konstantinovo" of the Penza region, two groups were formed from the number of retired animals: group I with a blood content of the Holstein breed of less than 75% and group II with a blood content of more than 75%. As a result of the studies, it was found that the lifetime productivity of cows in group I was 24908 kg, which is 3816 kg higher than that of cows in the second group (P>0.95). The indicator of the period of economic use of cows was also higher in animals with a blood content of less than 75% for the Holstein breed - 4.58 units versus 3.66 in cows with a blood content of more than 75% (P> 0.99). According to the analysis of the reasons for cow retirement, depending on bloodiness, it was revealed that with blood content in the Holstein breed of less than 75%, the highest dropout rate is low productivity and udder diseases; if the blood in the Holstein breed is more than 75%, the largest dropout rate is leg diseases and other reasons.

Key words*: duration of productive use, genotype, longevity, lifetime productivity, reasons for retirement.*

INTRODUCTION

The state program for the development of agriculture and regulation of markets for agricultural products, raw materials and food for 2013-2020 provides for an increase in milk production by 2020 to 38.2 million tons. Achievement of this goal can be ensured solely by increasing productivity and increasing the safety of the breeding stock of cattle, which is directly related to an increase in productive longevity (Shishkina et al., 2018). At the same time, in the last decade, a decrease in the terms of economic use of cows to 2-3 lactations has been noted by many authors (Abramova et al., 2018).

The milk production of cows is determined by a number of factors that can be divided into external and internal. External include feeding and keeping animals. The share of their influence, according to a number of authors, reaches 75%. The main internal factors should be considered genetic (breed and genotype of animals) (Shishkina, 2017; Shishkina et al., 2017). The degree of influence of these factors, cited by a number of scientists, is 25-30%.

Realization of the productive potential of livestock with an improved genotype is possible in appropriate conditions of feeding, housing and service (Kryukov, 2014).

Therefore, the dairy farming industry is being modernized: modern dairy farms are being built, existing farms and complexes are being reconstructed, progressive milk production technologies are being actively disseminated.

MATERIALS AND METHODS

To transform the genotype of black-and-white cows of local populations, reproductive and absorptive crossbreeding is widely used, while in most regions of Russia and in the Penza region in particular, the Holstein breed of dairy cattle is used as an improving one.

In connection with the above, we have set the goal to analyze the influence of the genotype for the Holstein breed on the longevity and lifetime productivity of black-and-white cows. The studies were carried out in the breeding farm of JSC Konstantinovo, Penza region.

At JSC "Konstantinovo" crossbreeding of related breeds is carried out, all the resulting hybrids are considered purebred black-and-white animals with different bloods according to the Holstein breed. From the number of retired animals, two groups were formed: group

I with a blood content of the Holstein breed of less than 75% and group II with a blood content of more than 75%. The selection of data for analysis was carried out from the cards of the breeding cow of the 2-MOL form.

JSC "Konstantinovo" is a pedigree reproducer for breeding black-and-white cattle. The total dairy herd of the farm is 1175 heads. Average milk yield per herd is 8881 kg of milk (Table 1).

At JSC Konstantinovo, a stall-walking system for keeping livestock has been adopted. The way of keeping is loose, boxed. The cows are kept in four barns with 250 stalls each. Two-row cowsheds with one aft aisle. Each row is divided into two sections. The section contains two rows of boxes. Walking areas are equipped to provide cows with exercise.

Table 1. Characteristics of cows in terms of milk productivity of JSC Konstantinovo

Groups animals	Total, heads	Milk yield, kg	Milk fat		Milk protein		Live weight, kg
			%	kg	%	kg	
All livestock	1175	8881	3.69	327.6	3.18	282.7	499
1 lactation	499	8446	3.68	310.7	3.18	268.9	480
2 lactation	313	9180	3.69	338.9	3.18	292.1	500
3 lactation	363	9221	3.70	341.2	3.18	293.6	524

The optimal microclimate parameters are set thanks to the movable side walls - "curtain system", which can be adjusted depending on the air temperature and wind.

The system consists of light-transmitting, UV-stable and low-temperature resistant tents that open from top to bottom. During the warmer months, the curtains can be fully opened, giving free access to fresh air.

In winter, the awnings are raised and protect the premises from cold winds and low temperatures.

To provide ventilation, as well as to enhance natural light, a light ventilation ridge (aerator) is installed in the barns. Drinking is carried out from automatic self-drinkers with heated water in the winter. The manure passages in the barns are shaped like a tray.

Manure removal is carried out three times a day during milking with the help of a bulldozer attached to tractors. After the manure is removed, the bedding material is covered with a spreader - straw cutting.

Milking of cows is carried out in two milking parlors on installations of the "Carousel" type for 36 milking places each. All milking equipment in the Westfalia Surge farm. Milking of cows - two times. The farm pays great attention to feeding and fodder quality.

The basic ration is corrected taking into account the level of productivity of cows in production groups. The diet includes the following types of feed: rump hay, oat straw, corn grains, beet pulp, corn, barley, sunflower cake, soybean meal, fodder treacle, protected fat, feed chalk, salt.

The breed and class composition of cattle for 2020 is presented in table 2. The total number of livestock on the farm is 2614 heads. Of these, 1683 are cows, 548 heifers, 101 heifers are up to one year old, 264 heads are heifers over one year old and 18 heads are heifers over 18 months old. The number of heads of cattle of the elite-record class is 1851. Of these, 1175 are cows. Class elite class includes 643 heads. Cows in this group were 495 heads, heifers - 83 heads. The total number of the 1st class reaches 120 heads. This group includes 83 heifers and 13 cows.

RESULTS AND DISCUSSIONS

As a result of the studies carried out, it was revealed that the lifetime productivity of cows in group I was 24908 kg, which is 3816 kg higher than that of cows in group II ($P>0.95$), in cows in group II this indicator was 21092 kg (Table 2).

Table 2. Lifetime productivity and the number of the last completed lactation of cows, M ± m

Holstein blood breed	Lifetime productivity, kg	Last number complete lactation
<75% (n = 43)	24908 ± 1173.9	4.58 ± 0.25
≥75% (n = 67)	21092 ± 882.6	3.66 ± 0.15
Δ - difference between comparative features (to determine if the means of two sets of data are significantly different from each other)	3816	0.92
td - Student's t-test	2.60	3.14

The indicator of the period of economic use of cows was also higher in animals with a blood content of less than 75% for the Holstein breed - 4.58 units versus 3.66 in cows with a blood content of more than 75% (P>0.99) (Figure 1).

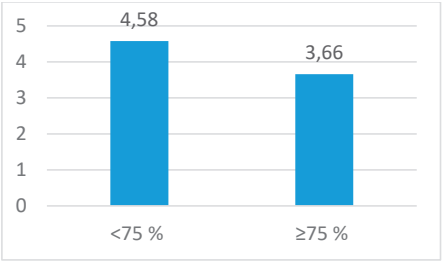

Figure 1. Number of the last completed lactation

Based on the research data, it can be concluded that in the analyzed herd, a tendency was revealed for a decrease in the economic use of animals with an increase in blood in the Holstein breed. Therefore, combinations of high milk yield and optimal periods of productive use of the broods are ideal.

Life span is the period from the birth of an animal to its natural death. Lifetime milk yield is the total milk yield for all lactations during the life of the animal. The genetic potential of cows' productive longevity is quite high and amounts to 12-15 years or 10-12 lactations or more.

The optimal duration of productive use in the Russian Federation can be considered a dairy cow, which, during five to six lactations, on average gives more than 6 thousand kg of milk, while maintaining normal fertility, that is, gives one calf per year, has good health and a strong constitution. In dairy breeds of intensive type, an average milk yield of 7-8 thousand kg for 3-4 lactations is acceptable. Lifetime milk yield from such cows will be at least 30 tons.

Longevity is a complex indicator that sums up the use of an animal in the production of offspring and the production of certain products. The heritability of this trait is very low. The heritability coefficient of longevity in animals is 3-13%.

The task was set - to study the duration of productive use of the breeding stock of JSC Konstantinovo.

As can be seen from Table 3, all animals in both control groups completed the first lactation. Already by the second lactation, the percentage of cows retired in the group of cows with a blood content of more than 75% was 18%, while in cows with a degree of "Holsteinization" less than 75%, only 5%. By the fourth lactation, the number of abandoned cows in group I was 53%, in group II - 81%, by 5 lactation - 72 and 91%, respectively. By the 6th lactation in the second group, 100% of the cows were eliminated, while in the first one animal reproduced milk production up to 9 lactation.

Summarizing the data of the study of the withdrawal of cows by JSC Konstantinovo of various degrees of Holsteinization, it was concluded that the best indicators for this parameter have cows with a blood blood level of less than 75%.

The increase in the terms of productive use of cows allows you to increase the lifetime productivity of cows, and, consequently, the profitability of production. Therefore, reducing the level of culling of cows is one of the leading values of the economic success of the enterprise. Knowledge of the main reasons for cow retirement and its decrease due to technological, organizational and various veterinary measures allows you to minimize this parameter.

In this regard, an analysis was made of the reasons for the withdrawal of the analyzed breeding stock of JSC Konstantinovo (Table 4).

Table 3. Intensity of retirement and safety of cows in controlled groups

Lactation by count	Indicator	By age periods		Disposal from the initial livestock	
		I Group	II Group	I Group	II Group
1	Number of cows at the beginning of lactation	43	67	-	-
	Successfully finished lactation, goal	43	67	-	-
	Lost cows, goal.	0	0	0	0
	Lost cows,%	0	0	0	0
	Livestock safety,% (percentage of disposal from the initial livestock)	100	100	-	-
2	Number of cows at the beginning of lactation	43	67	-	-
	Successfully finished lactation, goal	41	55	-	-
	Lost cows, goal.	2	12	2	12
	Lost cows,%	5	18	5	18
	Safety of livestock,%	95	82	-	-
3	Number of cows at the beginning of lactation	41	55	-	-
	Successfully finished lactation, goal	28	35	-	-
	Lost cows, goal	13	20	15	32
	Lost cows, %	32	36	35	48
	Livestock safety	68	64	-	-
4	Number of cows at the beginning of lactation	28	35	-	-
	Successfully finished lactation, goal	20	13	-	-
	Lost cows, goal.	8	22	23	54
	Lost cows, %	29	63	53	81
	Livestock safety, %	71	37	-	-
5	Number of cows at the beginning of lactation	20	13	-	-
	Successfully finished lactation, goal	12	6	-	-
	Lost cows, goal.	8	7	31	61
	Lost cows, %	40	54	72	91
	Livestock safety, %	60	46	-	-
6	Number of cows at the beginning of lactation	12	6	-	-
	Successfully finished lactation, goal	5	0	-	-
	Lost cows, goal.	7	6	38	67
	Lost cows, %	58	100	88	100
	Livestock safety, %	42	0	-	-
7	Number of cows at the beginning of lactation	5	-	-	-
	Successfully finished lactation, goal	3	-	-	-
	Lost cows, goal	2	-	40	-
	Lost cows, %	40	-	93	-
	Livestock safety, %	60	-	-	-
8	Number of cows at the beginning of lactation	3	-	-	-
	Successfully finished lactation, goal	1	-	-	-
	Lost cows, goal.	2	-	42	-
	Lost cows, %	67	-	98	-
	Livestock safety, %	33	-	-	-
9	Number of cows at the beginning of lactation	1	-	-	-
	Successfully finished lactation, goal	0	-	-	-
	Lost cows, goal	1	-	43	-
	Lost cows, %	0	-	100	-
	Livestock safety, %	100	-	-	-

As can be seen from Table 3, all animals in both control groups completed the first lactation. Already by the second lactation, the percentage of cows retired in the group of cows with a blood content of more than 75% was 18%, while in cows with a degree of "Holsteinization" less than 75%, only 5%. By the fourth lactation, the number of abandoned cows in group I was 53%, in group II - 81%, by 5 lactation - 72 and 91%, respectively. By the 6th lactation in the second group, 100% of the cows were eliminated, while in the first one

animal reproduced milk production up to 9 lactation.

Summarizing the data of the study of the withdrawal of cows by JSC Konstantinovo of various degrees of Holsteinization, it was concluded that the best indicators for this parameter have cows with a blood blood level of less than 75%.

The increase in the terms of productive use of cows allows you to increase the lifetime productivity of cows, and, consequently, the profitability of production. Therefore, reducing the level of culling of cows is one of the leading values of the economic success of the enterprise. Knowledge of the main reasons for cow retirement and its decrease due to technological, organizational and various veterinary measures allows you to minimize this parameter.

In this regard, an analysis was made of the reasons for the withdrawal of the analyzed breeding stock of JSC Konstantinovo (Table 4).

Table 4. The main reasons for the retirement of cows, depending on the degree of "Holsteinization"

Holstein bloodline	Low productivity		Gynecological diseases		Udder diseases		Diseases of the feet		Other reasons	
	Goal.	%	Goal.	%	Goal.	%	Goal.	%	Goal.	%
<75% (n = 43)	9	20.9	10	23.3	9	20.9	10	23.3	5	11.6
≥75% (n = 67)	12	17.9	16	23.9	11	16.4	17	25.3	11	16.5
Total for herd	21	19.1	26	23.6	20	18.2	27	24.5	16	14.5

According to the analysis of the reasons for cow retirement, depending on bloodiness, it was found that with a blood content of less than 75% for the Holstein breed, the highest dropout rate is low productivity and udder diseases; if the blood in the Holstein breed is more than 75%, the largest dropout rate is leg diseases and other reasons.

Thus, the main reasons for the loss of cows in the analyzed herd were leg diseases (24.5%), gynecological diseases (23.6%), low productivity (19.1%), udder diseases (18.2%) and other reasons (14.5%).

CONCLUSIONS

Based on the analysis of the influence of the genotype for the Holstein breed on the longevity and life-long productivity of black-and-white cows in the conditions of the breeding farm of JSC Konstantinovo of the Penza region, we made the following conclusions:

Among the studied cows of various degrees of blood in the Holstein breed, the best indicators of milk productivity in the first lactation were animals with a blood content of less than 75%, by the third lactation the best indicators were in cows with a blood content of more than 75%. It should be noted that in terms of milk yield and milk fat, cows with a blood content of more than 75% were more stable in the first and third lactation. By the third lactation, the values according to these indicators in cows with a blood content of less than 75% decreased;

By the second lactation, the percentage of retired cows in the group of cows with a blood content of more than 75% was 18%, while in cows with a degree of "Holsteinization" less than 75%, only 5%. By the fourth lactation, the number of abandoned cows in group I was 53%, in group II - 81%, by 5 lactation - 72 and 91%, respectively. By the 6th lactation in the second group, 100% of the cows dropped out, while in the first one animal reproduced dairy products up to 9 lactation. The main reasons for leaving cows in the analyzed herd were leg diseases (24.5%), gynecological diseases (23.6%), low productivity (19.1%), udder diseases (18.2%) and other reasons (14.5 %);

Lifetime productivity of cows with a blood content of less than 75% was 24908 kg, which is 3816 kg higher than that of cows of the second group (P>0.95) The indicator of the period of economic use of cows was also higher in animals with a blood content of less than 75% for the Holstein breed – 4.58 units versus 3.66 in cows with a blood count of more than 75% (P>0.99). Under the prevailing conditions, high-blooded Holstein animals poorly realize their genetic potential. We recommend to prolong productive longevity, as well as other indicators, to carry out careful selection and selection, directed rearing of young animals and the creation of a good forage base.

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SELECTION AND BREEDING VALUE ON TROPICAL HORSES: CASE STUDY ON POTENTIAL BREEDING DEVELOPMENT ON INDONESIAN RACE HORSES

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Abstract

The study aimed at estimating variance components of racing ability traits in Minahasa racehorses as a contribution to defining the breeding value for this population. Data were provided by Indonesian Horse Racing Assosiate/PORDASI (1) contained more than 150 placings at finish by horses running in 907 races from 2010 to 2017. Age of horses ranged from 2 to 7+ years, and the distances were from 600 to 1800 m. Speed of horses was derived from the distance of racetrack in the racing time of the winner due to only the horse wins finish time was recorded. Horses were from stables, from private breeders and from foreign breeding. Speed and Variance components were estimated by the descriptive and animal genetics measurement method. Statistical analysis accounted for fixed effects of year, age, race, sex and weight carried, and for the random effects of rider, permanent environment, and animal additive genetics. Speed rate was 14.62 m/s to 15.45 m/s or around 66 km/h (approx.) and heritability coefficients were 0.22 to 0.82 and repeatability 0.16 to 0.69, respectively. The highest breeding value (PBV) = 0.09 m/s of male H and MPPA (Most Probable Producing Ability) in the population = 0.19 m/s (individual B010) where the average MPPA value of the entire population is = 0.0239 m/s.

Key words*: EBV (breeding value), Minahasa, MPPA (Most Probable Producing Ability), race horses, speed traits.*

INTRODUCTION

As an animal of high economic value racehorses were maintained for the purpose of competing in a race, so that the development of the horse racing industry was inseparable from the improvement of the genetic quality of racehorses in every breeding program in each country (Edwards, 1994), no exception in Indonesia (Ambo et al., 2014). For maintenance in Indonesia racehorses are a pride for the owner (Oroh, 2004), because in addition to the advantages in terms of personal satisfaction can also provide a real advantage in the form of obtaining prizes on the achievements achieved by the racehorse (Sabeva, 2000a). Unfortunately, the development of the racehorse industry in Indonesia is not accompanied by efforts to improve the genetic quality of horses (Astuti et al., 2011). Cross pure breeds superior stud horses imported from out of the country, generally not accompanied by a proper and directed selection program, make racehorses in Indonesia less competitive in the international arena (Forum Sandalwood Indonesia, 2009). Australian horse breeds are mated to local pony females, producing local racehorses. The term local racehorse, known as G1 for the first, second (G2) races, and so on has a great posture (Soehardjono, 1990). Cross-produced offspring became the main prerequisite to take part in the classic horse racing held in Indonesia (Pordasi, 2003). Therefore, knowing the genetic potential of Minahasa (Indonesia) horses is a very strategic source of information to develop racehorses in Indonesia (Takaendengan et al., 2011). Racehorse speed performance according to Tolley et al. (1983) is an expression of a horse's running speed at a certain distance. Therefore, horse racing time records can be used as one of the best parameters for measuring the appearance of genetic quantitative properties of a superior mares/mares, so analysing the trait of running speed can be used as the main variable in selecting quality racehorse studs (Langlois, 2007). The selection method is a breeding step to improve the quantitative nature of the running speed of Indonesian racehorses, so that the characteristics of the running speed of Minahasa racehorses can be improved (Makalalag, 2014). The classic problem in

trying to improve the genetic quality of racehorses is currently due to the lack of breeding efforts based on the results of correct scientific studies from horse keepers in Minahasa (Takaendengan et al., 2011). Based on this problem, the authors wanted to conduct research to obtain information on the potential advantages of phenotypic and genetic racehorses in Minahasa based on horse running speed traits sourced from a collection of horse racing results organized by the Equestrian Sports Association North Sulawesi (PORDASI, 2003). This study was conducted to see and get an idea of how much genetic potential of Minahasa racehorse speed traits as basic information to make changes to the existing racehorse population through the selection stage. Breeding value is expected to be the initial benchmark of conducting a selection program based on males or mares selected as the parent stock of seedlings of superior racehorses in Minahasa.

MATERIALS AND METHODS

In this study, the time record of horse racing from 8000s of Indonesian racehorses that finish provided by the Indonesian equestrian sports association for the last 20 years. The information available for each horse includes gender, age, brood, body size, fur colour and breed. Horse pedigree information covers at least 3 generations (Takaendengan et al., 2011) and data from interviews with various parties involved in the championship. All of the horses recorded are from Minahasa (North Sulawesi). The available finish time data is processed to get the running speed figures, first analysed to get a descriptive picture of the spread of racehorse speed in Minahasa. Using the Paternal Half-sib Correlation method according to Becker (1994) obtained heritability (h^2) then continued by looking for breeding value (*EBV*) from Kennisnet (2017) and interclass correlation repeatability (r) to get the most probable producing ability (*MPPA*) value according to Hardjosubrorto (1994).

$$h^2 = \frac{4\sigma_s^2}{\sigma_s^2+\sigma_w^2};$$

$$EBV_{mass\ selection} = h^2 \times [P - P_{mean}];$$

$$r = \frac{\sigma_w^2}{\sigma_w^2+\sigma_e^2};$$

$$MPPA = \bar{P} + \left[\frac{nr}{1+(n-1)r}\right][\bar{I} - \bar{P}]$$

Calculation and tabulation are performed manually by using Microsoft Office Excel and continued analysis of variance using SAS statistical applications. For the estimation of repeatability value, the data of foals (individuals) who have a record finish time of 5-6 times were used. The uniformity of the sample count is intended to avoid significant differences in the value of k (coefficient with number of children per male) resulting in irrelevant comparisons between individuals (half-siblings) (Becker, 1968).

RESULTS AND DISCUSSIONS

Mean, Standard Deviation and Coefficient Variation of Racehorse Running Speed in Minahasa based on Horse Studs

Based on the results listed in Table 1, shows that the average speed ranges from 14.62 ± 0.79 m/s to 15.37 ± 0.63 m/s or comparable to a speed of 52 km/h up to 55 km/h. Whereas, the top speed of the racehorse measured is 23.21 m/s and the lowest speed is 9.35 m/s with the average speed of the race being 15.04 ± 1.4 m/s.

Table 1. Description of Minahasa horse running speed (m/s) based on Horse Studs

Studs	Progeny (n)	Mean (m/s) ± SD	Max. (m/s)	Min. (m/s)	CV (%)
A	233	15.09 ± 0.75	19.89	9.35	4.98
B	165	14.62 ± 0.79	18.22	13.23	5.39
C	137	15.14 ± 0.63	16.31	10.43	4.15
D	132	15.37 ± 0.62	16.99	13.97	4.01
E	75	15.12 ± 1.25	16.64	9.75	8.27
F	56	15.02 ± 0.50	16.33	14.22	3.30
G	49	15.13 ± 0.35	15.88	14.41	2.34
H	52	15.45 ± 1.34	23.21	11.31	8.67
I	49	14.90 ± 0.55	16.33	12.90	3.71
J	46	15.30 ± 0.56	16.92	14.00	3.68
K	32	15.07 ± 1.13	16.77	11.33	7.53
L	35	15.15 ± 0.86	16.32	11.36	5.68
M	35	15.25 ± 0.53	16.66	14.12	3.49
N	28	15.22 ± 1.55	16.14	9.60	10.18

Males A to N are the order of males used in heritability analysis where the horse's real name is not written for privacy reasons.
m/s = meter per second; SD = standard deviation; max. = maximum; min. = minimum; CV = coef. of variation.

The average running speed of horses is higher when compared to the average speed of pure and thoroughbred Arabian racehorses in the Netherlands and Bulgaria reported by Schurink (2009); Sabeva (2000) was 13.80 m/s and 12.90 m/s. However, this result was lower than the average speed of a Turkish Thoroughbred racehorse of 15.30 m/s (Ekiz &Koçak, 2007) and the Brazilian quarter horse speed of 17.10 m/s (Corrêa &Mota, 2007). Many factors affect the running speed of racehorses such as the influence of horse breeds and nations, the climate of a country, the type of track/race and can be caused by variations in methods and types of data collection. The number of competitions conducted by each country, race recording equipment, and selection of racehorses are cited as factors that can also affect the running speed of horses (Ruhlmann C. et al., 2009). The "x" factor of jockeys and race business sometimes makes the estimation of the ability of racehorses in running to be biased (Bakhtiari & Kashan, 2009). The type of racehorse in Indonesia that was maintained in Minahasa, is generally the result of a cross between thoroughbred horses and local Indonesian horses over the past few decades (Komisi Peternakan & Kesehatan Veteriner, 2000). The lack of a targeted selection system of the running speed of racehorses developed in Indonesia therefore study data shows there was still a high genetic diversity in the trait of running speed that was between 2.34 to 10.18 percent (Martojo, 1992). The genetic diversity of Minahasa racehorse population shows the potential for future selection which was an opportunity to conduct breeding programs to obtain males or offspring that have superior genetic qualities based on running speed traits.

Estimating The Breeding Value Of A Stud Racehorse Running Speed Traits

Obtained the heritability value of the speed of running speed of a racehorse (Table 2), based on the method of correlation of half-siblings from low to high, which obtained male B has the lowest heritability value of 0.07 and male N has the highest heritability value of 0.48. According to Sulastri and Hamdani (2013), there are four males included in the low classification (0.0-0.1) and seven males in the medium classification (0.1-0.3); While the three males (K, M, and N) are included in the high classification (>0.3). Male H is the horse that has the highest EBV value (0.09) although the heritability value of this horse is relatively moderate (0.21). Meanwhile, compared to K males who only ranked 9th (0.01115), but had a relatively high heritability value (0.34). This is natural because according to Bailey, E (2014); Takaendengan et al. (2011); heritability value is not an absolute value but is the result of estimating the genetic potential of a trait in a population that is estimated or suspected based on measurable external traits (quantitative properties).

Table 2. Heritability of racing speed traits and estimated breeding value of Minahasa horse

Studs	h^2	S.E (h^2)	EBV	Rank
H	0.21	0.56	0.09	1
N	0.48	0.82	0.08	2
M	0.35	0.71	0.08	3
J	0.22	0.64	0.06	4
L	0.29	0.78	0.03	5
G	0.25	0.57	0.02	6
D	0.07	0.31	0.02	7
E	0.14	0.45	0.01	8
K	0.34	0.79	0.01	9
C	0.08	0.32	0.01	10
A	0.05	0.22	0.00	11
F	0.19	0.53	-0.00	12
I	0.20	0.62	-0.03	13
B	0.07	0.26	-0.03	14

Males A to N are the order of males used in this analysis where the horse's real name is not written for privacy reasons.
m/s = meter per second.

Looking at the results of the analysis of breeding values based on the performance data of individual males, it was obtained the result that horse H with EBV of 0.09 has the best genetic potential as a stud. The increase of 0.09 m/s in the following breeds certainly gives an advantage in terms of genetic quality that has an impact on the economic value of breeders (Dakhlan & Sulastri, 2002). When compared to the average population of only 0.03 m/s. The superior quality of males according to Hardjosubrorto (1994) puts the selected breed of horses in a more respectable position in the population.

Repeatability and Individual Most Probable Producing Ability

The repeatability value of the racing speed trait of the racehorse in Minahasa was explained in Table 3. Based on the results of this study,

some offspring of male J have higher genetic ability (R = 0.69) than some existing horse breeds, while male N has the lowest R value (0.15). The group of males with high R values are males C, I and J; medium male H and the rest of males A, B, F and N are included in the low classification.

Table 3. Repeatability of Racing Speed Traits and Most Probable Producing Ability of Minahasa Horse

Progeny	Avg Progeny (m/s)	Studs	R	S.E (R.)	MPPA (m/s)	Rank
B010	15.80	B	0.16	0.13	0.19	1
C007	15.52	C	0.42	0.20	0.16	2
J001	15.48	J	0.69	0.37	0.13	3
B013	15.42	B	0.16	0.13	0.13	4
A009	15.73	A	0.19	0.13	0.12	5
B006	15.35	B	0.16	0.13	0.12	6
B007	15.33	B	0.16	0.13	0.11	7
B011	15.30	B	0.16	0.13	0.11	8
C003	15.39	C	0.42	0.20	0.11	9
C004	15.38	C	0.42	0.20	0.11	10
C001	15.37	C	0.42	0.20	0.10	11
B008	15.24	B	0.16	0.13	0.10	12
B002	15.18	B	0.16	0.13	0.09	13
A007	15.51	A	0.19	0.11	0.08	14
H003	15.67	H	0.36	0.29	0.08	15
N001	15.74	N	0.15	0.30	0.08	16
F001	15.41	F	0.19	0.26	0.07	17
B003	15.08	B	0.16	0.13	0.07	18
A003	15.46	A	0.19	0.11	0.07	19
N002	15.66	N	0.15	0.30	0.07	20
I002	15.05	I	0.44	0.35	0.07	21
H002	15.63	H	0.36	0.29	0.07	22
B005	15.02	B	0.16	0.13	0.06	23
B009	15.01	B	0.16	0.13	0.06	24

[1]Males A to N are the order of males used in this analysis where the horse's real name is not written for privacy reasons.
[2]m/s = meter per second.

The MPPA (Most Probable Producing Ability) analysis in table 4 shows a horse's ability to maintain its genetic appearance continuously (Ardika et al., 2015). From the results of the study, it was seen that individual B010 has the highest MPPA value compared to other racehorse populations of 0.19 m/s. This indicates that B010 horses that are descendants of male B are thought to be able to inherit the genetic superiority of running speed properties by 0.19 meters per second faster in the next generation while describing the superiority of the brood of male B. Therefore, based on the results of the individual MPPA value, it is expected that the next generation racehorses will have a speed increase in accordance with the potential value of excellence measured today (Warwick et al., 1987). Although this is not absolute because it may be that the value is also influenced by the female/brood genes of the B010 mare. The accuracy of the horse's potential excellence based on the results of this analysis is highly dependent on the environmental factors of its successor (Ensminger, 1962; Blakely & Bade, 1991).

CONCLUSIONS

Speed rate was 14,62 m/s to 15.45 m/s or around 66km/h (approx.) and heritability coefficients were 0.22 to 0.82 and repeatability 0.16 to 0.69, respectively. The highest breeding value (PBV) = 0.09 m/s of male H and MPPA (Most Probable Producing Ability) in the population = 0.19 m/s (individual B010) where the average MPPA value of the entire population is = 0.0239 m/s.

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STUDIES ON THE INFLUENCE OF DIET FOODS ATHLETE IN THE COMPETITION STAGE

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Abstract

During the performance of any activity and especially on the effort in specific sports activities, the body expends energy in calories. Using an adequate diet we follow body energy reserves forming needed during effort and contests. Performance Athlete diet will be more strict to cope with the physical effort. Therefore we recommend that athletes must consume high caloric density foods like cereals, juices, honey, fruit yoghurt, hydrating beverages, vegetables, fruits, chicken, turkey and beef, fish, eggs, soy products etc. All these are required not to be absent from athlete diet. The research method was based on bibliographic study and experimental methods in order to determine the best food groups required in an athlete's diet.

Key words*: competition stage, diet, food, sports.*

INTRODUCTION

Because sports activity unfolds on stages which last longer (training) it is required to establish that diets based on this (Alexandrescu, 1994). A new problem arises, the necessity of finding a correct athletes diet, representing a succession of food rations, considering the consumption of energy necessary for the sportive activity; characteristics of the effort by sport branch and the preparation period; weather conditions; nutritive value of food; individual features (weight, age, gender, etc) (Craciun, 1996).

Since sports effort is achieved mainly with the help of muscles, muscle metabolism must be perfectly adapted to the body's effort, which can be achieved through methodical training, with numerous repetitions of movements in order to form stereotypes.

Food is made up of a number of nutritional factors. The well-defined substances from a chemical point of view and indispensable to humans are: proteins, lipids, carbohydrates, mineral salts, vitamins and water. From the point of view of the role they play in the body, they are divided into two groups: energetic or caloric and protective or maintenance. The first group includes carbohydrates and lipids. The second group can be divided into two subgroups: one with the role of recovery which includes proteins and some mineral salts (calcium, phosphorus, sodium, chlorine, potassium, etc.) and the other subgroup has a catalytic action to regulate some chemical reactions and this group includes vitamins, some mineral salts (cobalt, iodine, etc.) and water.

Meat in general and liver in particular have a strong erythropoietic action as these foods contain essential amino acids. Because it contains lysine, meat stimulates the growth process in general, especially muscle growth. Given their nutritional value and mainly their class I protein content, which is much needed during effort in sports, it is recommended that athletes consume a certain amount of meat or fish per day, especially during speed and strength exercises.

Also, vegetables and fruits are part of the group that includes all foods of vegetal origin containing lot of water. Vegetables and fruits are full of of vitamin C. The role of vitamin C

in the body is very important. It is involved in cellular respiration stimulating redox processes. Also, it enhances the antitoxic action of the liver and increases the overall resistance of the organism. For this reason, sport activity vitamin C should not only be used sporadically or before the start but systematically throughout the training and competitions in order to make use of all the benefits of this vitamin. Furthermore, vegetables and fruits are the most important source of carotene (provitamin A). Highest in carotene content belongs to: leafy greens, carrots, beets, tomatoes, radishes, cherries, cherries, peaches. Vegetables and fruits, in addition to their high vitamin content, also contain minerals. As food predominating alkaline miliequivalents providers they are indispensable for ensuring the acid-base balance of the ration for athletes. Fruit and vegetables are also a source of carbohydrates which, along with vitamins, increase glycogen reserves in liver and improve its functional status.

A balanced diet containing all of the previously mentioned types of food needs to be taken into account during the competitional stage in order to ensure that the athlete is in full shape.

MATERIALS AND METHODS

The most important elements of research methodology brings: bibliographic study method and experimental method.

The diet of the athlete must meet the following requirements:

1) The athlete performs sporting effort not so much on food ingested immediately before exercise as reserves in the body

2) To achieve the necessary neuromuscular effort for effective sports activities, it is necessary a rich blood irrigation to organs intensively used in effort in damage of blood irrigation of unsolicited ones, as is the particularly case of the digestive tract which must be left idle during exercise.

3) As body adaption to workout is done in time, adaptation to a modified diet should be done gradually.

4) Assimilation coefficient varies depending on the nature of food and every individual (Barbuica, 2015).

In general, in terms of energy requirements we can establish three major periods in the composition of athlete diet depending on the stage of training (Barbuica, 2015).

- Preparatory stage
- Competitive stage
- Recovery stage and rest (recovery rest)

RESULTS AND DISCUSSIONS

The paper, experimental research results consist of the presentations from different studies on diet depending on the stage of training.

Preparatory Stage

In preparation for the general development of general resistance, diet must contain an increased amount of vitamins, especially B group (B_1, B_2, B_6) and vitamin C that acts in metabolic processes (Craciun, 1996). For strength-duration efforts should be increased, and the amount of carbohydrates that is realized based on sugar, fruits and vegetables that contain vitamins along and minerals and less on the account of pasta and bread. Gelatine and vitamin zing jelly fruits consumption helps to improve the resistance in speed endurance mode. At the end of preparatory stage when prevail exercises for speed developing, we recommend increasing the amount of protide, especially the phosphorus compounds found in milk, cheese, meat, etc.

A special importance is the correct distribution of food and daily meals:

- breakfast food should provide 25-30% of caloric intake;
- lunch food should provide 40-45% of caloric intake;
- dinner food should provide 30-35% of caloric intake.

Content of evening meal will avoid exciting foods (chocolate, cocoa and coffee) and meat in large quantities (Banu, 2005).

Competitive Stage

Overall food remains as in the preparatory stage, but we introduce foods with a higher heating value and easier assimilate. During competitions diet should not differ too much with the previous one. Food rations higher in terms of quantity and easily assimilate will increase by focusing especially on

carbohydrates, dairy products, minerals and vitamins (B_1 and C) which helps substantial athlete effort, even if eaten the day of the contest (Alexandrescu, 1994).

In sports games or tests that require speed and endurance in equal measure, will ensure a balanced diet made up of carbohydrates (bread, pasta, rice, etc.), phosphorus (milk, cheese, eggs), vitamins (B_1, C), vegetables and fruits (juices and syrups).

The food required for this competitional stage needs to be rich in vitamins B_1 and C, carbohydrates and minerals (phosphorus, sodium, potassium).

The products with a high content of vitamins B_1 and C are found in Table 1 as well as the ones containing a significant amount of minerals (potassium, sodium, phosphorus, calcium, magnesium) in Table 2 (Rosoiu, 2003).

Table 1. Amount of vitamins B_1 and C present in 100 g of consumed product

Types of food	VITAMIN B_1 (mg)	VITAMIN C (mg)
Cow milk	45	2,0
Cheese	50	1,0
Eggs	120	-
Oatmeal	250	-
Whole wheat bread	250	-
Pasta	120	
Peppers	110	150
Cucumbers	70	8
Spinach	60	10
Tomatoes	35	10
Leek	140	200
Green beans	150	20
Blueberries	-	15
Rosehip	30	1000
Oranges	60	50
Grapes	50	3
Nuts	550	5

Metabolism is the transformation that takes place in living cells based on nutrients when the energy needed for these processes and the development of biological phenomena take place. Metabolism comprises two phases: anabolism and catabolism. Anabolism is the phenomenon of assimilation of nutrients from food and their incorporation in the forms of the human body. Catabolism is the phase of dissimilation and degradation of assimilated substances. The qualitative and quantitative balance of metabolism represents nutrition.

Table 2. The amount of minerals (mg) present in 100 g of consumed product

Type of food	Minerals (mg)				
	K	Na	Ca	Mg	P
Cow milk	160	50	125	12	90
Milk powder	1280	400	1000	76	760
Egg yolk	120	50	140	15	500
Egg white	150	180	5	10	35
Oatmeal	360	35	60	120	350
Pasta	140	200	22	35	110
Whole wheat bread	190	400	28	45	200
Parsley	900	35	300	50	120
Carrots	220	100	50	15	40
Green salad	320	60	55	40	50
Spinach	700	17	13	11	35
Tomatoes	310	25	15	20	30
White cabbage	400	30	72	70	60
Bananas	380	3	8	40	30
Strawberries	160	2	25	13	30
Chestnut	510	8	35	350	150
Cherries	220	3	18	10	20
Melon	320	14	20	20	30
Apples	260	2	36	16	50
Dried plums	800	12	55	30	90

It consists of all the phenomena that occur in the body after digestion and absorption of food in the intestine. Part of the energy provided by food is used in the form of mechanical, chemical, thermal, electrical energy, the other part is kept in reserve, and the rest is eliminated. If there is a balance between the two phases of metabolism, the body's nutritional status is good and no disorders occur.

Diet during the break has on one hand the role to replenish reserves spent, and on the other hand to eliminate organism fatigue. For this it is recommended consumption of liquid, sugar and minerals, especially potassium in order to recover losses during efforts. If muscle cramps occur is recommended to drink salt water (salt 1-2 g/l).

During competition days should be avoided indigestible foods like: venison, fatty meats,

bacon, beans and dry peas, cabbage, rye or wheat bread etc. Alcoholic drinks, acid and carbonated juices and syrups made with chemicals and incentives must be avoided too. In order to obtain the correct food ratio of an athlete's diet, the energetic requirements need to be met for 24 hours depending on the type of physical effort.

To help the body recover after exercise sportive must facilitate detoxification. Body's water balance must be maintained at constant limits. After the competition when in blood circulating, metabolite results from the effort required a greater amount of water to eliminate. Therefore after exercise it is not enough just managing salty water but we will add potassium for the diuretic effect). Detoxification can be achieved through a ration with a sufficient intake of water, sodium chloride, potassium chloride, alkaline salts and vitamins, especially B_1 and B_6 a moderate percentage of lipids and carbohydrates, but low in protein. It should be administered 24 hours after the competition.

Carbohydrates needed in the sportive body should be provided at a rate of 65-70% polysaccharides (starch), which gradually digest and does not cause hyperglycaemia and only in proportion of 30-35% of mono and disaccharide (glucose, fructose, lactose, sucrose, etc.). Vegetal foods also contain an important polysaccharide called cellulose. This accelerates the intestinal transit in large amounts shorten the time of action of enzymes on food and absorption during trophies. The amount of cellulose used needs to be higher in the preparatory stages and recovery stages. In competitive stage cellulose intake must be smaller in order to not disturb the digestion.

Hence, the average quantity of food required is presented in Table 3.

Table 3. The average quantity of food with the energetic value of the ratio around 4500 calories

Type of food	Average quantity (g)
Beef	350
Milk	300-500
Eggs	2 pieces
Pasta	50
Fresh vegetables	500
Fresh fruit	500
Oil	30
Bread	300

This food ratio helps at obtaining the required amounts of salts and vitamins in the body. In Table 4, as well as figure 1, the main types of food in average quantities are presented by mentioning how many proteins, lipids, carbohydrates and calories are found in each one of them.

Table 4. Types of food required in an athlete's diet ration

Type of food	Amount per week	Proteins	Lipids	Carbohydrates	Calories
Beef	3 days x 250 g = 750 g	150	37	-	975
Cow milk	7 days x 300 mL = 2100 mL	70	70	96	1340
Eggs	7 days x 60 g = 420 g	65	60	3	830
Pasta	4 days x 50 g = 200 g	26	2	148	732
Vegetables	7 days x 400 g = 2800 g	16	31	324	1280
Fruits	7 days x 400 g = 2800 g	16	31	324	1280
Oil	7 days x 30 g = 210 g	-	199	-	1850
Whole wheat bread	7 days x 250 g = 1750 g	148	26	375	3762

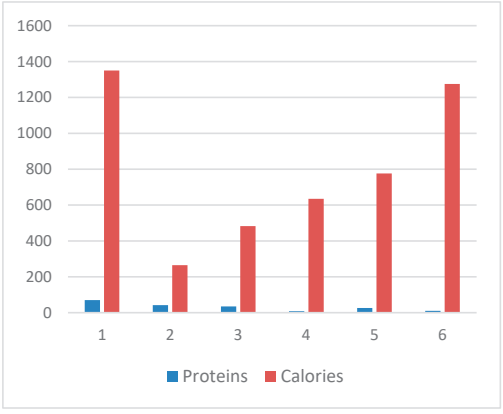

Figure 1. The amount of proteins and caloric value in the food ration in the competitional stage

Hypoglycaemia resulting from an intense and prolonged effort is accompanied by a decrease in plasma potassium. It is therefore recommended that after an exhausting effort to administrate to the athletes both carbohydrates and potassium.

The research results shows the nutritional values of meat, fish and their derivatives,

athletes food ration, but also the advantages and disadvantages of using these food groups in the diet of athletes. Meat, fish and their derivatives along with milk and cheese are a good source of protein with high biological value (Class I proteins). Thus light meat, especially beef, contains 17 to 22% protides, while weak fish contains 15-20% protides. Their association with cereal products raise cereal product's nutritional value. Meat, fish and their derivatives contain significant amounts of minerals. Meat, especially the viscera (liver, kidney) is the richest source of iron. Meat and fish are rich in phosphorus, potassium and sodium but low in calcium. Meat is the most important source of Vitamins PP, B_2 and B_6, while fish is a source of Vitamins A and D.

After the competition, the athlete loses a small amount of its reserves of fat. For that ratio to remain balanced and still respect the relationships between foods he can eat: butter, pasta or rice and oil in salads. For recovering of the potassium amount it is recommended the consumption of dried fruit at dinner. Other minerals (magnesium, calcium, iron, etc.) suffer certain changes, but losses may not be compensated immediately.

Carbohydrates reserve should be recovered avoiding massive ingestion of sugars. There are enough carbohydrates ingested at dinner table in form of pasta, rice, potatoes, fruit or fruit juice.

Dishes of meat and fish make up sources of vitamins of equal importance as foods originating from. Meat and fish are also a source of energy according to their fat content. The living organism needs food in order to cover energy costs. This energy is expressed by high calories. Depending on the energy requirements of the body we can talk about basal metabolism (basic) and effort metabolism (professional and sport).

Additionally, during sport activities the need for Vitamin C reaches an average of 150-200 mg per 24 hours, while during the competitional stage may reach 300-400 mg per 24 hours (even up to 500 mg per 24 hours on authors opinion). This dose must not be exceeded as it may cause various undesirable side effects including sleep, excitement, muscle cramps. In case of hypovitaminosis C muscle fatigue may occure. This happens more often in winter and spring due to lack fresh vegetables in the diet. Vegetables and fruits should provide 15% of the caloric value of the ration. If this percentage is not reached it is desirable to provide a supplement of Vitamin C athletes as juices. In some studies a correlation between vitamins is required as an excess of one vitamin may influence the effect of another. For example, provitamin A in excess leads to hypovitaminosis C.

Foods can also be divided into essential and non-essential. The essential ones are taken from the external environment because they cannot be synthesized by the body. For example, minerals, vitamins, the following amino acids (lysine, tryptophan, phenylalanine, methionine, cysteine, leucine, isoleucine, threonine, tyrosine, valine and some fatty acids, such as linoleic acid, linolenic acid and arachidonic acid. In order to be useful to the body, food is subjected to transformations, some outside the body and others inside it. Through the digestion process that takes place in the digestive tract, food is broken down under the action of various digestive ferments, first in the substances that are formed (proteins, lipids, carbohydrates, salts, etc.). They are further broken down into simple elements that are absorbed in the intestinal mucosa. After absorption the nutrients pass into circulation and reach the cells where they are metabolized.

Diet dominated by meat has the advantage that it allows muscle to increase their volume and strength. Animal proteins stimulates the the nervous activity and facilitates the transmission of nerve excitations which consequently helps to increase effort capacity especially in the speed contests. In high intensity effort sports (running, throwing, sports games) and in those in which force prevails by imposing a large muscle development (weightlifting, wrestling) 2.3-2.5g protein per kg of body weight per 24 hours are required. Of these 60 % must be of animal origin and 40 % of vegetable origin.

CONCLUSIONS

The sports performances obtained worldwide have reached values that years ago seemed inconceivable. For their achievement, the athletes are subjected to a complex training

process, in which the effort often requires the body to exceed its maximum physiological limits. To meet these requirements and in order to stay close to these sports activity for as long as possible, it must combine the training process with the observance of the sports life regime, in which the correct nutrition has a primordial role. Given their nutritional value and especially their Class I protein content (necessary during the effort), it is recommended that athletes consume at least 250-300 grams of meat or fish per day especially on speed and strength efforts. The diet of the preparatory phase change depending on the nature and intensity of effort.

Also, in general the athlete nutrition must not have a high volume but to consist of foods with high biological value. The assimilation of food is helped by correct ration composition and preparation of good food especially those vegetal. The manner in which of the feed ration for athletes whose caloric needs are up to 5000-5500 calories per 24 hours is made. To obtain the results were taken into account: 1 g of protein emits by burning 4.1 high calories; 1 g of fats emits by burning 9.3 calories; 1 g of carbohydrates emits by burning 4.1 calories. Since the ration is set weekly the respective amounts will be increased by 7.

Meat is recommended to be administered on meals before special effort. In the evening meat consumption should be reduced because it can adversely affect the sleep. Meat derivates and canned fish are more nutritious, have a high caloric value, but are harder to be digested. Consuming of large amounts of meat derivates and canned fish determine the change in internal pH to acidic, which is unfavorable for sport activities, especially after the finish of exercises.

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THE INFLUENCE OF NEW MICROBIAL ASSOCIATIONS ON THE SOME FUNCTIONAL PARAMETERS OF CALVES AND PIGLETS

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Abstract

In gnotobiological experiments, the influence of a new microbial associations developed by combining strains of beneficial microorganisms on the body mass, numerical value of enterococci and some biochemical blood indices of calves and piglets, and their effectiveness was investigated. The remedy under test was administered orally, immediately after the birth of the animals, 30 minutes before receiving colostrum and then once a day, over a day, until the age of 9 days. The animals were monitored for 30 days. It was observed that in animals with intestinal dysfunction the investigated remedy contributed to the disappearance of diarrheal symptoms, thus contributing to the increase of the general resistance of the organism, preventing dehydration of the organism and decreasing mortality.

Key words*: beneficial microorganisms, farm animals, intestinal microbiota.*

INTRODUCTION

Food security provides not only the provision of sufficient food to the population, but also the provision of high-quality food.

Milk, meat, and eggs, the "animal-source foods" though expensive sources of energy, are one of the best sources of high-quality protein and micronutrients that are essential for normal development and good health (Smith et al., 2013).

Thus, improvement of food quality is an important aspect of animal breeding and has become more important over time having as objectives the increase of the nutritional value (nutritional value), the assurance of the consumers' health, and optimizing processing characteristics.

Healthy animal or poultry is essential requirement for getting safe and good-quality foods of animal origin. Thus, a major objective in ensuring a quality animal production is the elimination of diseases (Collins & Wall, 2004).

Effective functionality of the gastrointestinal tract (GIT) and its health, are important factors in determining animal performance (Kogut & Arsenault, 2016).

By supporting gastrointestinal functionality of farm animals, the animal breeders can improve the weight gain of animals, leading to increased processing yields.

The concept of "intestinal health" has begun to attract interest in the field of the animal science and refers, in principle, to such aspects that promote the performance and welfare of animals as: diet, effective structure and function of the gastrointestinal barrier, host interaction with the gastrointestinal microbiota, effective digestion and absorption of feed and effective immune status (Celi et al., 2017).

It is believed that the appearance of many diseases in farm animals is due to disorders of the gut microbiota (Lee et al., 2016).

It is known that all cavities of the animal body at different ages have a certain obligatory microflora which fulfills a protective, enzymatic and synthetic functions. However, immediately after birth, the adult animals' specific microflora enters the sterile digestive tract of a newborn animals. In most cases this microflora is represented by putrefaction microorganisms, conditioned-pathogenic and pathogenic microorganisms, often leading to the death of young farm animals (Isaev et al., 2010).

According to statistics, the main gastrointestinal diseases, caused by pathogenic microorganisms, which are spread among piglets aged 5-15 and 26-40 days, cause great economic damage to pig farms, including those in the Republic of Moldova (Timoșco, 1990).

In infant piglets, these diseases were manifested by a microbial shock, and in weaned pigs - as dysbiosis (Carol-Dumitriu et al., 1981).

In this regard, further research aims to find ways to increase the natural resistance and productivity of young farm animals.

The positive role of the obligatory gut microbiota in increasing the bactericidal force of blood serum, of the function of macrophages and the general immunological reactivity of the animal organism it was elucidated (Park et al., 2018; Rovira & Melero, 2018).

Thus, the aim of this research was to study the effect of monocultures and associations of non-pathogenic microorganisms of the genera *Bifidobacterium*, *Enterococcus* and *Lactobacillus* on some parameters of body function, as well as their effect on the process of multiplication and development of intestinal enterococci in calves and piglets. Intestinal enterococci are part of the obligatory species of microorganisms of the intestinal flora which through their probiotic action have an influence on the health and resistance of farm animals, and respectively contribute to increasing the productivity of farm animals in industrial conditions.

MATERIALS AND METHODS

The microorganism strains selected and tested in gnotobiological experiments were subjected to verification of their effectiveness in experiments on calves and piglets, in farm conditions.

For this purpose, 30 calves were divided into three lots of 10 each. The first group included clinically healthy animals; group II - calves with intestinal dysfunctions (contamination with associations of bacteria from the conditioned-pathogenic genera: *Clostridium*, *Proteus*, *Staphylococcus* and *Escherichia*); group III - calves identical to group two (according to clinical status), but which received a remedy based on the association of microorganisms of five species required for calves with increased antagonistic capacity (selected in gnotobiological experiments: *Bifidobacterium longum* var. longum, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Streptococcus bovis* and *Streptococcus lactis*). The remedy under test was administered orally, immediately after the birth of the calves, with 30 min before receiving colostrum and then once a day, over a day, until the age of 9 days, i.e., 5 times. The animals were monitored during 30 days.

Strains of microorganisms were also tested on piglets, in the conditions of the technology-intensive enterprise. The experimental animals (400) were divided into five groups, 80 piglets each. Immediately after birth, before the first colostrum intake, they were orally injected with 10 ml of biomass of non-pathogenic microorganisms (obligatory types of intestinal microflora), containing 10^9 microbial cells in 1 ml, and then the same dose was administered six more times over a day, i.e., up to the age of 14 days. Animals from group I received a monoculture of *Bifidobacterium thermophilum*; animals from group II - *Streptococcus faecium*; animals from group III - association of *Bifidobacterium thermophilum* and *Enterococcus faecium*; animals from IV - *Bifidobacterium thermophilum* + *Enterococcus faecium* + *Lactobacillus acidophilus* + *Lactobacillus fermenti*; animals from group V did not receive cultures of microorganisms and served as the control group. All experimental piglets were raised according to the technology adopted in an industrial pig farm, with a closed breeding cycle, excluding chemotherapeutic drugs (sulfamides and antibiotics).

Biochemical analysis of blood in farm animals was performed according to the methods described by Vasilieva E. (1982) and Kondrahina I. (2004).

The content of enterococci was determined using classical microbiological methods (Garmasheva & Kovalenko, 2010).

Their inoculation was performed on agarized elective nutrient medium, recommended for enterococci (produced and marketed by the company "Himedia"). Over 72 hours after incubation of the inoculated samples on Petri dishes at 37 ± 1ºC, quantitative indices of enterococci were calculated at 1 g of intestinal contents (by multiplying the number of colonies by diluting the sample). The final results are expressed in decimal logarithms (log) (GOST 30518-97, 2000).

RESULTS AND DISCUSSIONS

As mentioned above, microbiota of digestive tract greatly affects the development of the host animal, mainly at an early age, and plays a very important role in the animal's resistance to infectious diseases. The formation of an obligatory microbiota as soon as possible, beneficial to host organism, consists in its barrier function and in the ability to prevent the implantation of allochthonous microbes in the gastrointestinal tract.

Thus, knowledge of the mechanism of bacterial interactions in an inevitable presupposition if optimization of the consumption of the gastrointestinal microbiota and stimulation of the beneficial effects of the latter on the host animal are desired (Stavric & Kornegay, 1995).

The greatest differences in the composition of the gut microbiota have been shown to occur between ruminants such calves and monogastric animals such piglets (Bomba et al., 2006).

In this aspect in experiments on calves, the action of the association of microorganisms of five obligatory species characteristic of these animals, with increased antagonistic capacity (*Bifidobacterium longum* var. Longum, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Streptococcus bovis* and *Streptococcus lactis*) was investigated.

First, the body mass of the calves at birth and after 30 days of administration of the tested associations of microorganisms was determined (Table 1).

Table 1. The body mass of the calves at birth and after 30 days of administration of the tested associations of microorganisms

Experimental groups of calves	Body mass of calves, kg	
	Newborn calves	30-day-old calves
I	28.5± 0.02	42.12 ± 0.2*
II	29.9 ± 0.01	42.1± 0.12
III	31.3 ± 0.04	46.33± 0.02*

* P≥0.05, ** P≥0.01

It was observed that the growth energy of calves from group III is higher compared to calves from the control group. The weight gain in group III of animals was 15.03 kg, in control group - 13.63 and in group II - 12.22 kg.

So, the daily weight gain is 454.33 g in the control group, in group II (the animals with intestinal dysfunction) - 407.33 g and in group III - 501 g.

Another parameter investigated was the numerical value of intestinal enterococci (Table 2).

Table 2. Quantitative level of intestinal enterococci in calves with and without dysfunctions caused by microbial factor, per 1 g of intestinal contents, decimal logarithms (log)

Groups of animals	Age (days)				
	1	4	7	15	30
I	1.25±0.1	3.34±0.12	5.78±0.1	6.19±0.11	5.47±0.12
II	4.12±0.09	9.76±0.12	9.9±0.11	9.49±0.14	9.21±0.11
III	6.5±0.1**	8.4±0.07*	8.7±0.09	6.9±0.13*	6.4±0.09*

**P<0.02; **P<0.05*

According to the data presented in the Table 2, there is a clear difference in the quantitative indices of enterococci of the experimental animals. Thus, in calves from group III, which received a remedy based on the association of obligatory microorganisms, the maximum content of enterococci is attested in the first 7 days of life (8.7 g (log)), after which their quantitative level reaches values characteristic of healthy animals (group I) - 6.4 g (log). At experimental animals from group II during the investigation period (30 days) an increase in the numerical value of enterococci was observed from 4.12 to 9.21 g/(log) (Table 2).

At the same time, it was established that in the first 7 days of life, in calves from group III, which received the association of obligatory microorganisms, the diarrheal symptoms disappeared. At animals from group II, diarrheal symptoms characterized by 100% dysmicrobism, manifested until the age of 20 days, causing weakening of general resistance, dehydration of the macroorganism, resulting in mortality of 30% of calves. This indicates the possibility of developing intestinal dysfunction, if the beneficial microbiota is not formed in time in young animals.

It was also found that in animals from groups I and II, the hemolytic streptococci predominated. In experimental group III prevailed bacteria from group D streptococci (enterococci), i.e., useful forms of the macroorganism, such as *Enterococcus faecium* which has the ability to produce strong bacteriocins (enterocin A and enterocin B), which is important for suppressing the excessive growth of the

opportunistic flora that forms in dysbiosis (Salvucci et al., 2012; Cotter et al., 2013).

The determination of the blood indices of general resistance of calves in the experimental groups reveals the prevalence of bactericidal activity of blood serum in calves from group III compared to that from group I and II at the age of 15 days (respectively 15.84% and 18.4%) and at 30 days (respectively with 5.84% and 11.36%) (Table 3).

Table 3. The blood indices of calves without and with intestinal dysfunctions caused by the microbial factor and under the influence of the association of microorganisms of the obligatory genera of the gastrointestinal tract

Studied indexes	Experimental groups of calves	Age of animals, days	
		15	**30**
Bactericidal activity of blood serum, %	I	83.3±1.28	94.2±0.82
	II	81.5±0.9	89±0.87
	III	96.5±0.93*	96±1.37
Bicarbonate, blood, mg%	I	370±7.06	376.7±12.4
	II	320±9.98	360±11.16
	III	355±12.33*	388±7.2*
Blood glucose level, mg %	I	96.7±0.95	103.8±1.4
	II	79.2±0.89	98.4±1.47
	III	104.9±1.06*	109.3±1.3*

*P<0.05

According to the data on the indices of the alkaline blood reserve, the differences between the experimental groups and according to the age of the animals were not non-essential. At the age of 30 days, this index was higher for calves of group III respectively by 3.03% and 7.78%, compared to those from groups I and II.

The quantitative level of glucose in animals receiving remedies based on the multi-component microorganism association obligatory for the gastrointestinal tract (group III) at calves of 15 days age was with 8.48% and 32.45% higher compared to this index at calves from groups I and II. After 30 days of administration of the tested remedy, this difference was 5.3% and 11.08%.

So, the influence of the remedy based on the association of microorganisms on the young calves (in the first 30 days of life) and on the intestinal microbiota is beneficial.

Piglets as monogastric animals differ from calves (ruminants) by the composition of the intestinal microbiota (Bomba et al., 2006).

It has been established that gradual changes in the composition of the gastrointestinal microbiota that take place within an animal species are related to age (Smith, 1965). At an early age the microbiota of the digestive tract of young animals is very similar. This largely depends on the fact that after birth the intestinal microbiota is determined by breastfeeding. Especially, milk constituents largely determined which microbiome can be implanted in the intestines (Bomba et al., 2006).

It should be noted that newborn piglets possess very efficient selection systems enabling it to favor certain bacterial species among the bacteria of different ecosystems. This selection is influenced by many factors: diet, environmental conditions such as hygienic stage, temperature, the microbial interactions in the digestive tract and the barrier effect of the dominant microbiota against the environmental bacteria (Jiang et al., 2019).

The monocultures and associations of microorganisms studied were tested on piglets for 30 days, during which the animals were monitored, recording weight gain, numerical indices of enterococci in their digestive tract, as well as non-specific resistance (bactericidal activity of blood serum, %, the hemoglobin, g/dL and erythrocytes content $\times 10^{12}$/L) according to classical methods (Vasilieva, 1982; Kondrahina, 2004).

The data regarding the action of investigated microorganism strains on body mass of piglets are given in table 4.

Table 4. Body mass of piglets in testing experiments of monocultures and new microorganism associations

Experimental groups of piglets	Body mass, kg	
	3-day-old piglets	25-day-old piglets
I	0.89± 0.06	6.25 ± 0.31*
II	0.93 ± 0.04	5.25 ± 0.24
III	1.18 ± 0.03	6.2 ± 0.22**
IV	1.01 ± 0.05	6.75 ± 0.27
V	1.22 ± 0.05	4.45 ± 0.25**

* P≥0.95, ** P≥0.99

The tested microorganisms had a positive effect on the productivity of experimental animals. In particular, the average weight of a 25-day-old piglet in group I was 6.25 kg; in group II - 5.25 kg; in group III - 6.20 kg; in group IV - 6.75 kg and in group V - 4.45 kg. Therefore, the daily weight gain of a piglet was 210 g in group I; 170 g - in group II; 204 g - in group III, 230 g - in group V and 134 g in group V.

Also, the same changes were noted regarding the data on the vitality per head of experimental

animals. Thus, in group I it was 95%, in group II - 85%, in III - 90%, in IV - 97.5% and in V - 65%.

The obtained data revealed that the administration of the association of microorganisms – *Bifidobacterium thermophilum* + *Enterococcus faecium* + *Lactobacillus acidophilus* + *Lactobacillus fermenti* had the most beneficial effect on the weight gain and vitality of experimental animals.

The effect of monocultures and associations of microorganisms on the multiplication and development of enterococci in the digestive tract of piglets was also investigated. It is known that the optimal content of enterococci in the intestine ensures the proper functioning of the intestinal microbiota.

It is known, that the enterococci are ubiquitous in GI tracts even though they constitute a small proportion of the gut consortium, typically comprising less than 1% of the adult microflora (Finegold et al., 1983; Sghir et al., 2000). Enterococci are primarily localized to the small and large intestine, where enterococci are prominent members of jejunal, ileal, cecal, and recto-sigmoidal consortia (Hayashi et al., 2005).

Therefore, the numerical value of enterococci was documented in 6 segments of the digestive tract: stomach, duodenum, small intestine, ileum, check and rectum.

The results on the numerical indices of intestinal enterococci in piglets are presented in Table 5.

Among the piglets of control group, bacterial gastroenteritis from dysbacteriosis was recorded, resulting in 35% piglet mortality. In the experimental groups, intestinal disorders at piglets were not observed.

Analysis of the numerical values of enterococci in all gut segments of piglet, in the first 30 years of life at administration of remedies based on monocultures and associations of microorganisms, revealed a lower content of enterococci in animals from group I (administration of *Bifidobacterium thermophilum* monoculture) and higher in group II of animals that received the monoculture of *Enterococcus faecium*.

It should be noted that in the stomach the content of enterococci was the lowest compared to the other segments of the digestive tract (gut), and in the check and rectum the highest values of these bacteria were detected.

When monitoring the numerical indices of enterococci during 30 days of administration of the remedies used in the study, it was established that a higher content is attested on the 7th day in animals of group II, III and IV in the stomach, duodenum, small intestine, ileum and check. In the rectum, the highest level of enterococci is revealed on the 3rd day of the experiment in animals from experimental groups II, III and IV.

Table 5. Quantitative indices of intestinal streptococci in suckling piglets that received a remedy, based on monocultures and associations of microorganisms of the obligatory genera of the gastrointestinal tract

Experimental groups of piglets	Age (days)		
	3	7	30
	Stomach		
I	0	0	0
II	5.98±0.13	6.77±0.15*	4.55±0.11**
III	5.17±0.09*	0	5.17±0.05*
IV	5.17±0.07*	5.75±0.09**	2.32±0.11**
V	4.29±0.17	3.88±0.2	3.00±0.21
	Duodenum		
I	0	2.26±0.07*	2.44±0.09*
II	6.71±0.12*	7.6±0.14**	2.24±0.08*
III	5.65±0.1*	6.72±0.11*	6.69±0.09**
IV	5.69±0.1*	6.26±0.08*	2.25±0.1*
V	6.35±0.19	4.67±0.15*	4.49±0.2*
	Small intestine		
I	2.48±0.07*	3.48±0.05*	3.26±0.08*
II	7.7±0.15*	7.82±0.13*	3.52±0.09*
III	6.74±0.12*	7.57±0.1*	2.89±0.05*
IV	2.88±0.13*	6.88±0.11*	3.32±0.06*
V	7.64±0.22	5.8±0.19	5.28±0.17
	Ileum		
I	3.59±0.09*	4.5±0.11*	4.74±0.1*
II	7.93±0.18*	8.29±0.14*	5.46±0.1*
III	7.69±0.13*	8.39±0.15*	4.39±0.1*
IV	7.76±0.09*	7.84±0.08*	4.79±0.1*
V	6.68±0.19	5.83±0.15	5.83±0.15
	Check		
I	4.6±0.11*	5.52±0.12*	5.87±0.1*
II	8.65±0.17*	8.44±0.15*	8.44±0.15*
III	7.69±0.13*	8.67±0.12*	6.07±0.1*
IV	8.59±0.12*	8.4±0.13*	6.83±0.11*
V	6.27±0.2	8.04±0.17	6.86±0.19
	Rectum		
I	4.15±0.1*	5.55±0.12*	5.67±0.11*
II	8.69±0.17*	8.38±0.15*	5.91±0.12*
III	8.62±0.12*	8.23±0.11*	5.45±0.13*
IV	8.55±0.08*	7.54±0.06*	5.82±0.07*
V	5.87±0.17	7.22±0.17	6.35±0.15

After 30 days of study, the numerical value of enterococci decreased in all animals of the experimental groups in all segments of the digestive tract, except for group I, where an increase in enterococci was observed after 30 days of monoculture *Bifidobacterium thermophilum* administration.

It was established that the associations of microorganisms tested in group III and group IV contributed to maintaining of the enterococci content as the same level as in control group in gut segments as: stomach, duodenum, small intestine and ileum

In check and rectum high numerical values of enterococci are characteristic for all experimental groups (except group I), including the control group.

Thus, based on the obtained data on the influence of monocultures and associations of microorganisms on the numerical indices of enterococci in gut segments of the digestive tract we can mention that:

- administration of the monoculture of *Bifidobacterium thermophilum* (group I) resulted in a lower content of enterococci during 30 days of testing in all segments of the digestive tract;
- the administration of the monoculture based on *Enterococcus faecium* (group II) determined the increase of the numerical indices of the enterococci, reaching the highest level in check and rectum (compared to the other experimental groups) in the first 7 days of testing;
- administration of the associations of *Bifidobacterium thermophilum* and *E. faecium* microorganisms (group III) also contributed to the increase in enterococcal contents in the digestive tract segments, in particular in the check and rectum during the first 7 days of testing.
- the administration of associations of microorganisms *Bifidobacterium thermophilum* + *Enterococcus faecium* + *Lactobacillus acidophilus* + *Lactobacillus fermenti* (group IV) more or less determined the maintaining of the enterococci content at the same level in gut segments, which reveals the beneficial action of this remedy on the homeostasis of the gut microbiota and respectively on the health of the digestive tract.

It should be noted that the microbes of the genus *Enterococcus* are mainly ancient and highly evolved members of GI tract consortia of various hosts (Lebreton et al., 2014). It is known that the content of enterococci in the digestive tract can vary, and their numerical value can determine either the positive effect or the negative effect.

Usually, many *Enterococcus* spp are regarded as commensals of the intestine tract in mammals, but some members (*E. durans* and *E. hirae*) have also been associated with diarrhoea in suckling animals. The indigenous commensal enterococci, can act as opportunistic pathogens and translocate across the mucosal barrier to cause systemic infection in immune-compromised hosts (Berg, 1996; Donskey, 2004).

Thus, we can assume that the associations of microorganisms studied, as well as the monoculture of *Bifidobacterium thermophilum* have the most beneficial effect, in terms of maintaining a constant or low level of enterococci in the digestive tract of piglets.

In order to highlight the action of remedies based on monocultures and associations of microorganisms on the general condition of piglets, some biochemical indices of blood that reflect nonspecific resistance (bactericidal activity of blood serum, the hemoglobin and erythrocytes content) were determined. The obtained data are shown in figure 1. The determination of bactericidal activity is important because this is a one of parameters of innate immunity.

As a result of the investigations, the positive action of the monoculture of bifidobacteria (group I) and the association of microorganisms (group IV) on the bactericidal activity of blood serum in piglets was found, the level of which increased by 9.6% and 6.7%, respectively at the age of 7 days, and by 3.9% and 6.7%, respectively, at the age of 25 days, compared to the indices of control group (figure 1A).

Bactericidal activity in group III increased compared to the control group by 5.6% in 7-day-old piglets and by 1.9% at the end of the experiment. The animals that received only monocultures of enterococci (group II), a decrease of bactericidal activity was observed, being at the level of the indices from the control group (Figure 1A).

The best results of the studied indices were found in the groups of animals that received monocultures of bifidobacteria and the association of microorganisms, both at the age of 7 days and at the age of 25 days (Figure 1A).

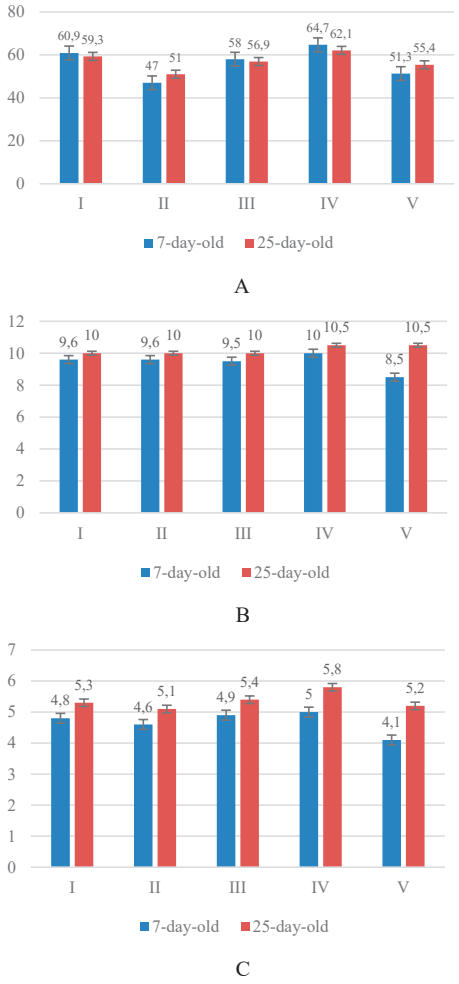

Figure 1. Bactericidal activity of blood serum, % (A), the hemoglobin, g/dL (B) and erythrocytes, $\times 10^{12}$/L (C) content of piglets under the influence of monocultures and association of microorganisms of the obligatory genera of the gastrointestinal tract

The hemoglobin content in the blood of experimental piglets in the first 7 days of investigation was higher compared to the control, but after 25 days, the hemoglobin content became identical in all experimental groups (including control) (Figure 1B). The content of erythrocytes has the same trend of modifications as of hemoglobin parameters: in the first 7 days it is higher in the experimental groups; after 25 days, it is established at the level of the indices identical to the control group (Figure 1C).

This indicates that remedies developed based on monocultures and associations of microorganisms have a more pronounced effect on the indices of nonspecific resistance in the first days of life of piglets. This is important in forming the body's general resistance to pathogens, which in turn causes a decrease in mortality in young animals and an increase their productivity, respectively.

Thus, intestinal integrity is fundamentally important for the growth and performance of food animals.

CONCLUSIONS

All types of tested microorganisms had a beneficial effect on the growth and development of farm animals in industrial farm conditions. In calves, the associations of microorganisms during the experiments ensured the increase of the body mass by 9.8-10%. A monoculture and new developed microorganism association ensured the average daily weight gain of piglets 1.26-1.7 times compared to the control and maintaining its vitality by 20-32.5%.

The investigated associations of microorganisms, as well as the monoculture of *Bifidobacterium thermophilum* have a homeostatic effect on the enterococci content in digestive tract of calves and piglets, which argues their beneficial action on the gastrointestinal microbial cenoses and respectively on the health of the digestive tract.

A direct relationship has been established between the indicators of natural resistance of farm animals in experimental groups and their productivity, which indicates the appropriateness of using bifidobacteria, enterococci and lactic acid bacteria to raise young piglets and calves in industrial farms to increase natural resistance and animal productivity, as well as for the prevention of bacterial gastroenteritis.

The beneficial effect of remedies based on monocultures and association of obligatory microorganisms argue the utilization of microbial strains as alternatives to antibiotics for sustainable food animal production.

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RESEARCH ON THE EFFECTIVENESS OF ENCAPSULATED UREA GRANULES IN POLYMERS

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Abstract

The encapsulation of fertilisers and pesticides represents an important process in agriculture and plays a significant role in the crop productivity. In literature, there is an explosion of data regarding the production and use of biodegradable polymers and the encapsulation of fertilizers, pesticides or their mixtures is also recorded. The aim is to control the release of the active substance over time, not only to extend the period of time during which it acts but also to prevent the pollution of ground water due to the washing processes. At the same time, it also aims to improve working conditions regarding the transport and use of fertilizers and pesticides.

Key words*: biodegradable polymers, encapsulation, urea.*

INTRODUCTION

The global demand for synthetic fertilizers which can affect crop productivity has increased during the past few years (Trenkel, 2010). The research based on biodegradable polymers used as coatings for slow-release fertilisers is increasing as they provide a more efficient alternative with a less negative environmental impact (Li et al., 2021). Despite the fact that the use of slow release fertilizers is still limited due to increased costs, they were shown to improve agricultural efficiency (Akelah, 1996). The absence of nitrogen in the soil tremendously affects the industry (Leghari et al., 2016). Hence, urea based fertilisers were developed to meet this demand (Azeem et al., 2014). The discharge of nitrogen from urea needs to be controlled in order to avoid losses, such as NH_3 volatilization, which can increase the level of pollution as well as the cost of fertilizers (Horrigan et al., 2002). The ability to coat urea with biodegradable materials can also improve the functioning of the soil (Matson et al., 1998).

The process of encapsulation consists of polymer synthesis followed by the addition of those on granules of fertilizers using different methods (Ni et al., 2011). Considering this, two polymers were used: one of them was synthesised by sulphonating C_2-C_3 rubber in order to encapsulate the granules of urea (spraying technique) and the other one was polyethylene rubber with a low molecular weight, also used for encapsulation.

MATERIALS AND METHODS

Synthesis of sulphonated C_2C_3 rubber, also known as S-EPDM (sulphonated ethylene propylene diene monomer rubber)
EPDM (30 g) and toluene (330 g) were added in a triple neck round bottom flask (1L capacity) with a magnetic stirrer, thermometer and condenser attached. The temperature of the mixture was kept to 60^0C with the aid of a thermostat. After the rubber was fully dissolved (2 hours), acetic anhydride (11 mL) was added and the solution was stirred for 30 minutes. Sulfuric acid (4 mL, 95%) was added and the

sulphonation occurred after 1 hour. The sulphonated polymer was neutralised with sodium hydroxide (6 g) in a methanol/toluene azeotropic mixture (methanol: toluene = 120: 46.5 g). The solution can be used as it is or dilluted with toluene in order to obtain a fine film of polymer on the urea granules (from 2 to 100 μm, with the most suitable film having a 2-20 μm thickness).

Urea granules (25 granules) were used to determine the barrier properties of polymer film used to encapsulate it. Hence, for the first type of polymer, the solution which was obtained after synthesis was sprayed on the granules of urea with a specialised glass sprayer. The process of spraying and drying was repeated three times to ensure that the layer of polymer film has a reasonable consistency. Additionally, the ethylene oligomer was melted and sprayed directly on the urea granules. To determine the barrier properties of the polymer film, the granules of urea were immersed in distilled water (200 cm^3, T = 20^0C). After 24 hours, the granules were separated using gravity filtration and immersed in fresh distilled water (200 cm^3).

The amount of urea released in 24 hours was determined using the method presented below, using two samples of aqueous solution of urea: one containing 20 g of this solution and another one containing 40 g. The analysis can be gravimetric, volumetric or colorimetric. An aqueous solution (100 mL) containing urea (40-50 mg) was added to a beaker. Anhydrous acetic acid (30 mL) and Xanhidrol (25 mL, 10% solution in anhydrous ethanol or 1% solution in acetic acid) were added whilst stirring. The solution was added in small portions (5 mL) every 20 and 10 minutes. The product of the reaction is kept for 24 hours and then filtered using a G-3 crucible glass filter with sintered disc. Initially, gravity filtration is performed followed by slow vacuum filtration using a water aspirator. The product obtained was washed with a saturated solution of 1,3-di(9H-xanthen-9-yl) urea in ethanol (20 mL) and dried at 100ºC for 1-1.5 hours before being weight.

RESULTS AND DISCUSSIONS

There are multiple advantages to encapsulating fertilizers, pesticides or the mixtures of those, such as economical advantages but also advantages which involve the current climate crisis. Hence, the process of encapsulating in order to control the release of the chemicals can have a significant influence of the environment:

- the release of the active substance into the soil is performed slowly, under control, which determines a prolonged biological activity of the fertilizers, pesticides or their respective mixtures;
- the pollution of the ground water which is determined by the washing processes caused by heavy rain is avoided;
- when both fertilisers and pesticides are applied at the same time, this causes serious energetical savings and the number of treatments applied needs to be reduced;
- superior work conditions are ensured during the packing, shipping and using periods.

Considering all of the previously mentioned advantages, the process of obtaining fertilisers, pesticides and their mixtures encapsulated in polymer film is not trivial at all. Hence, it is absolutely necessary to run a feasibility study which can help to determine one of the procedures that needs to be used. It is important to notice that for developing countries the cost (price) of the procedure is not as important as the final goal that needs to be achieved.

In order to perform the encapsulation of the fertilisers, pesticides and their mixtures there have been multiple studies. The results obtained by the multiple researching groups were published and they are starting to be applied in various industries.

Historically, microcapsules represent core/shell particles where the shell is manufactured from a semi-impermeable polymer film and the core is made of the active substance: the fertiliser, pesticide or the mixture.

There are multiple ways of encapsulating the previously mentioned active substances, such as:

- polymerization in emulsion, which is obtained by taking into consideration the core/shell principle;
- encapsulation through direct spraying of the molten mass of polymer on the fertiliser granules, called “Hot melt coating”;
- encapsulation by spraying a solution made of polymers in organic solvents;
- encapsulation aqueous solutions of fertilisers;
- encapsulation by forming the polymer “in situ” as a result of poly-condensation or poly-addition processes (Crosslinkable Coating);

- encapsulating fertilizers in the polymer gel;
- encapsulation by forming hydrogen bonds between the fertilizer and the polymer.

As a result of these processes, two different types of fertiliser granules are obtained:
- granules covered on the exterior by a semi-impermeable membrane made of polymers;
-monolithic granules in which the fertiliser is trapped in a polymeric matrix or dry hydrogel.

In this experiment, the granules of fertiliser are being sprayed using a solution of polymer in a solvent or in a solvent-cosolvent mixture, with the process being followed by evaporation of the solvent at a temperature, under a normal pressure or under vacuum, depending on its boiling temperature. Furthermore, the list of solvents is not limited at all. Hence, aromatic hydrocarbons (benzene, toluene, xylene, mesitylene), aliphatic hydrocarbons (hexane, toluene, n-octane, 2-ethyl-hexane, 2-ethylcyclohexane) as well as chlorinated derivatives (dichloromethane, trichloromethane, tetrachloromethane, trichloroethylene, tetrachloroethylene).

The spraying process is achieved in machines which function in fluidised layer. The method of obtaining the fertiliser capsules was described in the literature and allows the achievement of thin and uniform covering film. When it comes to polymers, a variety of polymers can be used and a lot of them are modified through sulphonation. In order to reduce the viscosity of the polymer solutions, it is advisable to introduce a polar cosolvent which is capable of solubilising pendant ionomeric groups. The hydrophilic cosolvent must possess a solubility parameter between 10-11 and its weight proportion in the system to be between 0.01% and 15% related to the total organic mixture.

The hydrophilic cosolvent is decided between the following classes of compounds: alcohols, amides, amines, acetamides etc. We are mentioning that aliphatic alcohols (methanol, ethanol, n-propanol, isopropanol, 1,2-propandiol, the monomethylether of ethylene glycol as well as n-ethylformamide or methyl-isobutyl carbinol) are preferred as polar cosolvents.

Extraordinary results can be obtained if, at the solutions of sulphonated polymer, a basic polymer is added, capable of forming complexes through acid-base interactions. A very interesting method relies on spraying granules of fertiliser in vacuum. According to this method, the granules added in the micro-encapsulation machine are vacuumed and, at the time when the spraying process of polymer solution takes place, it is fully absorbed into the pores which can be found on the surface of the granules. It is worth mentioning that this procedure can be used to spray aqueous dispersions of polymers (e.g. Copolymers of ethene with unsaturated carboxylic acids).

The mechanism of controlled release of fertilisers

Despite the chosen method of encapsulation, when the product is inserted into the soil, when it gets in contact with the humidity within it a controlled release takes place of the fertiliser or/and the pesticide. The polymers used for encapsulation can be biodegradable or not. Usually, polymers which contain backbones formed by C-C units tend to be not biodegradable. The insertion of heteroatoms onto the backbone can offer biodegradability. Most of the time, the controlled release of fertiliser occurs in the presence of water. When water passes through the polymer capsule, the hydrophilic polymer can expand which allows the controlled release of the active substance. The release can also be related to the increased osmotic pressure due to the penetration of water in the semi-permeable membrane of the polymer.

Tables 1 and 2 represent a comparison between the results obtained and the literature values. These values were used to obtain two graphs corresponding to the two different polymers analysed: EPDM and polyethylene (He et al., 2000). The analysis of the graphs suggests that the results are reliable, taking into consideration the type and the coating thickness of the polymer film. These influence the release rate of the urea into the soil (Hodosan, 2007). When two molecules of xanhidrol are added to urea (Figure 1) a hydrophobic condensation product is obtained which is used in its quantitative determination. It was found that 0.142 mg of urea correspond to 1 mg of 1,3-di(9H-xanthen-9-yl) urea. For correction, 0.5% of the value obtained is added to the result.

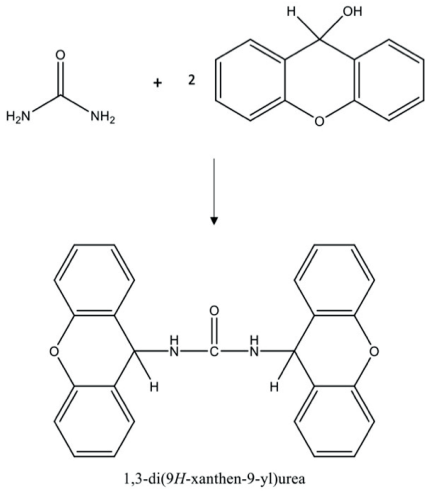

Figure 1. Reaction between urea and xanhidrol to give the desired product

Table 1. Results obtained in order to determine the barrier properties of the S-EPDM polymer film

No. Days	POLYMER: S-EPDM		
	g urea/ 20 g sample	g urea cumulated	% urea released
1	0.4804	0.4804	19.216
2	0.2302	0.716	28.424
3	0.1918	0.9024	36.096
4	0.2097	1.1121	44.484
5	0.0959	1.2080	48.320
6	0.1525	1.3605	54.420
7	0.0479	1.4084	56.336
8	0.1519	1.5603	62.412
9	0.1223	1.6826	67.304
10	0.1616	1.7842	71.368
11	0.1243	1.9085	76.340
12	0.0461	1.9546	78.184

Table 2. Results obtained in order to determine the barrier properties of the polyethylene polymer film

No. Days	POLYMER: POLYETHYLENE		
	g urea/ 40 g sample	g urea cumulated	% urea released
1	0.1108	0.1108	2.216
2	0.0642	0.1750	3.500
3	0.0352	0.2102	4.204
4	0.0150	0.2252	4.504
5	0.0369	0.2621	5.242
6	0.0629	0.3250	6.500
7	0.1572	0.4822	9.644
8	0.0478	0.5300	10.600
9	0.3904	0.9204	18.408
10	0.3998	1.3202	26.404
11	0.1901	1.5103	30.206
12	0.3458	1.8560	37.122
13	0.3761	2.2322	44.644

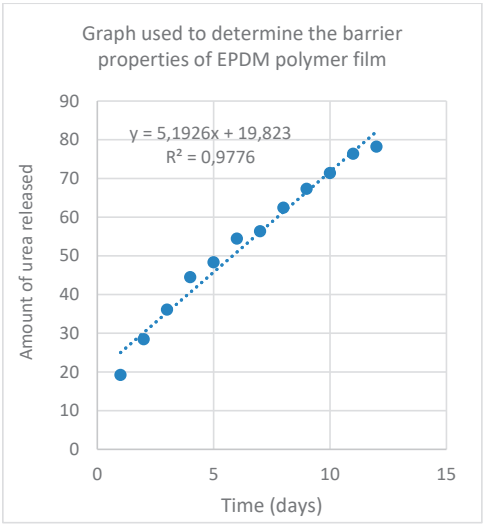

Figure 2. Graph used to determine the barrier properties of EPDM polymer film

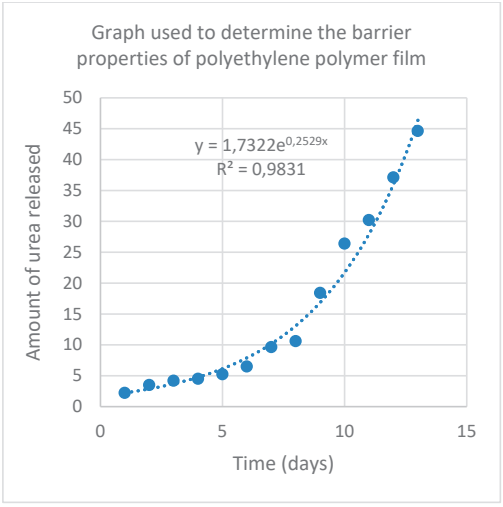

Figure 3. Graph used to determine the barrier properties of polyethylene polymer film

The direct encapsulation of polymers on the fertiliser granules using the Hot Melt Coating technique

The encapsulation of fertilisers using this procedure consists of spray coating the molten mass of polymer or mixture of polymers directly on the surface of the fertiliser granules in machines in fluidised layers or in spherical machines which rotate continuously.

When it comes to polymers, generally products with the melting temperature between 30° and 120° are used as well as mixtures of petroleum

wax with polyethylene with the molecular weight <10000, copolymers ethylene-vinyl acetate, copolymers ethylene-carbon dioxide, copolymers of ethene with α-olefins, aliphatic polyesters etc.

An interesting example of controlled slow-release consists of attaching the fertiliser to the main backbone through covalent bonds which hydrolyse easily in the presence of water, releasing the fertiliser. A polymer like this one is polyvinylic alcohol in which the hydroxyl groups have normal reactivity and react with the groups already present in the fertiliser.

In the literature, there are examples in which the fertiliser is attached to the polymer backbone through hydrogen bonds. In this case, the polymer must contain functional groups which are able to form these hydrogen bonds. These groups could be: -OH; -NH_2; -NH-; -SO_2NH-; -SO_2NH_2; -CONH; -$CONH_2$; -COOH; -CON<; =N-; -N=N-.

No matter what the nature of the mechanism of controlled release of the fertiliser or its mixture with pesticides is, it is important to ensure that the duration of the mechanism of action is prolonged for longer compared to the treatment with normal fertiliser which is not encapsulated. It is necessary to obtain these mixtures of fertilisers with pesticides and their encapsulation since this is the basis of modern and sustainable agriculture. This is required due to the significant changes in the economy as well as the impact on the environment since a lot of chemicals are toxic and could potentially harm humans over a longer period of time. Additionally, the pollution of the groundwater needs to be avoided.

Taking into consideration the results obtained in this experiment, it is undoubtedly necessary to encapsulate fertilisers and pesticides in order to improve their action but also to protect the environment.

CONCLUSIONS

The encapsulation of fertilisers and pesticides has been proven to efficiently and effectively improve the crop production as well as diminish the levels of chemicals found in the soil. There are multiple industrial procedures which can achieve the encapsulation and the most common one involves spray coating with a mixture of polymers.

In this experiment, urea was encapsulated using two types of polymers: sulphonated EPDM rubber (with the sulphonation being achieved in the lab using an original method) and polyethene with a low molecular mass. The analysis of the barrier properties of the products suggested that the results obtained are reliable by comparing them to literature values for similar polymers. Furthermore, the sulphonation of EPDM rubber can be achieved with a standard procedure, but the encapsulation of the urea granules is obtained by spraying whilst they were present in the solution (toluene/methanol with rubber) which requires the recovery of the toluene and methanol after the spraying process.

The presence of methanol in the system is vital to the solubilisation of the hydrophilic ionomer. The sodium present in the neutralised sulphonic groups can be replaced by zinc, one of the main microelements which aids the growth of plants. It is very effective to use ethylene oligomers to encapsulate urea due to the fact that the price is low and the spraying process is achieved using specialised machinery for powdering fertilizer granules in order to prevent their crowding. This process is considered to be the only suitable one considering the economical situation in our country.

The results obtained support the evidence that the encapsulation process is necessary and further research should be conducted in order to determine the most accurate and efficient procedure as well as the best polymers chemical compounds which can be used to encapsulate fertilisers.

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THE OMEGA-3 AND OMEGA-6 FATTY ACIDS POTENTIAL OF PUMPKIN, CANDLENUT AND NUTMEG SEEDS AS PHYTOADDITIVE FOR POULTRY. A REVIEW

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Abstract

Fatty acids are important constituents of plants and known to possess antimicrobial activities. The biological activity and the possibility of the therapeutic of fatty acids of plant extracts as antimicrobial agents is reviewed. Pumpkin seed, candlenut, and nutmeg are rich in omega-3 and omega-6 fatty acids that possess antimicrobial activities, have potential antioxidant, antiinflammatory, antihyperlipidemic properties in poultry feeding. Pumpkin seed, candlenut, and nutmeg are a rich source of fixed and essential oil, triterpenes, and various types of phenolic compounds. The literature about the benefits of plants seed potency as an alternative phytoadditive for poultry was rare. This article provides an overview that on the potency and biological activity of the omega-3 and omega-6 from pumpkin seed, candlenut, and nutmeg as a basis for exploring it as a phytoadditive for poultry. The method used is the synthesis matrix. From the review of the article, it was concluded that pumpkin seed, candlenut, and nutmeg have the potential to be developed as an alternative feed for poultry, and have bioactive constituents that promote health.

Key words*: antibacterial, omega-3 fatty acid, omega-6 fatty acid, phytoadditive, poultry.*

INTRODUCTION

The common fatty acids in plants are saturated or simple unsaturated compounds of C16 or C18 chain length. Palmitic acid (C16) is the major saturated acid in leaf lipids and some seed oils, while stearic acid (C18) is a major saturated acid in seed fats in several plant families. Unsaturated acids based on C16 and C18 are widespread in leaf and seed oils. The tri-unsaturated linolenic acid, linoleic and oleic acids are common (Harborne and Baxter, 1993), and according to Harwood (1980), quantitatively, the major fatty acids in plant are palmitic, linoleic and, in particular, α-linolenic acids.

Many fatty acids are known have antibacterial and antifungal properties (Russel, 1991). The antibacterial properties of antimicrobial lipids have been known since long, when it was shown that fatty acids, a class of antimicrobial lipid, inhibited growth of the *Bacillus anthracis* pathogen that causes anthrax (Thormar, 2010). Antimicrobial lipids such as fatty acids and monoglycerides are promising antibacterial agents that destabilize bacterial cell membranes, causing direct and indirect inhibitory effects (Yoon et al., 2018).

Fatty acids are released from lipids by the action of enzymes to become free fatty acids, which have potent biological activities (Desbois & Smith, 2010). Fatty acids (FAs) are potential therapeutic antimicrobial agents due to their potency, broad spectrum of activity and lack of classical resistance mechanism against the actions of these compounds (Desbois, 2012). Various long chain polyunsaturated FAs, which are found naturally at high levels in many marine organisms, have been shown to exert highly potent activity against Gram-positive bacteria, including eicosapentanoic acid (EPA; C20:5n-3) (Desbois, 2013) docosahexanoic acid (DHA; C22:6n-3) (Huang & Ebersole, 2010), γ- linoleic acid (GLA; C18:3n-6) and dihomo- γ - linolenic acid (DGLA: C20:3n-6). Similar to many other PUFAs, eicosapentaenoic acid (EPA; C20:5 n-3) exerts potent effects against Gram-positive species, including human pathogens *Bacillus cereus* and *S. aureus*.

Long-chain unsaturated fatty acids, such as linoleic acid, show antibacterial activity and are the key ingredients of antimicrobial food

additives and some antibacterial herbs (Zheng et al., 2005).

Long-chain unsaturated fatty acids are bactericidal to important pathogenic microorganisms, including Methicillin-resistant *Staphylococcus aureus* (Farrington et al., 1992), *Helicobacter pylori* (Sun et al., 2003), and *Mycobacteria* (Seidel & Taylor, 2004). These antibacterial actions of fatty acids are usually attributed to long-chain unsaturated fatty acids including oleic acid, linoleic acid, and linolenic acid, while long-chain saturated fatty acids, including palmitic acid and stearic acid, are less active (Sun et al., 2003; Seidel & Taylor, 2004).

Pumpkin, scientific name *Cucurbita moschata* is part of the Cucurbitales order, Cucurbitaceae family and *Cucurbita* genus that has long been applied in Asia for medicinal goals (Call et al., 2006). Pumpkin seeds has many nutrients including polysaccharides, essential fatty acid, carotenoids, mineral, active proteins, and essential amino acids. The seeds have a high nutritional value (Fokou et al., 2004). That seeds had medicinal properties for their biological effects such as antimicrobial activities (Abd EI-Aziz & Abd EI-Kalek, 2011).

Candlenut, scientific name *Aleurites moluccana*, belongs to euphorbiaceae family and grows widely in tropical and sub tropical regions. It is also known as Buah Keras in Malaysia, Kemiri in Indonesia, Indian Walnut in India, and Kukui in Hawaii. It is used in folk medicine to treat stomach in children, bad breath, skin sores, fever, headaches, tumors, diarrhoea, asthma and helps in rejuvenating the body after poisoning.

Candlenut seeds was classified as a type of stone fruit because they have physical characteristics of hard skin and shell shape, then the outer surface was roughly curved (Sinaga, 2016). Besides the consumption of core candlenut seeds is very large (Permana et al., 2017). Candlenut is a common spice that contains high levels of fatty acids.

Nutmeg, is dried kernel of broadly ovoid seed of *Myristica fragrans* Houtt (Family: Myristicaceae). It is widely used as spices in culinary preparations and in alternative medicine as aphrodisiac (Tajuddin et al., 2003), memory enhancer, antidiarrhea (Grover et al., 2002). This plant produces two spices: mace and nutmeg. Nutmeg is the seed kernel inside the fruit and mace is the red lacy covering on the kernel. Myristica species are natives of Moluccas, indigenous to India, Indonesia and Sri Lanka and now cultivated in many tropical countries (Pal et al., 2011). About 30-55% of the seed consists of oils and 45-60% consists of solid matter including cellulose materials.

Nutmeg seeds supplementation may improve blood lipids, ameliorate oxidative stress and this may be due to interactive or additive effects of the numerous bioactive constituents (Thomas & Krishnakumari, 2016). The medicinal use of nutmeg and its use as a spice suggest that it contains some constituents which are responsible for the reported biological activities (Al-Jumaily et al., 2012). Nutmeg extract ameliorates hyperglycemia and abnormal lipid metabolism in animal models (Arulmozhi et al., 2007).

This paper provides a comprehensive overview of various aspects of the species of pumpkin seeds, candlenut, and nutmeg in relation to its potential, which will be used as a benchmark for its use as phytoadditive.

MATERIALS AND METHODS

The technique and instrument used to present a comprehensive overview of the potential of pumpkin seeds, candlenut, and nutmeg is a synthesis matrix. The process is to integrate the results of the analysis of articles based on the similarities and differences of each article. Then make conclusions based on the identification and classification of the potential topic of pumpkin seeds, candlenut, and nutmeg (Ramdhani, et al., 2014). The synthesis matrix is a table/diagram that allows researchers to group and classify different arguments from several articles and combine different elements to get an impression / conclusion on the whole article in general (Murniati et al., 2018).

RESULTS AND DISCUSSIONS

The synthesis of research articles on the antimicrobial lipid potency of pumpkin seed, candlenut, and nutmeg shown in Tables 1, 2, and 3.

Table 1. Omega-3 and Omega-6 Potency of Pumpkin Seed

Descriptions/Issues of the Plant Seeds	References (Author, Year)
- seed oil showed that predominant unsaturated were linoleic (42%) and oleic (38%), - while the major saturated were palmitic (12,7%) and stearic (6%)	Esuoso et al., 1998
- oil showed the saturated fatty acids content was 27.73% and consisting of 16.41% palmitic acid and 11.14% stearic acid - unsaturated fatty acids value was 73.03% and consisting of 18.14% oleic acid and 52.69% linoleic acid	Alfawas, 2004
- Up to 60.8% of the pumpkin seed oil is from the fatty acids, oleic (up to 46.9%), linolenic (up to 40.5%) and palmitic and stearic (up to 17.4%)	Nakiae et al., 2006
- Excellent source of protein, minerals, vitamins and unsaturated fatty acids	Juranovic et al., 2003; Siegmund & Murkovic, 2004; Glew et al., 2006
- highly nutritional and rich nutraceutical components such as unsaturated fatty acids especially palmitic acid, stearic acid, oleic acid and linoleic acid	Stevenson et al., 2007
- The predominant fatty acids were stearic, palmitic, oleic acid and linoleic acid	Raharjo et al., 2011
- showed a high content of unsaturated fatty acids and the dominant fatty acids were palmitic, stearic, oleic, and linoleic acids - the seeds were well endowed in crude oil, protein, carbohydrates and crude fibre. - the oil contained unsaturated fatty acids and α- tocopherol	Karanja et al., 2013
- Those essential fatty acids are belonging to the ω-6 and ω-3 family which exert amazing nutritional functions and play important role in many metabolic pathways	Miura, 2013
- Polyunsaturated linoleic fatty acid was the predominant fat component in pumpkin seed oil. - In saturated fatty acids, palmitic and stearic acids predominated. - The antioxidant activity increased proportionally with the phenolic content	Kulaitienė et al., 2018

Pumpkin seed, candlenut and nutmeg have been used as an alternative feed ingredient in poultry production.

Pumpkin seeds proximate analysis revealed a higher crude protein, moisture and mineral content (Nworgu et al., 2007).

Hajati et al. (2011) indicated that supplementation of diets with 5 g pumpkin seed oil/kg dry matter in corn soybean meal-wheat based diet can be profitable because it reduced broiler chickens mortality and it did not have any adverse effect on the performance of birds.

Table 2. Omega-3 and Omega-6 Potency of Candlenut

Descriptions/Issues of the Plant Seeds	References (Author, Year)
- This plant possesses the antimicrobial activity against *S. typhi*, *Vibrio cholera*, and *E. coli*, lowering cholesterols and lipid absorption	Pedrosa et al., 2002
- anti-inflammatory and antipyretic	Niazi et al., 2010
- also as an analgesic	Quintao et al., 2011
- seed contain high proportions of polyunsaturated fatty acid (PUFA) such as ω-3, ω-6, and ω-9	Martin et al., 2010; Rohaida et al., 2014
- safe for internal uses, and its extract also has the potential to treat selected autoimmune inflammatory diseases by inhibiting the growth of the bacterial triggers	Mpala et al., 2017
- possess the antimicrobial activity against *S. typhi*, *Vibrio cholera*, and *E. coli*, lowering cholesterols and lipid absorption, anti-inflammatory, antipyretic, and an analgesic	Pedrosa et al., 2002; Niazi et al., 2010
- Based on spectroscopic analysis, these isolate of saponins may be predicted as triterpenoid saponins, diosgenin	Amalia et al., 2020

Table 3. Omega-3 and Omega-6 Potency of Nutmeg

Descriptions/Issues of the Plant Seeds	References (Author, Year)
- two types of oils extracted from the nutmeg seed, the essential oil and the fixed oil called the nutmeg butter	Forrest & Heacock, 1972
- As a plant seed, myristic acid is the main part of fat. Phenylalanine is the dominant amino acid in nutmeg	Pathak & Ojha, 1957; Maya et al., 2006
- the fatty acid composition of the triacylglycerol, the major lipid component, were myristic, palmitic, lauric, petroselinic, and stearic acids	Niyas et al., 2003
- Scientists reported that nutmeg have hypolipidemic and hypocholesterolemic effects, antimicrobial, antidepressant, aphrodisiac, memory-enhancing, antioxidant, and hepatoprotective properties	Jaiswal et al., 2009
- The major constituents fatty oil of Indian nutmeg were oleic acid, arachidic acid, palmitic acid - The major constituents fatty oil of Sri Lankan nutmeg were myristic acid and palmitic acid	Naher et al., 2013
- The major biological compounds in the methanol extract were 9,12-Octadecadienoic acid methyl ester, cyclododecyne, and octadecanoic acid, - the hexane extract constituents were margarinic acid, oleic acid, and 9,12-octadecadienlol.	Anaduaka et al., 2020
- Nutmeg has been reported to have antioxidant, anti-tumor, and antibacterial effects, and more	Olaleye et al., 2006; Acuña et al., 2016; Le et al., 2017; Zhang et al., 2016; Gupta et al., 2013
- Nutmeg extract posses antimicrobial antioxidant and anticancer activity. It support the fact that nutmeg extract can be used as future drug	Chakraborty et al., 2015
- Antioxidant activity of the mace essential oil was examined, β-carotene in linoleic acid and percent inhibition in linoleic acid (67.9 %) system.	Din et al., 2021
- Nutmeg seeds may improve blood lipids, ameliorate oxidative stress and this may be due to interactive or additive effects of the numerous bioactive constituents	Thomas and Krishnakumari, 2016

Pumpkin seed oil feed trials on broiler birds have also proved to lower bird mortality, as well as reduced cholesterol and triglyceride concentrations in blood plasma. Mart'ınez et al. (2010a, b) reported that 10% inclusion of pumpkin seed meal in broiler chicken diets served as a suitable substitute for soya bean meal as it enhanced the reduction in excessive abdominal fat, leading to increased production performance and improved organoleptic meat quality.

The inclusion of 0, 33, 66 and 100 g/kg of *Cucurbita moschata* in broiler diets, partially replacing soybean meal and vegetable oil, improved live performance and edible portions yield. In addition, abdominal fat and serum levels of harmful lipids were reduced, whereas serum levels of beneficial lipids increased. There was no effect on meat sensory quality (Aguilar et al., 2011).

Tabari et al. (2016) investigated that use of diet supplemented with pumpkin seed oil improved body weight and increased feed consumption in broiler chickens as a result of the positive effect of pumpkin seed oil on the intestine conditions leading to better digestion, absorption and utilization of nutrients and also due to the positive role of pumpkin seed oil on keeping a balanced microflora in the digestive tract.

That supplementary pumpkin seeds oils at a level 10 and 15 (g/kg) have a beneficial effect on productive trait, with no significantly effect of carcass characteristics, also pumpkin seeds oils in same levels reduced total plasma cholesterol concentrations and triglycerides in Japanese quail (Abbas et al., 2017). Pumpkin and flaxseed oils supplementation in feed mixtures of laying hens have a positive effect on the egg weight. Significantly higher average egg´s weight during experiment was found after dietary oils supplementation. Tendency of the highest egg´s weight was found after flaxseed oil supplementation (Herke et al., 2014).

Pumpkin seed extract is reported to be useful for immunomodulation, reproductive health, therapeutics over a wide range of disease conditions and stimulates metabolism of accumulated fats. Pumpkin seeds are a valuable source of protein and fat. Their bioactivity offers prospects for natural control of pathogenic/parasitic organisms, stimulate nutrition or enhance resistance to disease infections, and reduce abdominal fat and serum levels of harmful lipids, while increasing serum levels of beneficial lipids (Achilonu et al., 2018).

Broilers fed candlenut powder had significantly lower meat cholesterol content compared to basal diets. The use of candlenut powder as feed additive at the level of 1% is safely recommended to give better blood profile and reduce meat cholesterol content of broilers (Putri et al., 2018). Supplementing either treated or untreated candlenut meal at 2% level was shown to enhance the fatty acid profiles in broiler chickens meat (Rohaida et al., 2014). Supplementing 2.5% of various components of candlenut kernel in the diet did not improve growth performance, carcass yield, the chemical composition of broiler meat, and fatty acid composition of breast and thigh muscles of finishing broiler chickens (Rasid et al., 2019). The potential of the *A. mollucanas* nut as inhibitors of the growth of bacterial species associated with the onset of rheumatoid arthritis, ankylosing spondylitis and rheumatic heart disease (Mpala et al., 2017).

The *Myristica fragrans* seed meal supplementation at 0.25% enhanced the body weight gain, improved serum, and meat glutathione peroxidase and catalase, and reduced the broiler's meat cholesterol level and lipid oxidation (Adu et al., 2020). Supplementing either treated or untreated candlenut meal at 2% level was shown to enhance the fatty acid.

Supplementation 30% aqueous extract, 5% and 10% nutmeg increased weight and high profiles in broiler chickens meat (Rohaida et al., 2014).

That 30% aqueous extract, 5% and 10% supplemented nutmeg (*Myristical fragrance*) increased weight and high density lipoprotein (HDL) concentration and decreased blood glucose, low density lipoprotein (LDL), triglyceride and total cholesterol. Nutmeg diet exhibited significant anti-hyperglycemic in alloxan-induced diabetic rats (Oyindamola et al., 2017). The subchronic administration of 50, 100, and 200 mg/kg bw of nutmeg ethanolic extract did not cause the change of hematological parameters in rat (Bachri et al., 2017). *Myristica fragrans* ethanolic seed extract have hypolipidemic effect. *Myristica fragrans* ethanolic seed extract possess cardioprotective effect on experimentally

induced cardio toxic myocardial infarcted rats (Thomas & Krishnakumari, 2016)

The antioxidant and antiinflammatory activity possessed by nutmeg could be helpful in preventing or slowing the progress of various oxidative stress-related diseases and inflamematory diseases (Sethi & Dahiya, 2018). The subchronic administration of 50, 100, and 200 mg/kg bw of nutmeg ethanolic extract did not cause the change of hematological parameters in rat (Bachri et al., 2017). Seed of *M. fragrans* confirmed the anti-inflammatory properties and suggested that it may have deleterious effects on haemopoiesis at high doses (Bamidele et al., 2011).

As presented in this review, there is enormous potential for employing antimicrobial lipids to combat bacterial infections for animal health and human health. Over the past few decades, significant progress has been made towards understanding the relative potency and spectrum of antibacterial activity for different classes of antimicrobial lipids, in turn identifying particularly promising phytoadditive candidates through biological investigations.

CONCLUSIONS

The general conclusion of this literature study is that pumpkin seeds, candlenut, and nutmeg have bioactive constituents that promote health. By understanding how antimicrobial lipids function and the critical role of molecular self-assembly, need to begin to design new strategies to enhance therapeutic performance. Recognizing the challenges of antibiotic-resistant bacteria and taking advantage of the low cost and abundant supply of antimicrobial lipids, there is excellent opportunity to further explore antimicrobial lipids as next-generation antibacterial agents for animal health and human health. All of these findings bring us to the new idea in developing and innovating nutraceuticals, pharmaceuticals, and products from pumpkin seeds, candlenut, and nutmeg as phytoadditive for poultry.

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EVALUATION OF CORN FORAGE NUTRITION VALUE IN A COMPLETE FEED ON RABBIT PRODUCTION PERFORMANCE

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Abstract

This study aims to obtain the best ration using corn cane in complete feed for rabbit production performance in terms of feed consumption, weight gain and feed conversion. it used 30 male rabbits of the New Zealand White strain in the range of 435-1.037 g were used. The ration consists of a mixture of corn sugarcane and other feed ingredients, such as corn, coconut meal, pollard, bran, sugar drops, and minerals purchased from animal feed stores. The formulated feed ingredients have a complete feed without using grass. The treatments carried out were corn cane in complete feed in the form of pellets as follows: R0 = feed without corn cane, R1 = feed with 10% corn cane, R2 = feed with 20% corn cane, R3 = feed with 30% corn cane, and R4 = feed with 40% corn cane. Results showed that the highest feed consumption occurred in treatment R3, 72.81 g/head/day. The highest weight increment was observed in treatment R3, 23.24 g/head/day. However, treatment R3 gave the lowest food conversion. It indicates that this treatment is more efficient feed to use. This study also concluded that the use of 30% corn cane gave the best performance.

Key words*: conversion, consumption, corn cane, rabbit, weight gain.*

INTRODUCTION

The purpose of cultivating maize by breeders in North Sulawesi is dual function, namely obtaining corn kernels as food for pakok for rural communities or as a main source of food and forage corn as a source of animal feed. If the objective is prioritized for food sources and forage only as a by-product, it requires a high variable cost because the productivity of the seeds is highly dependent on fertilization. In this era, the people of North Sulawesi can hardly be found consuming corn kernels as a staple food source. Corn kernels are a very good source of carbohydrates for non-ruminant and ruminant livestock, therefore the selling value of these corn kernels will increase as a source of feed if converted into food sources (Tuturoong et al., 2020). Corn cliffs are all corn plants consisting of stalks, leaves, cobs and fruit which harvest at 45-65 days of age (Srichana et al., 2014). Because they are harvested at an early stage of growth, plant residues including stalks, leaves, husks and maize silk (corn silk) are a source of excellent quality, fresh and palatable feed for livestock. The breeder could take advantage by using plants as natural resource to improve its production (Rumokoy et al., 2014; Sumolang et al., 2020; Toar et al., 2020)

The minimum consumption of animal protein for Indonesian people by the Food and Agriculture Organization (FAO) is recommended as much as 6 gr/capita/ day but until now the protein consumption is still below the standard, namely 4.19 gr/capita/day. To achieve this target, the government is trying to improve and develop the livestock sub-sector, where one of the animal protein-producing commodities that is quite potential to be developed is rabbit livestock. The advantages of rabbits as a meat producer are good quality meat, namely 21% high protein content, 160 kcal calories, 8% fat, 70% water and 71 mg cholesterol (Winarno, 1992). Rabbits are suitable livestock to be kept in developing countries and are starting to use rabbits as a source of meat. In addition, rabbits also have potential, among others; small body size so that it does not take up much space; does not require a large investment in livestock and pen; short adult life which is about 4-5 months; high breeding ability and short fattening period, which is less than 2 months after weaning (Belabbas et al., 2019).

An innovative technological approach that enables effectiveness of growth and production,

namely through the application of complete forage-based feed using reinforcement feed (Rumokoy et al., 2021), through the complete feed formulation technique will be effective associative or complementary effects between the components of feed nutrients in forage grass with reinforcing feed, which in turn achieve the fulfillment of nutrition and rabbit cattle production.

Based on the description above, a study was conducted on the evaluation of the biological value of complete corn-sugarcane based feed on the performance of rabbit livestock production.

MATERIALS AND METHODS

Feeding trial used 30 male rabbits of New Zealand White strains with initial lived weight range of 435-1037 g obtained from public farm, corn cane, and other feed materials, such as corn, coconut cake, pollard, bran, sugar drops, and other materials bought from animal's feed material shops. These feed materials were formulated to make complete feed without the use of green feed.

Animal's cages of 50 x 50 x 60 cm^3 were prepared and facilitated with feeding and drinking spots, balance, room thermometer, and other supporting equipment.

The study was experimental with Group Randomized Design based upon the initial body weight of the rabbit. Variance coefficient (VC) was 23.77% (VC>10%) meaning that the initial body weight of the rabbit was not homogenous, so that they were separated into 3 groups, a) rabbit group of low body weight, 435 g, 456 g, 471 g, 480 g, 497 g, 535 g, 556 g, 574 g, 584 g, and 590 g (VC = 9.07%), b) rabbit group of medium body weight, 612 g, 620 g, 694 g, 708 g, 712 g, 721 g, 736 g, 747 g, 781 g, and 780 g (VC= 8.13%), and c) rabbit group of high initial body weight, 787 g, 814 g, 818 g, 830 g, 860 g, 878 g, 888 g, 960 g, 1016 g, and 1037 g (VC= 9.82%).

The treatment feed was separated into 5 levels of concentrations with 3 replications, so that there were 15 experimental units, and each experimental unit had 2 individuals, so that there were 30 individuals of rabbits used. The concentration levels of rumen content and sludge mixture fermented with *Cellulomonas* sp in the complete feed made in pellet form were set as follows:

R_0 = Feed without corn cane;
R_1 = Feed with 10% corn cane;
R_2 = Feed with 20% corn cane;
R_3 = Feed with 30% corn cane;
R_4 = Feed with 40% corn cane.

Table 1. Feed material nutritive content (in dry matter)

Ingredients	Nutrient Content						
	Dry matter (%)	Crude protein (%)	Crude fiber (%)	Ether Extract (%)	Calcium (%)	Phosporus (%)	Metabolizable energy (kcal/kg)
Corn cane	89.04	12.45	25.12	1.30	0.27	0.19	2,352
Pollard	88.54	16.50	14.90	4.00	0.14	0.32	1,300
Yellow corn	88.76	8.60	2.70	3.90	0.02	0.10	3,400
Coconut cake	87.92	18.50	15.00	2.50	0.20	0.57	2,200
Bran	87.82	11.56	13.36	7.00	0.04	0.16	2,860
Concentrate	88.78	38.00	11.14	5.90	1.40	1.20	2,600
Molases	87.50	3.00	0	0.10	0.90	0.10	1,960

Table 2. Composition and nutrient content of treatment feed

Materials	Treatment				
	R_0	R_1	R_2	R_3	R_4
Corn cane (%)	0	10.00	20.00	30.00	40.00
Pollard (%)	26.00	27.00	26.00	26.00	20.00
Corn (%)	22.00	19.00	16.00	10.00	8.00
Coconut cake (%)	15.00	8.00	7.00	6.00	5.00
Bran (%)	18.00	17.00	12.00	10.00	9.00
Concentrate (%)	17.00	17.00	17.00	16.00	16.00
Molases (%)	2.00	2.00	2.00	2.00	2.00
Total (%)	100	100	100	100	100
Dry Matter (%)	88.39	88.18	87.97	87.72	87.48

Materials	Treatment				
	R_0	R_1	R_2	R_3	R_4
Crude Protein (%)	17.56	17.30	17.36	17.29	17.07
Crude Fibre (%)	11.02	12.51	14.08	16.00	17.38
Ether Extract (%)	4.54	4.35	3.95	3.63	3.35
Calcium (%)	0.33	0.35	0.37	0.38	0.39
Phosporus (%)	0.43	0.40	0.40	0.39	0.38
Metabolizable Energy (kcal/kg)	2,412	2,447	2,473	2,470	2,579

Notes: Processed feed nutrient content.

The rabbits were divided into 3 groups of body weight, light body weight group (K1), medium weight group (K2), and heavy body weight group (K3), each of which was given treatment R_0, R_1, R_2, R_3, and R_4. The placement of each animal group and treatment in each group was randomly done using random numbers.
The *in vivo* research activities were divided into 3 phases as follows:

Adaptation phase.

- Male rabbits of New Zealand White strain of 8-11 weeks old were selected, weighed, and separated into 3 groups as small, medium, and large initial weights.
- The selected rabbits were put into a 50 x 50 x 60 cm metabolic cage using battery system and the placement of the experimental units was randomly set using random number with one individual per experimental unit. After the rabbits had been caged, they were adapted to experimental feed for 10 days.
- The need of each feed material was calculated following the treatment feed formulation.

Preliminary feeding adaptation

All experimental rabbits were fed 3 times a day at 07.00 am, 12.00, 17.00 pm of local time. This preliminary study was intended to eliminate the previous feed effect and to determine the amount of feed given. This activity was done for 5-10 days.

Data collection

The amount of the treatment feed was prepared a day before feeding, while weighing the remaining feed was carried out in the next morning (at 06.30 - 07.30 am) and each unfed feed was placed in prepared buckets. The data were used to know the amount of feed consumed and the feed was stocked for 3-5 days feed treatment. Data collection was conducted for 40 days.

Measurements

Feed consumption (g/head)
Daily feed consumption = amount of feed given - the amount of uneaten feed.
Body weight increment (g/head).
Body weight increment = final lived body weight - initial body weight during the study.

Feed conversion
Feed conversion was obtained from ratio of feed amount consumed and body weight increment in weight and time unit.

$$\text{Feed Conversion} = \frac{\text{Amount of feed consumed}}{\text{Body weight increment}}$$

Statistical analyses

The study used Group Randomized Design in linear model as follows:

$$Y_{ij} = \mu + T_i + \beta_j + \epsilon_{ij}$$

where:
Y_{ij} = observed value of treatment *I,* group *j;*
μ = general median;
T_i = effect of treatment *I;*
B_j = effect of group *j;*
ϵ_{ij} = experimental error of treatment *i*, group *j;*
$_i$ = treatment;
$_j$ = group.

The observed values obtained by the model above were analyzed with ANOVA and then continued with Duncan's Multiple Range test if there was significant effect in order to know the difference between treatments.

RESULTS AND DISCUSSIONS

Table 3 demonstrates the outcomes of the use of corn cane in the complete feed on the rabbit livestock production performance.

Table 3. Mean feed consumption, weight increment, and food conversion

Variables	Treatments				
	R_0	R_1	R_2	R_3	R_4
Feed consumption (g/head/day)	66.64^{ab}	66.83^{ab}	71.34^{ab}	72.81^{b}	64.55^{a}
Weight increment (g/head/day)	16.65^{ab}	16.24^{ab}	17.24^{ab}	23.24^{b}	15.20^{a}
Conversion	4.00	4.11	4.13	3.13	4.24

Notes: different alphabets indicate highly significant difference (P<0.01)

Effect of corn cane in the complete feed on feed consumption.

The growth of rabbit, like other animals depends on feed consumption and utilization. Feed consumption is very important factor in rabbit livestock productivity determination (Fadare, 2015; Maidala et al., 2016; Moningkey et al., 2016). Results showed that the highest feed consumption occurred in treatment R_3, 72.81 g/head/day (Table 3). Nurhayati et al. (2006) found that one of the benefits of fermentation was to raise the feed quality and palatability. According to Khan et al. (2017), there are several internal or external factors affecting the consumption level of the livestock, environmental temperature, palatability, taste, physiological status, nutrient concentration, feed shape, and livestock body weight.

Effect of corn cane in the complete feed on daily weight increment.

Bhatt et al. (2017) stated that one of the factors influencing the body weight increment is feed consumption. High feed consumption and digestibility will yield higher weight increment. It could result from increasingly numerous nutrients absorbed by the livestock's body. This is supported by Pascual (2014) that the livestock body weight is directly proportional to the feed consumption level. It means that feed consumption will illustrate the nutrients taken by the livestock affecting the livestock's weight increment. According to Bhatt et al. (2017), there is close correlation between growth and food consumption. Increasingly higher feed consumption will increase protein consumption so that higher livestock growth could occur and will consequently raise the meat production. The nutrients taken by the rabbit are utilized to fulfil the major living needs and growth.

Effect of cern cane in the complete feed on food conversion.

Food conversion is the ratio of feed amount consumed by the livestock and body weight increment. Food conversion is inversely proportional to feed efficiency, in which the lower the food conversion is, the higher the feed efficiency, so that it will affect the production costs (Belabbas et al., 2019).

Based on Table 3, it is apparent that mean food conversion be 4.00 (R_0), 4.11 (R_1), 4.13 (R_2), 3.13 (R_3), and 4.24 (R_4).

Mean food conversion of the rabbit in the present study is higher than that of previous study (Habib et al., 2004), 2.62-3.46, with mean value of 3.00, that employs feed containing 16% PK, DE of 2,500 kcal/kg and 0.1% biovet. Rabbits are efficient converter of forage in to good quality animal protein compared to other livestock (Pasupathi et al., 2015).

Food conversion could also be influenced by several factors, such as gene, feed shape, temperature, environment, feed consumption, fresh weight, and sex. Moningkey et al. (2016) stated that food conversion is affected by consumption of dry matter, and daily weight increment.

CONCLUSIONS

The supply 30% mixture of corn cane in complete feed showed the best results related to the consumption, body weight gain and feed conversion of rabbit livestock ration.

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THE INFLUENCE OF THE DAIRY COWS FEEDING ON THE METHANE AND CARBON DIOXIDE EQUIVALENT EMISSIONS FROM MANURE

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Abstract

The research purpose to demonstrate that feed rations can be a tool that would help the husbandry sector reduce greenhouse gases. The researches were realized on the Moara Domneasca farm on a flock of 27 dairy cows at different stages of Montbeliarde's lactation between November 2019 and September 2020. The dairy cows have been divided into lots which have been given different breeding flocks. The rations in the 6 variants were administered during the summer and winter seasons. The dairy cows are kept in free stabulation in a shelter and management system of manure were considered paddock, solid system and lagoon/slurry. The methane emission from manure management was calculated using tier 2 from the IPCC 2006, specific farm data are available for GE, DE, VS, and for B_0 and MCF were used default values. The results shows that methane and carbon dioxide equivalent emissions are the highest in variants 6 and 4 and the lowest in variant 1 with green fodder. Experimental variant of ration no. 1 and no. 5, which contain a large amount of green mass, show that methane emissions from manure management decrease compared to ration variations in which animals receive more concentrates feed.

Key words*: carbon dioxide equivalent, emissions, manure, methane.*

INTRODUCTION

Methane is the most abundant organic chemical in the earth's atmosphere, and its abundance is increasing with time and has reached levels not seen in recent geological history. Methane is produced both naturally and anthropogenically. One of teh sources of anthropogenic methane is manure from domesticated animals (Steed & Hashimoto, 1994).

Biogenic emissions of methane (CH_4) and nitrous oxide (N_2O) from animal manure are stimulated by the degradation of volatile solids (*VS*) which serves as an energy source and a sink for atmospheric oxygen (Someret al., 2004). Reducing short-lived climate pollutants is key to limiting global warming to 2°C above preindustrial levels (Shindell et al., 2012).

Arndt et al. (2018) shows that methane is a short-lived climate pollutant with 3 times the global warming potential (84 vs. 28) in the short term (20 yr) than the long term (100 yr; IPCC, 2013).

Consequently, the contribution of CH_4 to anthropogenic greenhouse gas is greater in the short term than in the long term (28 vs. 11%; US EPA, 2017b).

MATERIALS AND METHODS

The paper purposes to demonstrate that feed rations can be a tool that would help the husbandry sector reduce greenhouse gases. The researches were realized on the Moara Domneasca farm on a flock of 27 dairy cows at different stages of Montbeliarde's lactation between November 2019 and September 2020. The dairy cows have been divided into lots which have been given different breeding flocks. Each variant respect the physiological and productive needs of the animals. The rations in the 6 variants were administered during the summer and winter seasons. The dairy cows are kept in free stabulation in a shelter and management system of manure were considered paddock (0.6 which means 7.2 months for these manure system), solid system (0.35 which means 4.2 months only inside) and lagoon/slurry (0.05, half of months until is mixed with other type of manure for degraded

into a manure composting platform). When the weather is favorable (more than 7 months per year), the animals are taken into the paddock.

The methane emission from manure management was calculated using *method* 2 from the IPCC 2006, specific farm data are available for GE, DE, VS. To estimate the methane emission according to method 2 (box 4 of the decision tree), we also using default values for B_0 and MCF.

For the calculation of methane emissions from animal waste management systems, ***equations 10.22., 10.23*** and ***10.24*** of the *IPCC Good Practice Guidance and Uncertainty Management in National Greenhouse Gas Inventories, 2006* were used.

$$CH_{4\ MANURE} = \sum_{(T)} \frac{(EF_{(T)} * N_{(T)})}{10^6} \text{ (Ec. 10.22)}$$

where:

$CH_{4Manure}$ = CH4 emissions from manure management, for a defined population, $GgCH_4$/year;

$EF_{(T)}$ = emission factor for the defined livestock population, kg CH_4 /head/year;

$N_{(T)}$= the number of head of livestock species/category T in the country;

T = species/category of livestock.

$$EF_{(T)} = \left(VS_{(T)} * 365\right) * \left[B_{O(T)} * 0.67 kg/m3 * \sum_{S,k} \frac{MCF_{S,k}}{100} * MS_{(T,S,k)}\right] \text{ (Ec. 10.23)}$$

where:

$EF_{(T)}$ = annual CH4 emission factor for livestock category T, kg CH_4 /animal/year;

$VS_{(T)}$ = daily volatile solid excreted for livestock category T, kg of dry matter/animal/day;

365 = basis for calculating annual VS production, days/year;

$B_{o(T)}$ = maximum methane producing capacity for manure produced by livestock category T, m^3CH_4/kg VS excreted;

0.67 = conversion factor of m^3 CH_4 to kilograms CH_4;

$MCF_{(S,k)}$= methane conversion factors for each manure management system S by climate region k, %;

$MS_{(T,S,k)}$ = fraction of livestock category T's manure handled using manure management system S in climate region k.

$$VS = \left[GE * \left(1 - \frac{DE\%}{100}\right) + (UE * GE)\right] * \left[\left(\frac{1-ASH}{18.45}\right)\right] \text{ (Ec. 10.24)}$$

where:

VS = volatile solid excretion per day on a dry-organic matter basis, kg VS/day;

GE = gross energy intake, MJ/day;

DE% = digestibility of the feed in percent (e.g. 60%);

(UE*GE) = urinary energy expressed as fraction of GE. Typically, 0.04 GE can be considered urinary energy excretion by most ruminants (reduce to 0.02 for ruminants fed with 85% or more grain in the diet or for swine).

ASH = the ash content of manure calculated as a fraction of the dry matter feed intake.

18.45 = conversion factor for dietary GE per kg of dry matter (MJ/kg). This value is relatively constant across a wide range of forage and grain-based feeds commonly consumed by livestock.

In the calculation of (UE*GE) fraction, urinary energy expressed as a fraction of the gross energy was made based on the equation developed by Hoffmann & Schiemann (1974):

Urine Kcal (% of GE) = $3.30 + 0.233\ x_1 + 0.016\ x_2 - 0.00002\ x_2^2 \pm 0.7$

X_1 = the GP (crude protein) content of the ration (% of dry matter);

X_2= body weight (in kg).

X_1 value is calculated based on an extremely simple algorithm consisting in reporting the crude protein amount to the amount of dry matter in the ration, expressing the result as a percentage. The amount of protein and dry matter in the ration are essential parameters in calculating its structure, and the values for each type of fodder can be taken from the tables with the chemical composition and nutritional value of various forage categories (Stoica, 1997).

Thereafter, the result is converted into MJ (1 kcal = 239 MJ) to enter in the equation10.24.

For other parameters necessary for calculating the methane emissions from manure management systems, i.e.B_0 and MCF, will use default values in order to meet the conditions for method 2 of IPCC 2006 implementation (p.10.52).

The equation for calculating the entheric CO_2 emission shall be (Users' guide for estimating carbon dioxide, methane, and nitrous oxide emissions from agriculture using the State inventory tool, 2019):
CO_2 (kg/an) = (emission of CH_4 x 25 GWP) / 1,000,000,000

RESULTS AND DISCUSSIONS

The calculation of the GE and DE parameters was carried out on the basis of the rations administered in the 6 experimental variants using the chemical composition of the specified feed (Stoica, 1997).
The formula for calculating GE is:

$$GE\ (kcal/kg) = 5.72 \cdot PB + 9.5 \cdot GB + 4.79 \cdot CelB + 4.17 \cdot SEN$$

where:
GE = gross energy intake (kcal/kg);
PB = crude protein (%);
GB = raw fat (%);
CelB = crude fiber/cellulose (%);
SEN= non-nitrogenous extractive substances (%).
The rations have been formulated earlier according to the animal feeding schedule and the values of crude protein, crude fat, crude cellulose and non-nitrogenous extractable substances (Table 1) have been obtained from analyzes carried out in its own laboratory, i.e. by calculation (SEN).
Digestible energy (DE) is used to express the nutritional value of feeding stuffs and rations, especially for grazing animals.
Mathematical equations have been used to establish it by calculation, as in the case of raw energy, but in this case the digestibility content of nutrients is taken into account, taking into account the digestibility factors specific to each feed and species, namely taurine (Dragotoiu et al., 2017), then multiplied by the energy equivalents for digestible energy, which are different by species. In the table 1 are detailed the ration given to the dairy cows during the experimental period, according with lactation phases (upward, plateau and down ward phase).
The values obtained for methane emissions from manure management and the equivalent CO_2 emissions are given in Table 2, Figures 1 and 2, respectively.
For calculating methane emissions from manure management, the default value of B_0 (0.24), i.e. default values for MCF (0.01 for paddock, 0.02 for solids storage and 0.25 for liquid/sludge fraction) was used.

Table 1.The ration given to the dairy cows during the experimental period

Lactation phase	Up phase				Plateau phase						Down phase	
Exp. variant	V1		V2		V3		V4		V5		V6	
Feed	Feed (Kg)	Feed (%)	Feed (Kg)	Feed (%)	Feed (Kg)	Feed (%)	Feed (Kg)	Feed (%)	Feed (Kg)	Feed (%)	Feed (Kg)	Feed (%)
Lucerne hay	0	0	3.00	7.16	6.00	15.13	5.80	15.55	4.70	7.86	7.00	19.80
Hay clover	4.50	7.03	0	0	0	0	0	0	0	0	0	0
Corn soiled	0	0	27.33	65.23	15.00	37.83	25.00	67.04	0	0	17.30	48.95
Fodder beet	0	0	0	0	7.00	17.65	0	0	0	0	0	0
Beer Brewery	0	0	3.00	7.16	5.00	12.62	0	0	0	0	5.00	14.14
Spring bowl	28.50	44.55	0	0	0	0	0	0	33	55.23	0	0
Clover	23.60	36.89	0	0	0	0	0	0	17	28.45	0	0
Maise grain	5.70	8.91	2.30	5.49	3.10	7.82	4.60	12.34	2.80	4.68	2.50	7.07
Barley grain	1.00	1.56	2.20	5.25	2.20	5.55	0	0	2.20	3.68	2.20	6.22
Wheat bran	0.60	0.94	1.50	3.57	0	0	0	0	0	0	0	0
Sunflower meal	0	0	2.50	5.97	1.30	3.28	1.80	4.83	0	0	1.30	3.68
$CaCO_3$	0.07	0.11	0.07	0.17	0.04	0.10	0.06	0.16	0.03	0.05	0.04	0.11
$CaHPO_4$	0.01	0.01	0	0	0.01	0.02	0.03	0.08	0.03	0.05	0.01	0.03
Total	63.98	100.00	41.90	100.00	39.65	100.00	37.29	100.00	59.76	100.00	35.35	100.00
Ration contribution												
DM (kg)	17.45		17.91		19.82		17.31		15.86		16.94	
NEM (Mj)	104		102		99		98		99		94	
DEIN (g)	1728		1751		1587		1607		1623		1645	
DEIE (g)	1645		1601		1541		1575		1522		1526	
Ca (g)	159.68		111		115.28		114.72		155.01		123.02	
P (g)	61.86		74.81		54.34		57.65		70.00		53.91	

Table 2. The emission of CH_4 and CO_2 equivalent from manure management

Exp. variant	Head no.	GE (MJ/day)	DE (MJ/day)	Ash (%)	VS	EF	CH_4 (kg/an)	Emission CO_2 x 10^{-12} (t/year)
1	10	370.68	269.17	8.01	5.06	7.58	75.78	1894.54
2	10	333.9	216.61	8.24	5.85	8.76	87.56	2188.95
3	12	314.81	205.16	8.52	5.47	8.19	98.23	2455.68
4	12	322.16	205.34	9.56	5.83	8.72	104.65	2616.22
5	10	329.51	218.68	8.35	5.53	8.27	82.74	2068.42
6	12	315.52	198.20	10.51	5.85	8.76	105.10	2627.41
							554.05	13851.23

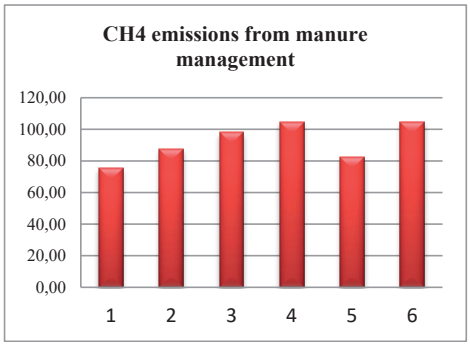

Figure 1. Methane emissions from manure management

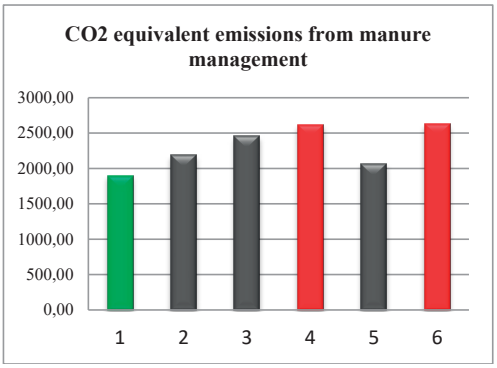

Figure 2. CO_2 equivalent from manure management

Methane and carbon dioxide equivalent emissions are the highest in variants 6 and 4 and the lowest in variant 1 with green fodder (75.78 kg CH_4/year, equivalent CO_2x10^{-12} 1.89 kg/year). Variant 2 with the highest amount of contaminated fodder used in the ration has determined an intermediate value, which is consistent with the research undertaken by several authors (Dhiman & Satter, 1997; Groff & Wu, 2005), which showed that the inclusion of contaminated maize in lactating cows' ration can improve animal production.

Experimental variant of ration no. 1 and no. 5, which contain a large amount of green mass, show that methane emissions from manure management decrease compared to ration variations in which animals receive more concentrates feed.

CONCLUSIONS

Together with the aspects of milk production, a number of measures are needed on the use of feed and feeding techniques that take into account the digestibility, quality and composition of the feed ration, which can reduce the methane generated during digestion.

The methane emission from enteric fermentation has the highest values for the variants 6 and 4, which contains rots (sunflower and soybean), maize, and wheat bran and the lowest emissions are recorded for the ration variant 1 which is rich in green fodder. In variant 2 with a reduced proportion of fibrous fodder a value of 1294 kg/year has been obtained. It is a middle value of CH_4 emissions from enteric fermentation.

Emission reductions can be made available to producers in the steer farming sector and the adoption of current best practices and technologies for the rearing and health of animals, feed rations can be a tool that would help the dragline sector reduce greenhouse gases.

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of greenhouse gases, expressed in tonnes of CO_2 equivalent".

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CONTRIBUTION OF NEW TROPICAL TREE LEGUMES SPECIES TO ENHANCE CARRYING CAPACITY OF KORONIVIA GRASS PASTURE IN COCONUT BASED FARMING SYSTEM

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Abstract

Indonesia is one of the larger producer copra in the world, so coconut still economic backbone of society. A tropical grass namely koronivia grass well adapted under mature coconuts, crude protein content lower than minimum requirement in the ruminant diet, carrying capacity able to provide only the needs of total digestible nutrient for cattle with bodyweight no exceed to 250 Kg. Needs inorganic nitrogen fertilizer application but costly and occurred negative impacts on the environment. Herbaceous legume integrated as mixed pasture failed to persist due to the aggressiveness of this koronivia grass. This research aims to study the ability of tree legume Indigofera zollingeriana as a source of protein and dry matter to enhance the carrying capacity. Koronivia grass solely could provide dry matter, crude protein and TDN for cattle with body weight 250 Kg, and I. zollingeriana as well, but the latter provides crude protein more, almost double than koronivia. Both species altogether in the same space and management could provide feeds to fulfil the requirement of cattle more than 250 kg body weight.

Key words*: capacity, coconut, enhance, I. zollingeriana, koronivia.*

INTRODUCTION

Indonesia is currently the largest copra producer in the world (FAO, 2009) therefore coconut commodity is still one of the economic backbone of society. Integrated land with industrial plantations include coconut that can be used for the development of forage crops (Anis et al., 2015) still a model farming systems applied in North Sulawesi eastern part of Indonesia. However, this kind of integration is faced with competition for nutrients, water, and sunlight.

Ruminant productivity in Indonesia is determined by forages availability throughout the year. Mostly of forage fed to animals is derived from local grasses species which is low quality contains only around 5% of crude protein (CP) lower than ideal protein content on ruminant diet. A kind of tropical grass namely *koronivia* well adapted under mature coconuts, persist under free-grazing as practice by the farmers, drought resistant and good performance as tropical pasture but crude protein content fluctuated, and in some case that content lower than minimum requirement in ruminant diet. Furthermore, carrying capacity pasture based on *koronivia* in coconut plantation able to provide only the needs of total digestible nutrient for cattle with bodyweight no exceed to 250 kg. Therefore, to increase crude protein content needs nitrogen fertilizer application but costly and in some circumstance occurred negative impacts on the environment. Herbaceous legume integrated as mixed pasture failed to persist due to the aggressiveness of this grass. On the other hand, there are some kinds of tree legumes available in Indonesia i.e., *Indigofera zollingeriana*, highly relished by the ruminant. This kind of tree legume alternate to replace *Leucaena leucocephala* which is susceptible to psyllid (Heteropsylla cubana) as kind of insect pests has been attack *Leucaena* all over the world. However, this *Indigofera* as single plant is widely studied in full sun environment, while growth and performance of this plant in shade conditions such as underneath mature coconut area has been reported yet, both in single plant or in mixed pasture with any tropical grasses.

Plant morphology can be seen and measured in several parameters including plant height, stem diameter, leaf number, branching, and root development. Morphological development is

observed both as a growth indicator and as a parameter used to measure environmental influences or treatments applied. Thus plant growth is an increase in size that can be known by the increase in length, stem diameter, plant-covered area, volume or biomass, wet and dry weight of plants (Abdullah, 2010). This type of legume contains relatively higher crude protein ranging from 22-29% compared to other tree legumes, and fiber content (NDF) is low between 22-46% (Abdullah & Suharlina, 2010), and has been used in improving diets of fattening goats (Anis et al., 2020).

The limited land for forage planting is a common problem in the development of ruminant animals (Sumolang et al., 2020; Rumokoy & Toar, 2014). Along with the increase of human population, participation in the availability of land for the development of extensive forage fodder is decreasing, because it is used for the development of food agriculture and other infrastructure. Therefore, there needs to be an effort to provide land for growing forage since the efficiency of land use in producing nutrition for animals becomes an important issue in a populated region. Evaluation of rows pacing is needed to find an appropriate row spacing for planting *Indigofera* over existing *koronivia* pasture to produce the highest forage yield and quality. There is needs to elaborate from animal nutrition aspect studied on dry matter intake, total digestible nutrients (TDN) and predicted carrying capacity.

MATERIALS AND METHODS

The study was conducted in the experimental station of Assessment Institute Agricultural Technology (AIAT) of North Sulawesi, located 12 km from Manado City. Experimental site receives an average rainfall of 2700 mm, and the distribution fairly even, except for the period of lower rainfall by 100-150 mm monthly, from July to September. The pH of the fertile sandy loam soil is around 6. Light transmission at 10.00 am on a sunny day as PAR underneath mature tall coconuts averaged 73%. This research consists of two experiments separately, from agronomic traits and animal nutrition aspect. *Indigofera* seeds sown on land that has been processed as a nursery. Seedling that has grown well are then moved into a 2.5 kg plastic bag that has already been filled with soil, one plant / plastic bag. After growing for 2 months in a plastic bag medium, the plant was then transferred to an experimental site. Treatments were different in plant spacing configurations of *I. zollingeriana* on pasture-based on *koronivia* grass. Three treatments of planting spacing (PS) namely PS1: 1.0 m x 0.5 m, PS2: 1.0 m x 1.0 m, and PS3: 1.0 m x 1.5 m corresponding to the number of plants of 21, 12 and 9 plants per plot respectively, placed randomly to the experimental sites in a plot size of 3x4 meter and distribute into 18 plots to accommodate those treatments, thereby 252 plants population has been used. Variable measured were forages yield and quality. Harvesting *I. zollingeriana* was done by cutting the plant sample one meter above ground level, then the leaves and stems are separated. Sample of *koronivia* was taken the plants in a square of 0.5 x 1.0 meter in the middle of each experimental plot. Five hundred grams of samples of both species were then dried in an oven at a temperature of 105^0C for 24 hours to get dry weight. For these morphological traits, data analysis used a Completely Randomized Design consisting of 3 treatments of planting distance and 6 replications. Data were then statistically analyzed by using analysis of variance (ANOVA) utilizing MINITAB (Version 16). Honestly, Significance Difference (HSD) was applied to determine the difference among treatments. Differences were considered at $P<0.05$. From animal nutrition aspect studied has been done on dry matter intake, total digestible nutrients (TDN) and predicted carrying capacity. Forages material used for biological value evaluations the only take from the treatment planting spacing PS3 which have higher forages yield production. This value obtained from the apparent digestible coefficient (ADC) of nutrients and finally total digestible nutrient (TDN). Total digestible collection methods have been used to determine the ADC of dry matter, crude protein, crude fiber, ether extract, and nitrogen-free extract. This trial has been done in two periods of time, where 7 days as preliminary periods for adaptation the animals to the new rations and to stay individual in metabolic cages. The second period of 5 days as feces and intake data collecting. Total feeds on-offered

and refused was measured each day during collecting periods, and drinking water for animal was available freely. At the present step of this research just only to study the contribution of *I. zollingeriana* to increase the carrying capacity of pasture-based on *koronivia* grass pasture.

Experimental site

The study was conducted in the experimental station of Assessment Institute Agricultural Technology (AIAT) of North Sulawesi, located 12 km from Manado City. Experimental site receives an average rainfall of 2700 mm, and the distribution fairly even, except for the period of lower rainfall by 100-150 mm monthly, from July to September 2018. The pH of the fertile, sandy loam soil is around 6. Light transmission at 10.00 a.m on a sunny day as PAR underneath mature tall coconuts averaged 73%. The soil color was dark brown clay. Precipitation peaks took place in January, with high rainfall intensity. This caused high relative humidity (80%). Air temperature ranged from 25^0C to 37^0C.

Experimental design

Experiment 1

Legume seeds *I. zollingeriana* were obtained from the Agrostology Laboratory of the Faculty of Animal Science, Bogor Agricultural University. *Indigofera* seeds sown on land that has been processed as a nursery. Plant seeds that have grown well are then moved into a 2.5 kg plastic bag that has already been filled with soil (one plant/plastic bag). After growing for 2 months in a plastic bag medium, the plant was then transferred to experimental site in a plot size of 3 m x 4 m that had been processed and divided into 18plots to accommodate the 3 treatments of planting spacing (PS) with row spacing 1 m apart and planting spacing varied from 0.5 to 1.5 m, namely PS1: 1.0 m x 0.5 m, PS2: 1.0 m x 1.0 m, and PS3: 1.0 m x 1.5 m, corresponding to the population densities of 21 plants/plot (1.75 plant/m^2), 12 plants/plot (1 plant/m^2), and 9 plants/plot (0.75 plant/m^2), corresponding 5714 plant/ha, 10.000 plant/ha and 13.333 plant/ha respectively. Each plot of, treatment had a size of 3 x 4 m (12 m^2) was then placed individually. Since the distance between plots of treatments were 1 meter apart, caused the space of land utilized of each plot enlarge up to 4 x 5 m (20 m^2) in each 10 x 10 m of square pattern planting of coconuts. Thereby the number of plots of treatments in each space of coconut of 100 m^2 were then 5 plots. The variables measured were: leaf dry weight (DW), wood DW, total DW (kg/ha), and leaf/wood ratio. Harvesting was done by cutting the plant canopy, then the leaves and stems are separated. Samples of 500 g were then dried in an oven at a temperature of 105^0C for 24 hours to get dry weight. This study used a Completely Randomized Design consisting of 3 treatments of planting spacing and 6 replications. Data were then statistically analyzed by using analysis of variance (ANOVA) utilizing MINITAB (Version 16). Honestly, Significance Difference (HSD) was applied to determine the difference among treatments. Differences were considered at $P<0.05$.

Experiment 2

From animal nutrition aspect studied has been done on dry matter intake, total digestible nutrients (TDN) and predicted carrying capacity. Forages material used for biological value evaluations the only take from the treatment planting spacing PS3 which have higher forages yield production. This value obtained from the apparent digestible coefficient (ADC) of nutrients and finally total digestible nutrient (TDN). Total digestible collection methods have been used to determine the ADC of dry matter, crude protein, crude fiber, ether extract, and nitrogen-free extract. This trial has been done in two periods of time, where 7 days as preliminary periods for adaptation the animals to the new rations and to stay individual in metabolic cages. The second period of 5 days as feces and intake data collecting. Total feeds on-offered and refused was measured each day during collecting periods, and drinking water for animal was available freely. At the present step of this research just only to study the contribution of *I. zollingeriana* to increase the carrying capacity potential of pasture-based on *koronivia*, thereby the feeding trial has been done separately between grass and legume leaves. Eight male goats with average body weight $\pm$ 15 kg has been used. Two treatments were arranged in 4 x 4 Latin Square Block design. Data were analyzed statistically with Analysis of Variance Test (ANOVA).

RESULTS AND DISCUSSIONS

Table 1 below presented data on the effects of treatments on biomass dry weight production based on population density or number of plants per hectare. Actually, this data come from our initial activity in this experiment with the treatments of population densities of 21 plants/plot (1.75 plant/m^2), 12 plants/plot (1 plant/m^2), and 9 plants/plot (0.75 plant/m^2). Those populations in hectare were 5,710 plants, 10,000 plants, and 13,333 plants, corresponding to PS1, PS2, and PS3, respectively. Leaf dry weight of treatment PS2 and PS3 were significant ($P<0.05$) higher than treatment PS1, but both treatments were not different significantly. The wider spacing of PS2 and PS3 showed plant height, stem diameter and number of branches were significantly superior compared to narrower spacing PS1. The increase in plant height in spacing (PS2) is probably due to the high rate of stem elongation. Stem elongation is related to the light competition among plants in narrow planting spacing (Widodo et al., 2016), followed by a taller plant compared to those in wider spacing (Crain & Dybzinki, 2013).

Table 1. Leaf (L), wood (W), L/W ratio and DW yield of *I. zollingeriana* under difference planting spacing in the coconuts plantation area

Items	Treatments groups Number plant (ha^{-1})		
	PS1 (5,710)	PS2 (10,000)	PS3 (13,333)
Leaf DW (kg/ha^{-1})	13.6[b]	16.59[a]	15.75[a]
Wood DW (kg/ha^{-1})	7.54[b]	9.31[a]	9.14[a]
Leaf/Wood ratio	1.81	1.78	1.72
Total.DW(kg/ha^{-1})	21.2[b]	24.1[a]	24.9[a]

[a, b]Means in the same row with different letters show differences($P<0.05$)

The increasing this plant height in PS2 treatment followed by increasing in stem diameter (1.18) and several branches (11.60). This founding is in agree with (Kumalasari et al., 2017) reported that narrower row spacing at 1.0 m x 0.5 m (PS1) reduces the number of branches. The greater spacing between adjacent plants within rows likely enhances the abilities of the plants to convert the intercepted solar radiation to leaf production (Telleng et al., 2016). Nevertheless, the leaf/wood ratio was not affected by all plant spacing treatments. It means that this plant could produce the same number of leaves at 12 weeks after planting for all treatments. This probably due to the age of tree legume plant at 12 weeks still in vegetative development stages which is leaves component grown dominantly (Anis et al., 2016). Plant parts that are preferred by livestock and have higher nutritional quality are leaf fractions (Kaligis et al., 2018) so that the ratio of leaves/stems becomes important. The highest dry weight production (24.1 kg/ha/harvest) resulted from the treatment of planting spacing 1.0 m x1.0 m (PS2) and 24.9 kg/ha/harvest at planting distance 1.0 m x 1.5 m (PS3), and both treatments were significantly higher ($P<0.05$) compared to treatment PS1 (21.2 kg/ha/harvest). This research has been done under shading environment in coconut plantations. Even though the number of plant populations increased per hectare but dry weight has not increased linearly. Total dry weight, as well as leaves and wood dry weight, increased up to the treatment PS2, and then almost reached plateau at PS3. This phenomenon is probably due to the light of the shortage in coconuts plantation.

Quality of Forage

Table 2 showed that the quality of both forages grown underneath coconuts plantations were varied markedly especially crude protein in *I. zolingeriana* 27.88% more than double compared to *koronivia*. Tully by 11.47%. Contrary nitrogen-free extract 45.85% higher than *I. zolingeriana* 25.39%, and slight differences in total digestible nutrients content. Pasture-based on *koronivia* under coconut plantation needs to enriched protein with tree legume, since integrated herbaceous or creeping legume not able to persist in mixed pasture due to aggressiveness of *koronivia* (Anis et al., 2015). Integrated of *Indigofera* in pasture underneath mature coconuts is the potential to enhance livestock productivity, but has to be precisely elucidated.

Table 2. Nutrient content of *I. zollingeriana* and *koronivia* grass

Plant Species	Nutrients content (%)					
	CP	CF	EE	NFE	Ash	TDN
Indigofera zolingeriana	27.88[a]	32.73	1.48	25.39[b]	12.51	66.41
Koronivia grass	11.47[b]	31.16	1.87	45.85[a]	9.65	62.41

[a,b] Means in the same columns with different letters show differences($P<0.05$)
Crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen-free extract (NFE), total digestible nutrient (TDN)

Average digestible coefficient of Forages

Table 3 below showed that differences in both forages feed attribute were CP intake of *I. zolingeriana* 163.89 g/head/day markedly superior than *koronivia* only 71.88 g/head/day. Both forages solely could provide only the needs of cattle with bodyweight around 250 kg. In mixed pasture would be provided nutrient requirement for more bodyweight.

Table 3. Feed intake and digestible coefficient

Variable	Components					
	DM	CP	CF	EE	NFE	TDN
I. zolingeriana.						
Intake (g/head/day)	607.64	163.89	194.24	9.10	151.75	66.41[a]
Apparent digestible coefficient (%)	58.14	87.47	75.88	87.69	63.22	
Total Digestible Nutrient /TDN (%)	-	24.25	24.87	1.30	15.99	
***Koronivia* grass**						
Intake (g/head/day)	599.23	71.88	185.69	8.86	152.08	
Apparent digestible coefficient (%)	56.87	67.26	78.18	76.30	63.36	62.41[b]
Total Digestible Nutrient / TDN (%)	-	7.71	24.36	1.42	28.88	

[a,b] Means in the same columns with different letters show differences($P<0.05$)
Dry matter (DM), Crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen-free extract (NFE), total digestible nutrient (TDN). Apparent digestible coefficient (ADC).

CONCLUSIONS

From animal feeds point of view *koronivia* solely could provide dry matter, crude protein and TDN for cattle with body weight around 250 kg, and *I. zollingeriana* as well. Moreover the latter has crude protein content almost double more than *koronivia*. Both species in mixed pastures be expected could provide feeds to fulfil the requirement of cattle more than 250 kg body weight.

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ACCUMULATION OF ITP-Hi AND GROWTH PERFORMANCE OF *HERMETIA ILLUCENS* PREPUPAE REARED IN TWO DIFFERENT MEDIA

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Abstract

This study aimed to observe the comparison of productivity of ITP-Hi in the fifth instar stage of the Hermetia illucens (BSF) prepupa reared in two different organic media: dry media with a composition of 200 grams of rice bran, 50 grams of coconut pulp and 50 grams of fish meal; the wet medium consisted of a mixture of 100 grams manure of cow farm and mixed with wet garbage fruits (300 grams). The media was placed in a cylindrical container with a dimension of 15 cm high and 8.5 cm of diameter. A total of 100 larvae aged 5 days old were placed in each media. The parameters of this study were growth performance and accumulation of ITP-Hi which consists of body weight, body length, body thickness, body width. The results showed that there was no significant difference (P>0.05) on body weight of H. illucens width of the two media. In addition, the growth performance of these insect larvae had a significant difference (P<0.05) higher from papaya fruit media compared to dry media. We concluded that papaya fruit is a good growth medium for BSF compared to media using coconut pulp and rice bran.

Key words*: antigens, Hermetia illucens, growth performace, rearing media*

INTRODUCTION

The purpose of this study was to determine the effect of different media on concentration of crude extract of antigen at larvae stage especially to its immungen thoraxial of prepuae of *Hermetia illucens* (ITP-Hi), beside that the effect of the media treatment on growth performace of *H. illucens* prepuae was also evaluated. Insects have immunogen antigens as reported by Rumokoy et al. (2017a) and Toar et al. (2019) underpinning the use of treatment material in this study. There are other hints that: insect salivary glands can be used for the immune system of mammalian organisms as related to the report of Breijo et al. (2018).

The antigens of several insect species as in Diptera order have been tested to stimulate the synthesis of IgG antibodies, which are natural substances that are used as alternatives in dealing with various pathogenic diseases, especially in newborn individuals (Toar et al., 2017). A study of Choi et al. (2012) used the extract of *Hermetia illucens* larvae as anti Gram-negative bacteria. A study conducted by Park et al. (2015) showed a novel peptide from *H. illucens* as an antibacterial. The positive effect of *H. illucens* larvae meal on broiler immunity has been reported Lee et al. (2018).

The high mortality rate in goats is caused by various factors as connected to the work of Ershaduzzaman et al. (2007), for example: genetic factors (Nguluma et al., 2013), environmental factors and the arrangement of the agricultural system (Phocas et al., 2016), where in a well-managed agricultural system zone will be able to provide opportunities for livestock (Oliveira et al., 2016). In addition, management of disease prevention and control is also a very determining factor in goat health (Goolsby et al., 2017). According to Fukuda et al. (2019), an unclean environment can be a source of pathogenic microbes that can be transmitted to livestock by transmitters such as flies.

The progress of several studies using the immunomodulatory antigen Diptera fly gives hope to improve the immune system in mammals as related to reported of Toar et al. (2019). Utilization of insects as a source of antigens can stimulate the production and circulation of antibodies in goat kids (Toar et al., 2017). Immunogens from the thoraxial extracts of Diptera (Muscidae) have been tested as immunogens as reported (Rumokoy et al.,

2020; Rumokoy et al., 2017b) have a potential benefit to overcome immunity and mortality problems in livestock such as local breed goats.

MATERIALS AND METHODS

Insects: *Hermetia illucens* were bred from the larva stage (maggot) in two different rearing media. The eggs come from adult flies that carry out an ovipositor in a special box.
Rearing: This study used a dimensioned rearing box (30 x 30 x 30 cm) to place the media containers. Larvae 5 days old were placed in two types of media in containers with dimensions of 15 cm in diameter and 8.5 cm in height. There are two different media, namely dry media (A) and wet media (B) where each container contains 100 larvae:
Media A as dry media was consisted by a mixture of 200 g of rice bran, 50 g of coconut dregs in the fermentation process and 50 g of fish meal and then were sprayed sufficiently with a clean water.
Media B as wet media, was used organic materials mixture: 100 g of cow manure and 300 g of wet mixture of garbage fruits like unconsumed of ripe papaya and waste ripe banana fruits.
The observation of the accumulation of ITP-Hi and growth performance was observed at the 4th instar. The prepupas were transferred to a transparent tube placed in a rearing box equipped with porous paper and moistened to maintain moisture. Before using all prepupae were washed with a clean water then placed in a box equipped with a filter then moved to a box equipped with a tissue for drying before measuring the growth performance. To measure the body weight: ten larvae were weighed in each weighing, and then continued with an extraction stage and freezing.
Immunogen crude extract preparation:
The *H. illucens* collection was carried out from each media unit and placed in a net bag, then moved in a 1 liter measuring glass then placed in a refrigerator at -4ºC for 10 minutes. The next stage was thoracic dissection using a three-dimensional photonic microscope.
Isolation of thoracic cavity by using a spatula and tweezers on a Petri dish by separating the exo-skeleton. The substance obtained was added with a 10% phosphate buffered saline (PBS) solution of 0.2 ml with a pH of 7.4 then crushed, filtered, centrifuged and separation substances. Then centrifuged at 5000 rpm for 3 minutes, followed by sediment accumulation and then a dilution and filtration using a 0.22 µm micro-filter to sterilize ITP-Hi against microbe-pathogenic substance and other materials. The level value of ITP-Hi substances obtained in this step of work were detected under a %Brix value by using portable spectrometry instrument.
The data was analyzed by using t-test of paired two samples for means.

RESULTS AND DISCUSSIONS.

The results as presented in the following Figures 1-5.

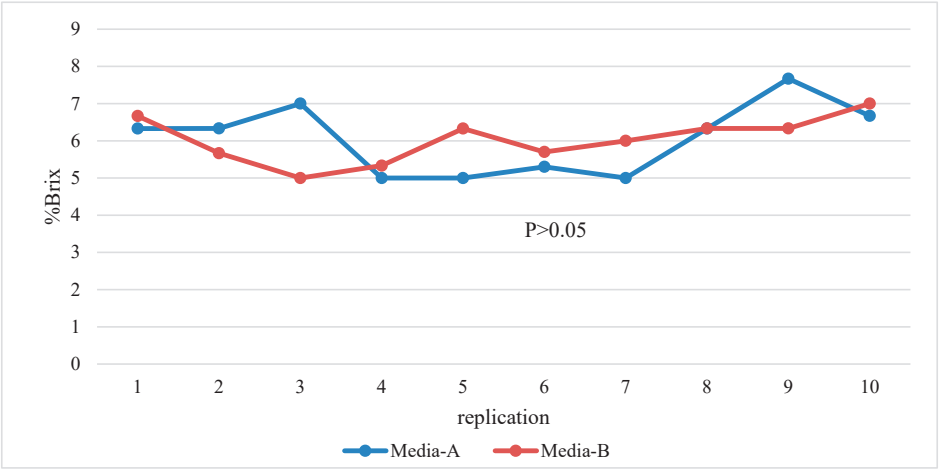

Figure 1. The ITP-Hi of *H. illucens* propotional level

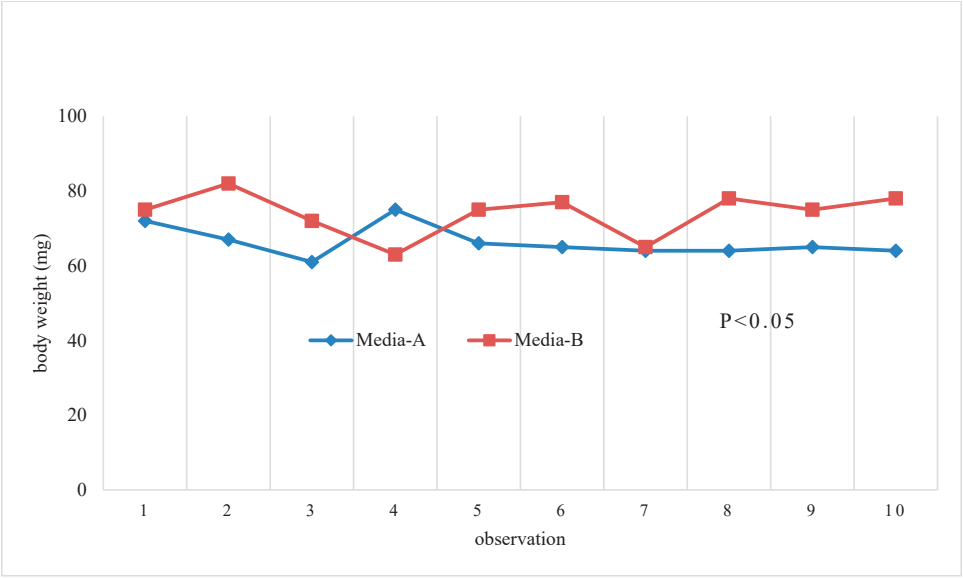

Figure 2. The weight of *H. illucens* in media-A and media-B

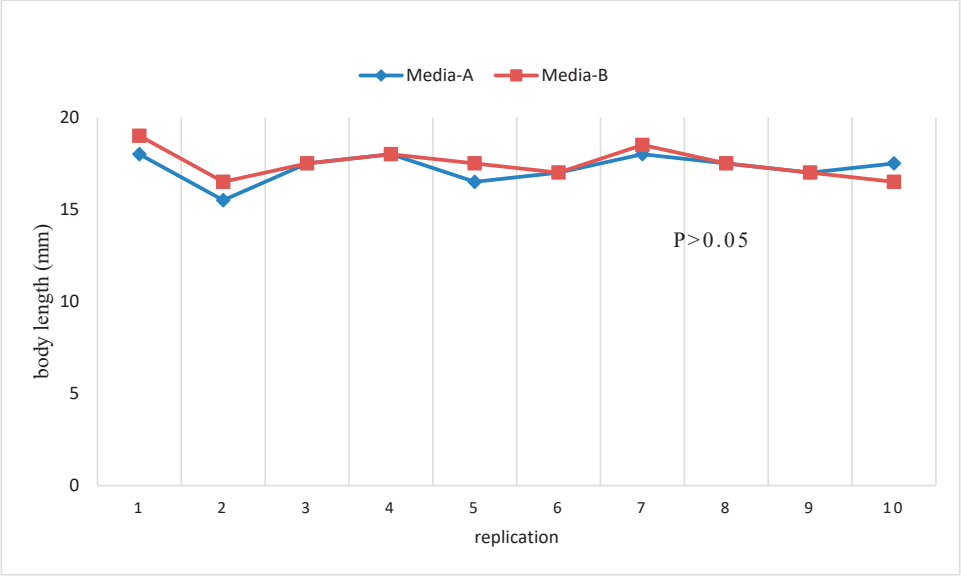

Figure 3. The body length of *H. illucens* in media-A and media-B

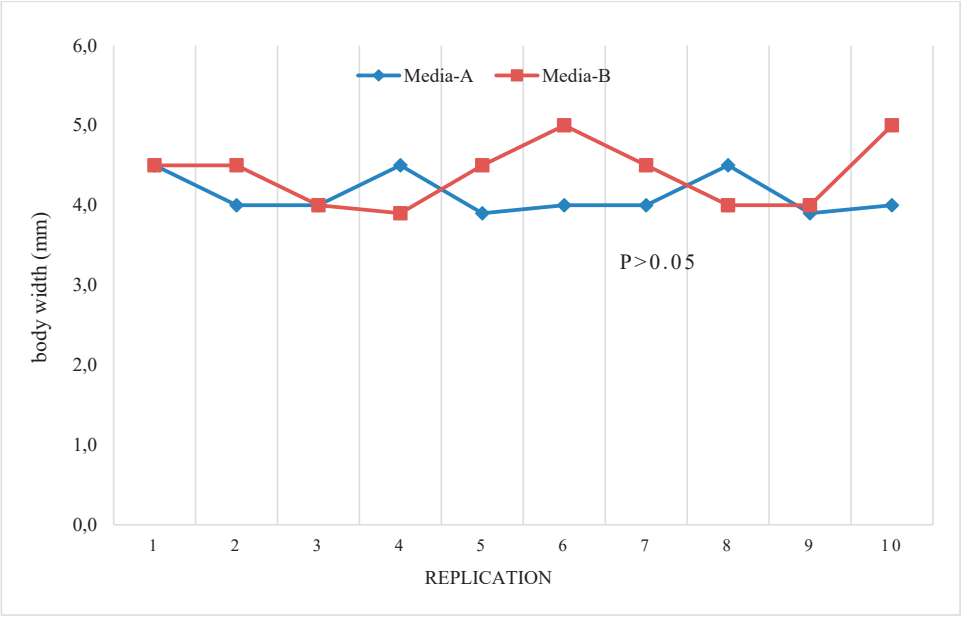

Figure 4. The body width of *H. illucens* in media-A and media-B

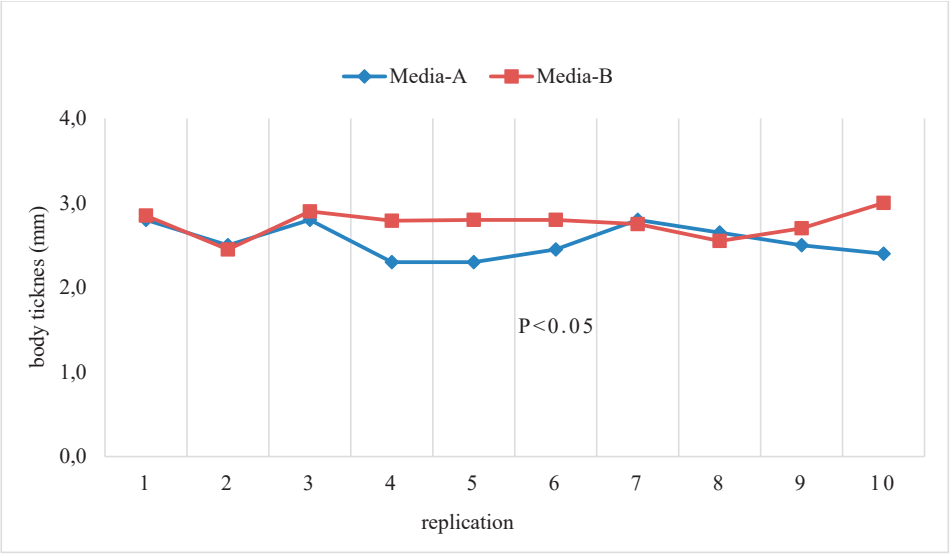

Figure 5. The body tickness of *H. illucens* in media-A and media-B

The treatment in media-A caused a non-significant effect on proportional level of ITP-Hi compared to the control media-B (P>0.05) as shown in Figure 1. The lowest level (%Brix) was found in prepupae both reared in media-A and media B which was at 3.9% Brix while the highest value was found in media-B. This non-significant difference could be caused by the ability of the larvae of *H. illucens* to profite the organic matter to converted to body protein mass of larva as connected to a scientific report of Kawasaki et al. (2020) and Klammsteiner et al. (2021). We considered this substance to be an immunogen for ruminant immunity as linked to the previous researcher reports (Lee et al., 2018; Rumokoy et al., 2017b) and also to

overcome the health problems of goat livestock in uncontrolled spread of pathogenic agent environment as described by Aldridge et al. (2018). The non-significant effect of media treatment showed in growth performances were in body length and body width. These results may due to genetic factor of this species: the situation of two types of media were negligible to body development especially to its body length and body width of larvae. Various organic material were suitable to *H. illucens* (Manangkot et al., 2014), when an attractant found in the media then they refused to use it (Toar et al., 2013). The larvae of *Hermetia* had a same adaptation to the experiment media as related to Khamis et al. (2020). The body weight of experiment larva in media-B was significant higher ($P<0.05$) then in media-A.

CONCLUSIONS

The difference of rearing media could be adapted by the larvae of *Hermetia illucens* as long as the conditions of media could be tolerated by the individuals for their ITP-Hi level (in %Brix) their body width and body length development. In this case the larvae were more able to profite media-B than media-A to improve their body weight and body tickness.

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PREDICTION OF NUTRIENT REQUIREMENTS AND COMPARISON OF ENERGY AND PROTEIN DIGESTIBILITY OF LOCAL AND IMPORTED FEEDS ON YOUNG RACEHORSES

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Abstract

The purpose of this study was to determine the standard needs of Indonesian racehorses for feed dry matter and protein, and also to determine the energy and protein digestibility of both local and imported feeds. 34 horses, body weight between 180-205 kg, 12-24 months of age were used in this study. The horses were fed daily, while feed intake and feces defecation were measured daily. The feed intake (DMI and PI) and metabolic body weight ($W^{0.75}$) (MW) data obtained were fitted to equation Y = a + bX, where Y = DMI and PI as the dependent variable (Y), and X = MW. The result shows that intake averages of DMI and PI were 5.75 kg and 494.66 gr, respectively, while MW was 51.42 kg. There was a positive correlation between DMI and MW of the horses, where r = 0,98 and R^2 = 95,86% ($p<0.01$). Likewise, between the PI and MW, where r = 0.96, and R^2 = 0.92. It is concluded that the standard requirements of dry matter and protein of Indonesian yearling racehorse can be predicted based on feed intake and metabolic body weights. Although the local feed digestibility is lower than imported feed, it is able to meet the energy and protein requirements of young racehorses.

Key words*: digestibility, feed intake, local and imported feeds, metabolic body weight, young racehorse.*

INTRODUCTION

The racehorse animals have their own uniqueness in the process of maintenance, because these animals are classified in pets. Maintenance management is different from other livestock, because the purpose of production is the achievement during the race.

Foals requires special attention in feeding because errors in feed handling will have an impact on growth and appearance while on the race.

The need for feed and nutrients for Indonesian young racehorse until now has not been standardized, so the feeding for the young racehorse by the breeders is only based on the custom which is the inheritance of the previous horse breeders. In the meantime, feed requirement standardization of Indonesia yearling racehorse (that is thoroughbred crosses with local horse) to date have not existed, so it still refers on thoroughbred racehorses feed requirement, which different of both posture and weight from Indonesia racehorses. Whereas, feed is a crucial factor in the growth process, as well as the fulfilment of nutrient feed needs is very decisive for the condition of the horse to follow the training program when prepared for the race. Another thing that needs to be studied is that until now the management of horse feeding in Indonesia, in general, still relies heavily on imported feed, both for maintenance, or in preparation for training for competitions, so an evaluation is needed to compare the effectiveness between the use of local feed and imported feed source through biological evaluation, namely digestibility tests. Based on this problem, this research was held with purpose to determine both feed dry matter and protein standard requirement of young Indonesia racehorse through a prediction model approach, and also testing the biological effectiveness of local feed and imported feed.

MATERIALS AND METHODS

This study consisted of two experiments, namely: the first experiment was conducted to predict the nutrient requirement of young racehorses; the second experiment was for biological evaluation between local feed and imported feed, through digestibility tests. This

research was conducted during six months in Tompaso district of Minahasa regency, which is the centre of racehorse farm in North Sulawesi.

Materials

34 horses, body weight between 180-205 kg, 12-24 months of age were used in this study, where 17 of them were selected for use in the first experiment, while for the second experiment all of these horses were used.

The diet applied in this first experiment was commonly used by local racehorse farmers consisted of 40% local concentrate and 60% forages (local feed), while in the second experiment, this local feed was used as treatment diet 1 (F1), and the treatment diet 2 (F2) consisted of 40% imported concentrate and 60% forages (imported feed). The forages consist of: *corn forage, and field grass* and the concentrate ingredients i.e: *corn, rice bran, coconut cake, soybean, green beans, and unhulled rice*. The treatment diets composition given in this study is shown in Table 1.

Table 1. Composition of treatment diets

Diets	Ingredients	Proportion (%)	DM (%)	Prot (%)	GE (Kcal /kg)
Local Feed (F1)	Local Concentrate	40	89.87	9.43	3.66
	Forages	60	88.46	8.26	3.56
Imported Feed (F2)	Imported Concentrate	40	91.24	11.04	3.91
	Forages	60	88.46	8.26	3.56

Note: DM (dry matter), GE (Gross Energy)

Methods

First experiment. The approach model used for data analysis was a simple linear regression method (Steel & Torrie, 1991). In this case, the feed intake (DMI and PI) and metabolic body weight (W0.75) (MW) data obtained were fitted to equation:

$$Y = a + bX$$

where:

Y = feed intake (DMI and PI);

X = metabolic body weight (MW).

The regression analysis also tested the regression coefficients, which were intended to test the significance of the relationship between feed intake (Y variable) and the feed requirement based on MW (X variable) of young racehorse, or to test whether feed intake actually affected the horse feed requirement. The test was performed using t - test on the hypothesis of the research:

H0: $\beta = 0$ (there is no relationship between variables X and Y)

H1: $\beta \neq 0$ (there is a relationship between variables X and Y)

Second experiment. It was arranged in two treatment groups according to t-test (assuming unequal variance). The treatment diets consisted of F1 = 40% local concentrate + 60% forages and F2 = 40% imported concentrate + 60% forages The variables measured were crude protein and energy digestibility.

Procedure of the study

First experiment, the horses were fed ad libitum three times a day, and fresh water was available all times during the whole experiment. Feed intake were measured daily in order to obtain both of dry matter intake (DMI) and protein intake (PI) averages per head.

Second experiment, the horses were fed restricted, and fresh water was available all times during the experiment. Feed intake and feces defecated were measured daily in order to obtain the energy and protein digestibility of both experimental diets. Measurement of feed digestibility based on the formula:

$$Digestibility = \frac{Nutrient\ Intake - Nutrient\ in\ Feces}{Ntrient\ Intake}\ X\ 100\%$$

RESULTS

The first experiment

The metabolic weights and feed consumption of dry matter and horse protein intake for 6 months of study were presented in Table 2.

Regression analysis of metabolic body weight (X) and feed intake (Y) were performed in Table 3.

Table 2. Metabolic body weight, dry matter intake (kg) and protein intake (g)

	Body weight (BW) (kg)	Metabolic weight (MW) (kg)	Dry matter intake (DMI) (kg)	Protein intake (PI) (g)
Minimum	180	49.14	5.42	462.57
Maximum	205	54.18	6.11	528.00
Mean ± SEM	191.24 ± 1.90	51.42 ± 0.38	5.75 ± 0.01	494.66 ± 4.45

Table 3. Regression analysis of metabolic body weight and feed intake

Assay		Equation	Significance	Multiple R (r) (%)	R^2 (%)	RSD (g)
1.	DMI vs MW	Y = 127.31X - 791.08	P<0.01	97.90	95.86	43.27
2.	PI vs MW	Y = 11.19X - 80.52	P<0.01	96.12	92.39	5.23

Notes: r = Correlation; R^2= Coefficient of Determination; RSD = residual standard deviation

Metabolic weight versus dry matter intake

The regression analysis towards metabolic body weight and dry matter intake turned out that values of multiple R (r), R^2, and RSD were 97.90%, 95.86% and 43.27 g respectively, with equation Y=127.31X–791.08 that indicates estimated regression line (Figure 1). The regression coefficient test also shows t-stat value (18.64) > t - table (1.74), which means H0 is rejected, whereas H1 is accepted.

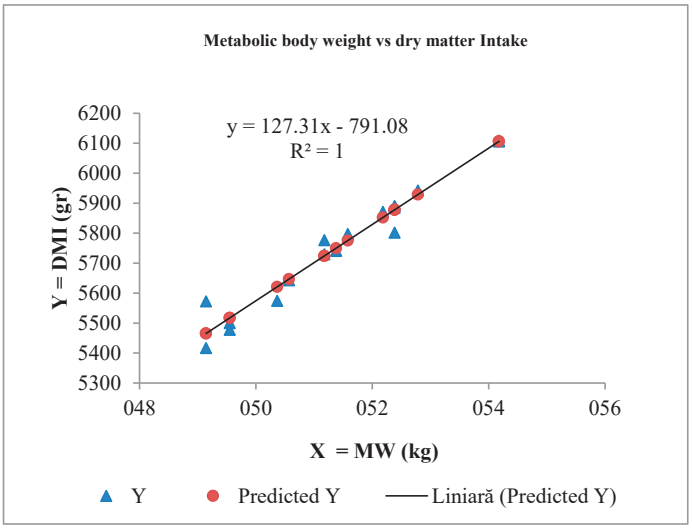

Figure 1. Estimated regression line of MW and DMI

With the value of multiple R (r) of 97.90%, the level of correlation between the metabolic weight and dry matter intake variables were very close. Meanwhile, from the value of coefficient of determination (R^2) shows that 95.86% of variation in DMI can be explained by the value of metabolic body weight of yearling racehorse. Furthermore, the RSD value explains that the value of the distribution around the Y variable was 43.27g, where the smaller the RSD value the smaller the real Y spreads the regression line.

Metabolic weight versus protein intake

The regression analysis on metabolic body weight and crude protein intake shows that values of multiple R (r), R^2, and RSD were 96.12%, 92.39% and 5.23 respectively, with equation Y=11.19X – 80.522 that indicates estimated regression line (Figure 2). The regression coefficient test also shows t-stat value (13.50) > t - table (1.74), which means H0 is rejected, whereas H1 is accepted.

With the value of multiple R (r) of 96.12%, the level of correlation between the metabolic

weight and the protein intakes variables were very close. Meanwhile, from the value of coefficient of determination (R2) shows that 92.39% of variation in intake protein can be explained by the value of metabolic body weight of yearling racehorse. Furthermore, the RSD value explains that the value of the distribution around the Y variable was 5.23g, where the smaller the RSD value the smaller the real Y spreads the regression line.

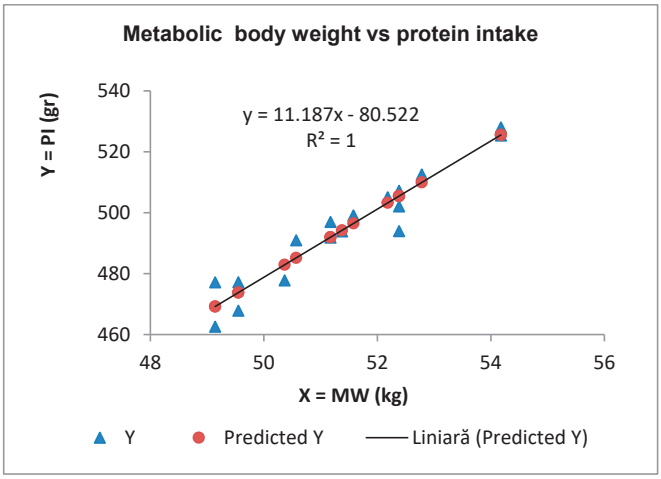

Figure 2. Estimated regression line of MW and PI

Second experiment

Energy and protein digestibility: Energy and protein digestibility data of the two experimental diets are presented in Table 4.

Table 4. Average energy and protein digestibility of the two experimental diets

Digestibility	Local diet (F1) (%)	Imported diet (F2) (%)
Energy	72.67[a]	84.74[b]
Protein	64.55[a]	75.20[b]

Table 4 shows, the average energy digestibility of local feed was 74.67%, while imported feed was 84.74%. The t test results showed that the energy digestibility of imported feeds was higher and significantly different ($P<0.05$) compared to the local feed. Likewise, protein digestibility, where the protein digestibility of imported feed reached 75.20%, was higher than that of local feed.

DISCUSSIONS

First experiment

Dry matter intake: The results showed that the average of dry matter intake of 1-2 years old horses in this study was 5.75 kg of tail^{-1} day^{-1} or 2 to 3% of body weight, in which comparison of forage and concentrate consumption were 30:70%, with the pattern of feeding 3 times a day, that is morning, afternoon and afternoon. Pilliner (1992), suggests that the amount of feeding of thoroughbred horse is 2 to 3% of body weight. Further, Anderson & McIlwraith (2004) suggests that the amount of dry matter intake of foal is 3% of body weight with a balanced nutrient content. This is similar to Ralston (2016), who reports that the average maximum daily dry matter intake is 2.5% -3% body weight (although some breeds and age groups, notably ponies and weanlings, can exceed those maximums if on good pastures). Thus, the feeding program conducted by horse farmer in North Sulawesi has similarities with feeding programs in some countries of the world that are already very advanced in race horse farming.

Figure 1 clearly shows that with increasing metabolic weights the consumption and requirement of dry matter also increases according to the equation Y = 127.31X-791.08.

Protein Consumption

The average of protein intake of 1 to 2 years horses was 494.66g of tail^{-1} day^{-1}. Slade et al.

(1970) suggests that the protein requirement for horse staple life varies from 0.49 to 0.68 g/kg weight/day. Based on the estimated needs of Ralston (2016), for growing horse and pony age between 1.5 and 2 years requires protein around 320-545 g. Meanwhile, Nutrition Requirement of Horses (2007) recommends the protein requirement for foals 750-860 g of tail^{-1} day^{-1}. The higher requirement according to the NRC recommendation is because the standard protein requirement for foals is the standard of thoroughbred horse needs, whereas this study uses crosbred horses between thoroughbred and local horses that have differences in weights. Johnson et al. (2009) who states that horses need more protein when the network is being set for growth (foal), that is in a rapid growth phase. This is in line with the opinion of Freeman et al. (1988) that for the feeding of high protein content for adult horses is unfavourable because it will lead to increased body weight that affects the decrease in performance when race, vice versa. Holand (2010), Measurement of crude protein content provides an assumption about the protein requirement of feed, but does not provide much information about the quality of the protein.

Second Experiment

Energy Digestibility

The difference in energy digestibility values may be due to differences in the dry matter and protein content of the feed, where imported feed has a higher content, which in turn affects the effectiveness of energy metabolism in young racehorses. The average energy digestibility value of local feed shows 72.67%, this means that the digestible energy of young racehorses using local feed reaches 15.07 Mcal per day, where this value actually exceeds the energy adequacy recommendation by NRC (2007), which is 12.20 Mcal. Gibs et al. (2009) stated that wheat and oat feed ingredients provide carbohydrates that can be used directly or stored in muscles and liver in the form of glycogen for later use. In terms of quantity and quality, imported feed is indeed superior to local feed. Tulung (2012) states that the biological value of local feed is lower than imported feed so that even though there is a high amount of protein consumption, only a few are ready for use because of their low biological value. Gibs et al. (2009) suggest that racehorses need a lot of energy to achieve and maintain optimal body condition when participating in training and competitions. According to Lawrence (2004), horses use 80-90 percent of feed for energy metabolism by utilizing carbohydrates and fats in feed. Furthermore, it is said that during routine training, race horses take advantage of the energy supply from fat in the body. Potter et al. (1990) said that while exercising, horses are able to get enough oxygen to the tissues to burn fat as an energy source while during the race, horses cannot rely entirely on fat but they get the main energy supply stored in blood glucose and liver and muscle glycogen produced from dietary carbohydrates. Oldham et al. (1990) argued that it is very important to note that racehorses receive sufficient available energy from carbohydrates in feed to maintain blood sugar levels and store energy in the form of muscle glycogen because this is the main source of energy (fuel) for horses.

Protein Digestibility

The results showed that the protein digestibility value of local feed was lower than imported feed. This is because imported feed has a relatively higher protein content. The protein digestibility value in this study is in line with the research of Mende et al. (2015), who also used local feed and imported feed on race horses in their study, where the protein digestibility results for local feed and imported feed were 62.37% and 72.30%, respectively. Meanwhile, Manarisip et al. (2017), in their study reported higher digestibility values of local feed protein and imported feed in their study on young racehorses, 75.87% and 86.89%, respectively. Tulung (2012) states that the biological value of local feed is lower than imported feed so that even though the amount of protein consumption is high, only a few are ready for use because of their low biological value. In general, horses in the growth phase need a higher percentage of protein than adult horses. Johnson et al. (2009) stated that horses need more protein when the tissue is being regulated for growth (foals), that is, in a rapid growth phase. This is in line with the opinion of Freeman et al. (1988) stated that feeding a high protein content for adult horses is not

beneficial because it will result in an increase in body weight which results in decreased performance when driven, and vice versa.

CONCLUSIONS

It is concluded that the standard requirements of dry matter and protein of Indonesian young racehorse can be predicted based on feed intake and metabolic body weights, so that these results can be used as the basis references for feeding standard of Indonesian racehorse. Although the energy and protein digestibility of local feed is lower than imported feed, it turns out that local feed is able to meet the energy and protein requirements of young racehorses.

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AGRO-BIOLOGICAL PECULIARITIES AND THE FORAGE QUALITY OF *ASTRAGALUS GALEGIFORMIS* L. UNDER THE CONDITIONS OF MOLDOVA

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Abstract

Efficient animal-based agriculture is sustained by the cultivation of legumes. Forage legumes have the unique attributes of producing high-quality forage to enhance animal performance and the ability to utilize symbiotic nitrogen. Perennial legumes of the genus Astragalus are promising subjects in the search of new species and forms for introduction and breeding. We investigated some agro-biological peculiarities and the forage quality of Astragalus galegiformis, cv. 'Vigor' grown in an experimental field of the National Botanical Garden (Institute), Chişinău. We found that the first cutting green mass contained 21.9% dry matter, but second and third cutting green mass – 31.9-34.2% dry matter. The dry matter of whole plant contained 231-234 g/kg CP, 111-126 g/kg ash, 247-248 g/kg ADF, 378-401 g/kg NDF, 31-38 g/kg ADL, 209-217 g/kg Cel, 131-153 g/kg HC, 188 g/kg TSS,83.2- 87.52% DMD, 75.3- 82.3% DOM and RFV = 161-171, 13.6 MJ/kg DE, *11.2* MJ/kg ME, *7.2* MJ/kg NEl. *The fermented fodder from Astragalus galegiformis 'Vigor', contained 227-194 g/kg CP, 320-335 g/kg CF, 127-143 g/kg ash, 334-343 g/kg ADF, 518-524 g/kg NDF, 39-43 g/kg ADL, 34-92 g/kg TSS, 295-300 g/kg Cel, 175-190 g/kg HC, with nutritive value: 70.6-74.0% DMD, 63.0-66.0% OMD, 12.26-12.39 MJ/kg DE, 10.07-10.17 MJ/kg ME and 6.09-6.40 MJ/kg NEl. The cultivar 'Vigor' of Astragalus galegiformis can be used as natural and fermented fodder for farm animals.*

Key words: *Astragalus galegiformis, biochemical composition, forage quality, cv. 'Vigor'*

INTRODUCTION

In recent years, the advances in plant and animal breeding, the introduction of new plant species and the development of new management approaches have made it possible to increase animal performance. Forage production in all of its ramifications is of major importance to agriculture. The use of high-quality forage crops will increase the efficiency of livestock programs, encourage the adoption of good soil fertility and pasture management techniques and hasten the movement away from practices inimical to the protection of soil resources and the environment. Efficient animal-based agriculture is sustained by legumes.

Perennial forage legumes are particularly valuable components of permanent and temporary grasslands, have the unique attributes of producing a high-quality forage and nutritional value to enhance animal performance and the ability to utilize atmospheric symbiotic nitrogen fixation, decrease the need for nitrogen fertilization and reduce forage costs. Nitrogen produced by legumes has the advantage of being available over a longer period, possesses a stimulating effect on crop yields and plays an important role in organic farming.

The genus *Astragalus* L. is one of the most important genera in the *Fabaceae* family, it is widely distributed throughout the temperate and arid regions of the world, but is particularly abundant in the temperate regions of North America, Europe and Asia. It has been estimated to contain about 3000 species as annual or perennial herbs, subshrubs, or shrubs. These invaluable plants are widely used as medicines, food, fodder, fuel and as ornamental plants in different ethnobotanical practices throughout the world. In pharmacological studies, *Astragalus* species showed anti-inflammatory, immunostimulant, antioxidative, anti-cancer, antidiabetic, cardioprotective, hepato-protective, and antiviral activities. (Li et al., 2014; Amiri et al., 2020). Recently, a lot of research and work has been done on the introduction and breeding of various species of the genus *Astragalus*. Among them, the most promising

forage species are: *Astragalus adsurgens* Pallas, *Astragalus asper* Jacq., *Astragalus brevidens* (Gand.) Rydb., *Astragalus cicer* L., *Astragalus davuricus* (Pall.) DC., *Astragalus dasyanthus* Pall., *Astragalus galegiformis* L., *Astragalus falcatus* Lam., *Astragalus inopinatus* Boriss, *Astragalus falcatus* Lam., *Astragalus membranaceus* (Fisch.) Bunge, *Astragalus onobrychis* L., *Astragalus ponticus* Pall., *Astragalus schelichowii* Turcz., *Astragalus sulcatus* L., *Astragalus uliginosus* L. (Ye et al., 1996; Ostapko & Shinkarenko, 2003; Boraeva & Bekuzarova, 2010; Yu et al., 2010; Asaadi, & Yazdi, 2011; Xu et al., 2011; Phelan et al., 2015; Cacan et al., 2017; Naseri et al., 2017; Kornievskaya & Silanteva, 2018; Rakhmetov et al., 2018; Bondarchuk, 2019; Lardner et al., 2019; Dmitriev, 2020).

Astragalus galegiformis L., syn.: *Astragalus galegifolius* L., *Tragacantha galegiformis* (L.) Kuntze. is a caulescent, herbaceous perennial, native to the Caucasus Mountains, occurs in Eastern Europe, it grows in subalpine meadows, deciduous forests, scrublands and on river banks. Plant develops light green or grayish erect stems, which usually grow about 150-200 cm tall. The compound leaves are grey-green, glabrous on the upper side and sparsely hairy on the lower side, 8-15 cm long, oblong-ovate, 12-25 mm long; the stipules are 3-10 mm long, linear-lanceolate. Peduncles 7-14 cm long. The flowers are shortly pedicellate, pendulous, in lax, cylindrical, 30-40-flowered racemes. The bracts are 6-8 mm long, linear. The calyx is about 5 mm long, campanulate, with sparse and short black hairs and with 2-3 mm long, subulate to triangular teeth. The corolla is pale yellow; the keel 14-16 mm long. It blooms in May-June, is cross pollinated, produces fruits in July. *Astragalus galegiformis* is an excellent source of nectar and pollen for honeybees, melliferous potential is 200-300 kg of honey per hectare. The pods are 12-15 mm long, plano-convex, laterally compressed, glabrous, long-stipitate, mucronate, containing 4-6 seeds. The seeds are kidney-shaped or elliptical, strongly compressed on the sides, seed scar rounded with pale surrounded by 3.7-3.9×2.5-2.7 mm. The surface is smooth, shiny, greenish-brown or brown. The weight of 1000 seeds are 9-13 g. It produces a strong tap root, reaching a depth of 150-200 cm in the soil.

The aim of this study was to determine some agro biological peculiarities and the forage quality of *Astragalus galegiformis* under the conditions of Republic of Moldova.

MATERIALS AND METHODS

The cultivar 'Vigor' of *Astragalus galegiformis,* created at the "Alexandru Ciubotaru" National Botanical Garden (Institute), registered in the Catalogue of Plant Varieties* and cultivated in monoculture in the experimental plot, Chişinău, latitude 46°58′25.7″N and longitude 28°52′57.8″E, served as subject of the research, and the traditional leguminous forage crop alfalfa, *Medicago sativa* was used as control.

The green mass, in the second year, was harvested manually, at 10 cm stubble height. The first cutting samples were collected in early flowering stage, May, 14, the second cutting – July, 27, the third cutting – October, 22. The green mass productivity was determined by weighing the yield obtained from a harvested area of 10 m^2. The leaves/stems ratio was determined by separating leaves from the stem, weighing them separately and establishing the ratios for these quantities; samples of 1.0 kg harvested plants were used. For chemical analyses, the samples were dried at 65±5°C. The dry matter content was detected by drying samples up to constant weight at 105°C. For ensiling, the harvested whole plants were shredded and compressed in well-sealed containers. The haylage was prepared from wilted green mass, shredded and compressed in well-sealed glass containers. After 45 days, the containers were opened, and the organoleptic assessment and the biochemical composition of the silage and haylage was determined in accordance with the Moldavian standard SM 108**. Some assessments of the main biochemical parameters: crude protein (CP), crude fibre (CF), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), digestible organic matter (DOM) have been determined by near infrared spectroscopy (NIRS) technique PERTEN DA 7200 of the Research and Development Institute for Grassland Braşov, Romania. The concentration of hemicellulose

(HC) and cellulose (Cel), the digestible energy (DE), the metabolizable energy (ME), the net energy for lactation (NEl) and the relative feed value (RFV) were calculated according to standard procedures.

RESULTS AND DISCUSSIONS

As a result of the performed research, it has been found that, in order to germinate abundantly, the seeds of *Astragalus* need to be scarified. In the first year of vegetation, the growth and development rates of *Astragalus galegiformis* were low in comparison with *Medicago sativa*, *Lotus corniculatus* and *Onobrychis viciifolia,* the shoots were prostrate, thin, and about 47-91 cm long at the end of the growing season. In the second year, the growth and development of *Astragalus galegiformis* plants began when the average temperature was above 0°C, in the end of March. It was established that the revival of *Astragalus galegiformis* 'Vigor' plants from dormant buds was uniform, characterized by faster grow and development rates. At the time when the first cutting green mass was harvested, the erect shoots reached 118 cm, the yield was 65.54 t/ha green mass or 10.58 t/ha dry matter, containing 53.5% leaves in the harvested mass (Table 1). After the first cut, under unfavourable weather conditions, the plants grew back slower, shoots developed from large buds, which were located just above the collar. *Astragalus galegiformis* plants were cut for the second time in late July, obtaining 15.97 t/ha green mass or 5.79 t/ha dry matter, the harvested mass was poor in leaves (31.7%). The lack of rainfall and the very high air temperatures during August to the first half of September affected the regeneration and development rate of *Astragalus galegiformis* 'Vigor' plants. The formed shoots were prostrate, thin and over 33-41 cm long, with moderate proportion of leaves (54.5%) and dry matter content (26.8%). The yield, at the third cut, decreased essentially in comparison with the previous cuts. Other researchers (Boraeva & Bekuzarova, 2010; Chibis et al., 2011) have also mentioned the slow regrowth of *Astragalus galegiformis* plants after mowing.

The annual productivity of *Astragalus galegiformis* 'Vigor', in the second growing season, reached 87.33 t/ha green mass or 17.93 t/ha dry matter.

As a result of a research conducted by Rakhmetov et al. (2018) in the Right Bank of Forest-Steppe of Ukraine, it has been found that the yield of *Astragalus galegiformis* was 126.6 t/ha green mass or 26.53 t/ha of dry mass; *Astragalus ponticus* - 98.7 t/ha green mass or 21.34 t/ha of dry matter; *Astragalus cicer* - 56.7 t/ha green mass or 11.21 t/ha of dry matter.

Table 1. Some agrobiological peculiarities of *Astragalus galegiformis* and the structure of the harvested mass depending on the harvest time

Harvest time	Plant height, cm	Stem, g		Leaf, g		Productivity, t/ha		Content of leaves in fodder, %
		green mass	dry matter	green mass	dry matter	green mass	dry matter	
First cut	118	105.0	14.4	87.5	16.6	65.54	10.58	53.5
Second cut	56	8.1	2.8	3.2	1.3	15.97	5.79	31.7
Third cut	36	4.2	1.0	4.0	1.2	5.82	1.56	54.5

Forage plants are valuable because they serve as food for wild and domesticated herbivores and sustain the production of meat, milk and other commodities. Forage plants contain different quantities of fibre, lignin, minerals and protein, and vary in the proportion of their tissue that can be digested by herbivores. These nutritive components are important determinants of consumer growth rates, reproductive success and behaviour (Lee, 2018). The productivity, the quality and the seasonal distribution of forage may be of great importance to the livestock farmers. The chemical composition, protein and carbohydrate fraction profiles, and predicted nutritional and energy values for *Astragalus galegiformis* green mass forages are shown in Table 2. Analysing the quality of the green mass of the cultivar 'Vigor' of *Astragalus galegiformis*, we found that the dry matter content and its chemical composition varied in comparison with alfalfa. Protein is a key

nutrient that must be considered both in terms of amount and type for various animal diets. It has been found that the cultivar 'Vigor' is characterized by high content of protein in dry matter (231-234 g/kg) and low content of crude fibre (224-233 g/kg) in comparison with *Medicago sativa.* The presence of minerals in animal nutrition is indispensable for their growth and health, because they are essential components of all tissues and organs that maintain osmotic pressure at a constant level, participate in the regulation of acid-base balance, activate a number of enzymes, moderate the neuromuscular activity and prevent the emergence and development of diseases in animals (McDonald et al., 2010). We could mention that the first cutting green mass of *Astragalus galegiformis* contains high amounts of minerals (126 g/kg), but the second cutting green mass - low amounts of minerals (111 g/kg), in comparison with traditional leguminous forage crops. The total amount of soluble sugars in *Astragalus galegiformis* forage reached 188 g/kg, which was twice more than in *Medicago sativa* forage.

Table 2. The biochemical composition and forage quality of the green mass

Indices	*Astragalus galegiformis*		*Medicago sativa*
	First cut	Second cut	First cut
Crude protein, g/kg DM	234	231	207
Crude fibre, g/kg DM	233	224	270
Ash, g/kg DM	126	111	121
Acid detergent fibre, g/kg DM	248	247	280
Neutral detergent fibre, g/kg DM	401	378	398
Acid detergent lignin, g/kg DM	31	38	42
Total soluble sugars, g/kg DM	188	-	92
Cellulose, g/kg DM	217	209	238
Hemicellulose, g/kg DM	153	131	118
Dry matter digestibility, %	87.5	83.2	73.9
Organic matter digestibility, %	82.3	75.3	66.4
Digestible energy, MJ/kg	13.58	13.61	13.15
Metabolizable energy, MJ/kg	11.15	11.17	10.79
Net energy for lactation, MJ/kg	7.17	7.18	6.81
Relative feed value RFV	161	171	157

Plant cell walls provide mechanical strength, maintain cell shape, control cell expansion, regulate transport, play important roles in plant responses to various abiotic stresses, such as drought, flooding, heat, cold, and salt and are essential for the storage of food reserves. Cell wall components such as NDF, ADF, cellulose, hemicellulose and lignin are very important limiting factors to the feeding processes and to the ability of the animal to utilize the consumed forage. The amount of neutral detergent fibre in the first cutting green mass of *Astragalus galegiformis* did not differ significantly from *Medicago sativa,* but it was substantially lower in the second cutting green mass of *Astragalus galegiformis*. It was found that the concentrations of cellulose and lignin, in the green mass of *Astragalus galegiformis*, were low and those of hemicellulose – high, which had a positive effect on dry and organic matter digestibility, relative feed value and energy content. Besides, the *Astragalus galegiformis* forage had 83.2-87.5% DMD, 75.3-82.3% OMD, RFV=161-178, 13.6 MJ/kg DE, 11.2 MJ/kg ME, 7.2 MJ/kg NEl, but the *Medicago sativa* forage - 73.9% DMD, 66.4% OMD, RFV = 157, 13.20 MJ/kg DE, 10.8 MJ/kg ME and 6.8 MJ/kg NEl, respectively.

Some authors mentioned various findings about the green mass quality of *Astragalus* species. Davis (1982) remarked that *Astragalus galegiformis* contained 34.7% dry matter with 15.5% crude protein, 15.1% crude fibre, 7.7 mg/g tannin and 0.34% oxalate. According to Ostapko & Shinkarenko (2003), the chemical composition of *Astragalus cicer* natural fodder was 18.1% crude protein, 4.1% crude fats, 26.4% crude cellulose, 9.9% sugars; *Astragalus falcatus* fodder contained 19.1% crude protein, 4.7% crude fats, 21.9% crude cellulose, 11.6% sugars; *Astragalus galegiformis* - 25.1% crude

protein, 3.2% crude fats, 26.2% crude cellulose, 8.6% sugars and *Astragalus onobrychis* - 17.5% crude protein, 2.9% crude fats, 21.1% crude cellulose, 11.8% sugars. Kshnikatkina et al. (2005) mentioned that the chemical composition of the dry matter of *Astragalus galegiformis* was: 17.39% crude protein, 1.76% crude fats, 25.56% crude cellulose, 4.56% ash, 1.2% calcium and 0.8% phosphorus. Boraeva & Bekuzarova (2010) remarked that *Astragalus galegiformis* contained 18.77% crude protein, 43.88% crude cellulose, 3.11% sugars, but *Galega orientalis* - 16.48% crude protein, 35.78% crude cellulose, 2.7 % sugars. Xu et al. (2011) mentioned that, a whole plant of *Astragalus adsurgens*, harvested in the budding stage, contained 290 g/kg dry matter with 14.4% crude protein, 45.5% NDF, 30.3% ADF, 8.1% ADL, 4.7% WSC. Asaadi &Yazdi (2011) found that under the climatic conditions of dry rangelands of Iran, the chemical composition and the energy value of *Astragalus brevidens* harvested in the flowering stage were 14.45% crude protein, 47.85% ADF, 50.22 % DMD and 6.54 MJ/kg metabolizable energy, respectively. Chibis et al. (2011) found that the concentrations of nutrients and energy in the first cutting green mass of *Astragalus galegiformis* were 17.2% crude protein, 2.7% crude fats, 25.7% crude cellulose, 8.8% minerals, 45.6% nitrogen free extract, 0.78 nutritive units/kg, 11.9 MJ/kg metabolizable energy and 144 g digestible protein per nutritive unit, but in the second cutting green mass – 20.89% crude protein, 2.9% crude fats, 22.4% crude cellulose, 8.7% minerals, 45.2% nitrogen free extract, 0.79 nutritive unit/kg, 11.3 MJ/kg metabolizable energy and 149 g digestible protein per nutritive unit. Amiri et al. (2012), remarked the forage quality of *Astragalus macropelmatus* growing in Zagros semi-arid rangeland centre in Iran: 13.12% protein, 3.33% fat, 8.54% ash, 47.27% NDF, 28.64% ADF, 65.57% DMD, 9.32 MJ/kg metabolizable energy and RFQ=131. Teleuţă et al. (2015) found that the natural fodder from *Astragalus galegiformis* contained 17.64% crude protein, 3.69% crude fats, 23.21% crude cellulose, 48.47 % nitrogen free extract, 7.00% ash, *Galega orientalis* -17.80% crude protein, 3.55% crude fats, 30.50.21% crude cellulose, 39.47 % nitrogen free extract, 8.69% ash and *Medicago sativa* -16.16% crude protein, 1.88% crude fats, 34.74% crude cellulose, 37.22% nitrogen free extract, 10.00% ash. Hou et al. (2017) mentioned that, under the conditions of meadow steppe in China, the chemical composition of *Astragalus melilotoides* harvested in full-bloom stage was as follows: 36.51% DM, 95.91% OM, 12.08% CP, 1.97% fats, 59.73% NDF, 48.95% ADF. Naseri et al. (2017) mentioned that *Astragalus fridae* green mass harvested in the flowering stage contained 7.8% protein, 38.96 crude fibre, 45.20% NDF, 26.98% ADF, 6.98% ash, 66.08% DMD, 9.23 MJ/kg metabolizable energy. Uskov et al. (2017) found that the concentrations of nutrients and energy in harvested green mass of *Astragalus galegiformis* were 46.1 g/kg crude protein, 32.5 g/kg digestible protein, 11.6 g/kg crude fats, 59.3 g/kg crude cellulose, 95.8 g/kg nitrogen free extract, 22.8 g/kg minerals and 2.34 MJ/kg metabolizable energy. Cacan et al. (2017) mentioned that the green mass of *Astragalus onobrychis* harvested in the flowering stage contained 20.07 % crude protein, 0.63 crude fats, 47.14% NDF, 18.3% ADF, 7.05% ash, 55.48% DMD, 8.01 MJ/kg metabolizable energy. Lardner et al. 2019 remarked the chemical composition of *Astragalus cicer* fresh forage, which contained 31.8-33.4% dry matter, 16.1-16.9% crude protein, 2.1-2.5% crude fats, 41.8-46.1% NDF, 34.0-37.5% ADF, 7.2-7.7% ADL, 7.8-8.0% ash, 1.14-1.27% calcium, 0.18-0.22% phosphorus, RFV=127-139, but *Medicago sativa* fresh forage – 32.4% dry matter, 14.1% crude protein, 2.4% crude fats, 54.2% NDF, 43.2% ADF, 8.3% ADL, 7.4% ash, 1.41% calcium, 0.18% phosphorus, RFV=93. Makarov (2017) revealed that the species *Astragalus uliginosus* contained 26.75-30.48% crude protein, 1.94-2.53% crude fats, 17.76-22.71% crude cellulose, 9.91-10.34% minerals, 1.90-1.93% calcium, 0.29-0.32% phosphorus, 34.88-41.95% nitrogen free extract, 10-15 mg/% carotene. *Astragalus sulcatus* contained 25.6% crude protein, 2.5% crude fats, 16.5% crude cellulose, 7.5% minerals, 1.2% calcium, 0.3% phosphorus, 47.0% nitrogen free extract. *Astragalus davuricus* contained 26.2% crude protein, 1.4% crude fats, 21.5% crude cellulose, 10.9% minerals, 1.3% calcium, 0.5% phosphorus, 40.2% nitrogen free extract.

Astragalus inopinatus contained 25.6% crude protein, 3.5% crude fats, 13.8% crude cellulose, 10.9% minerals, 2.2% calcium, 0.3% phosphorus, 45.5% nitrogen free extract. *Astragalus onobrychis* contained 25.5% crude protein, 2.3% crude fats, 20.6% crude cellulose, 7.8% minerals, 1.1% calcium, 0.3% phosphorus, 41.1% nitrogen free extract. Bondarchuk (2019) mentioned that, under the conditions of the Right-Bank Forest-Steppe of Ukraine, the biochemical composition of *Astragalus galegiformis* was: 20.91% crude protein, 455% crude fats, 4.69% ash, 34.84% crude cellulose, 45.6% nitrogen free extract, 0.65% calcium and 0.11% phosphorus.

It has been commonly accepted that one of the pre-requirements for the high productivity of farm animals in the developed regions of the temperate climate zone was the introduction and utilization of efficient methods of forage conservation. Farmers often prefer to feed the livestock with silage and haylage, because rainfall often hinders hay production. Under year-round uniform feeding, silage and haylage are the most effective types of the diet. Vegetables are good sources of quality protein for livestock. When forage legumes are adequately preserved, silage and haylage can help reduce subsequent feed costs and improve animal performance.

When opening the glass vessels with silage and haylage made from green mass of *Astragalus galegiformis*, obtained after the first cutting, a little gas was released, but there was no juice leakage from the preserved mass. The preserved forage materials had pleasing colour and aroma, the consistency was retained in comparison with the initial green mass, without mould and mucus. During the sensorial assessment, it was found that, in terms of colour, the silage had specific dark green leaves and greenish stems, with pleasant smell, specific to pickled vegetables, while the haylage contained homogeneous dark green leaves and brownish yellow stems with pleasant smell like pickled apples.

The fermentation quality of the silage and haylage from *Astragalus galegiformis* is demonstrated in Table 3. It has been determined that the amounts of organic acids reached 41.1- 55.3 g/kg and the pH was 4.31-4.78, most organic acids were in fixed form. The butyric acid was not detected in the fermented fodder. *Astragalus galegiformis* haylage was characterized by optimal pH values, high content of lactic acid and low content of acetic acid.

Table 3. The fermentation quality of the silage and haylage from *Astragalus galegiformis,* first cutting

Indices	silage	haylage
pH index	4.31	4.78
Total organic acids, g/kg	41.1	55.3
Free acetic acid, g/kg	5.3	2.6
Free butyric acid, g/kg	0	0
Free lactic acid, g/kg	5.3	11.5
Fixed acetic acid, g/kg	7.8	6.9
Fixed butyric acid, g/kg	0	0
Fixed lactic acid, g/kg	22.7	34.3
Total acetic acid, g/kg	13.1	9.5
Total butyric acid, g/kg	0	0
Total lactic acid, g/kg	28.0	45.8
Acetic acid, % total acids	31.87	17.18
Butyric acid, % total acids	0	0
Lactic acid, % total acids	68.13	82.82

The results of the biochemical studies (Table 4) indicate that the fermented fodder from *Astragalus galegiformis* 'Vigor' contained 227-194 g/kg CP, 320-335 g/kg CF, 127-143 g/kg ash, 334-343 g/kg ADF, 518-524 g/kg NDF, 39-43 g/kg ADL, 34-92 g/kg TSS, 295-300 g/kg Cel, 175-190 g/kg HC, with nutritive value: 70.6-74.0% DMD, 63.0-66.0% OMD, 12.26-12.39 MJ/kg DE, 10.07-10.17 MJ/kg ME and 6.09-6.40 MJ/kg NEl.

Table 4. The biochemical composition and the forage quality of the silage and haylage from *Astragalus galegiformis*

Indices	silage	haylage
Crude protein, g/kg DM	227	194
Crude fibre, g/kg DM	320	335
Ash, g/kg DM	143	127
Acid detergent fibre, g/kg DM	334	343
Neutral detergent fibre, g/kg DM	524	518
Acid detergent lignin, g/kg DM	39	43
Total soluble sugars, g/kg DM	34	92
Cellulose, g/kg DM	295	300
Hemicellulose, g/kg DM	190	175
Dry matter digestibility, %	74.0	70.6
Organic matter digestibility, %	66.0	63.0
Digestible energy, MJ/kg	12.39	12.26
Metabolizable energy, MJ/kg	10.17	10.07
Net energy for lactation, MJ/kg	6.40	6.09
Relative feed value	112	112

The concentrations of crude protein, ash and hemicellulose were high in *Astragalus*

galegiformis silage. In haylage, the content of total soluble sugars was significantly higher.

Several literature sources have described the quality of fermented fodder from *Astragalus* species. According to Ye et al. (1996) the Chinese milkvetch (*Astragalus sinicus*) silage had pH = 4.1 and contained 18.9% CP, 54.9% NDF, 38.0% ADF, 3.3% ADL. Yu et al. (2010) found that the dry matter content and the concentrations of nutrients in the silage prepared from fresh mass of *Astragalus cicer* were: 21.92-23.22% DM, 17.80-17.91% CP, 47.05-48.84% NDF, 37.79-39.98% ADF, 10.30-11.75% ash, 3.88-3.95% fats, pH = 4.17-5.48, 6.2-19.9 g/kg lactic acid, 1.1-11.0 g/kg acetic acid, 0-0.6 g/kg butyric acid, but in the silage prepared from wilted mass – 36.50-37.97% DM, 18.16-19.39% CP, 47.55-48.45% NDF, 39.40-40.38% ADF, 10.07-10.08% ash, 3.91- 4.11% fats, pH = 4.51-4.85, 8.2-17.6 g/kg lactic acid, 2.6-8.2 g/kg acetic acid, butyric acid was not detected. Xu et al. (2011) revealed that dry matter content and the biochemical composition of silage from erect milkvetch (*Astragalus adsurgens*) treated with distilled water was: 259 g/kg, pH = 5.48, 17.6 g/kg lactic acid, 10.8 g/kg acetic acid, 5.8 g/kg butyric acid, 142 g/kg CP, 467 g/kg NDF, 315 g/kg ADF, 86 g/kg ADL, 7.1 g/kg WSC, 530 g/kg DMD, but the silages treated with inoculant and enzymes – 260 g/kg, pH = 4.83, 34.5 g/kg lactic acid, 7.8 g/kg acetic acid, 1.3 g/kg butyric acid, 161 g/kg CP, 407 g/kg NDF, 272 g/kg ADF, 74 g/kg ADL, 7.1 g/kg WSC, 602 g/kg DMD. Uskov et al. (2017) remarked that the silage from *Astragalus galegiformis* green mass conserved with benzoic acid contained 23 % dry matter with pH 4.2, 8 g/kg lactic acid and 4 g/kg acetic acid, 41.6 g/kg crude protein, 12.7 g/kg crude fats, 56.3 g/kg crude cellulose, 97.5 g/kg nitrogen free extract and 2.34 MJ/kg metabolizable energy in silage.

CONCLUSIONS

The cultivar 'Vigor' of *Astragalus galegiformis*, in the second growing season, was characterised by high growth rate and moderately regenerative capacity after being mowed, making it possible to cut it three times per season, obtaining 87.33 t/ha green mass or 17.93 t/ha dry matter.

The dry matter of the harvested whole plant contained 231-234 g/kg CP, 111-126 g/kg ash, 247-248 g/kg ADF, 378-401 g/kg NDF, 31-38 g/kg ADL, 209-217 g/kg Cel, 131-153 g/kg HC, 188 g/kg TSS, 83.2- 87.52% DMD, 75.3-82.3 % DOM and RFV= 161-171, 13.6 MJ/kg DE, 11.2 MJ/kg ME, 7.2 MJ/kg NEl.

The fermented fodder from *Astragalus galegiformis* 'Vigor', contained 227-194 g/kg CP, 320-335 g/kg CF, 127-143 g/kg ash, 334-343 g/kg ADF, 518-524 g/kg NDF, 39-43 g/kg ADL, 34-92 g/kg TSS, 295-300 g/kg Cel, 175-190 g/kg HC, with nutritive value: 70.6-74.0% DMD, 63.0-66.0% OMD, 12.26-12.39 MJ/kg DE, 10.07-10.17 MJ/kg ME and 6.09-6.40 MJ/kg NEl.

The cultivar 'Vigor' of *Astragalus galegiformis* can be considered as a promising forage legume to restore degraded soils and create temporary grasslands, and the harvested biomass can be used as natural and fermented fodder for farm animals.

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OPTIMIZATION OF TECHNOLOGICAL PARAMETERS OF CRYOPRESERVATION OF BULL AND CARP SEMEN

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Abstract

Increasing the efficiency of reproduction of farm animals is possible through the use of progressive cryotechnology. Numerous studies have been devoted to this problem. However, a significant number of cells do not restore their functional activity after deconservation, which hinders a fuller use of the genetic potential of high-value breeding animals. Therefore, the purpose of the research was to determine the optimal technological parameters of cryopreservation. To achieve the intended goal, experiments were carried out to determine: the optimal rate of cooled semen up to 4°C, the temperature of the fluoroplastic plate, the duration of cooling of bull sperm after diluting it with mediums containing various cryoprotective agents. It has been established that when developing new mediums, it is necessary to use the optimal modes of cryopreservation of sperm of different types of farm animals. The results presented in this paper allow us to understand that cryotechnologies should be developed taking into account the features of the frozen object and the physicochemical properties of the components of protective mediums.

Key words*: cryoprotective mediums, cryoprotectants, cryotechnologies, gametes.*

INTRODUCTION

The cryobanks of reproductive cells and tissues benefits agriculture, livestock programs and biomedical research (Gunasena et al., 1997). Cryopreservation may even exclude the need to maintain a large number of breeding animals, since the genetic material is still available for future use. The cryobank of embryos, sperm, and oocytes is becoming very important both for reducing maintenance costs and for improving the dissemination of highly efficient farm animals (Agca et al., 2005; Oh et al., 1998).

The development of effective cryoprotective medium is one of the essential aspects of a successful solution to the issue of storing animal semen in a frozen state (Курбатов, 1988; Милованов, 1962; Наук, 1991; Шапиев, 1998; Balan, 2013; Watson, 1995). However, an equally important condition is the development of the optimal parameters of cooling, freezing and thawing, at which the protective effect of the components of the medium is maximally manifested (Борончук et al., 2003; Наук, 1991; Осташко, 1978; Шапиев, 1998; Balan, 2013; Roşca, 2000).

The procedure of sperm preservation differs among different species due to their inherent characteristics. There are noticeable species differences in the size and morphology of spermatozoa. In addition, there are more subtle differences in the composition of membrane phospholipids and sperm metabolism (Barbas et al., 2009). Despite the species differences, there are common stages in any sperm freezing procedure. All technologies include sperm collection and distribution, the addition of cryoprotective agents and cooling above 0°C, freezing below 0°C, storage and thawing (Curry et al., 1994). At all these stages, spermatozoa are exposed to a number of potentially destructive stresses, such as changes in temperature, osmotic and toxic stresses caused by exposure to high molar concentrations of cryoprotectants, as well as the formation and dissolution of ice crystals in the extracellular space (Watson, 2000). The success of cryopreservation depends on the resistance of spermatozoa to these influences (Salamon et al., 2000; Watson, 2000). Mediums, cryoprotectants, optimal cooling and thawing rates are essential for successful cryopreservation of sperm (Curry, 2007;

Hammerstedt et al., 1990; Mazur, 1984; Purdy, 2006). The composition of medium and the cooling rate have a significant effect on sperm viability, and there is a strong interaction between medium and the cooling rate (Woelders et al., 1997). If the cooling rate is lower or higher than optimal, it can cause irreversible damage of the spermatozoa (Fiser, et al., 1990; Koshimoto et al., 2000; Mazur, 1970). The optimal cooling rate should be low enough to allow water to leave the cells to avoid the formation of intracellular ice, and fast enough to avoid severe cell dehydration and cryo-damage (Mazur, 1970).

It is generally accepted that at least 50% of spermatozoa die during the freezing and thawing procedure (Watson, 2000), known as cold shock. Thus it may be necessary to eight times more cryopreserved sperm compared to fresh sperm to achieve *in vivo* fertilization (Shannon et al., 1995).

Since in previous studies (Борончук et al., 2003) a number of substances were identified that quite effectively stabilize the studied indicators of the functional-biochemical status of gametes in the process of conservation, naturally, the question arose about working out the optimal technological parameters for each of them.

MATERIALS AND METHODS

Special experimental studies were carried out at private and state breeding enterprises in compliance with the proper zoo-veterinary requirements. In the experiments used the semen of bulls of Black and White breed, as well as carp. Freezing of the experimental material was carried out in the form of open granules with a volume of 0.1-0.2 ml, in liquid nitrogen vapor. The temperature was measured using a thermocouple and mercury thermometers.

Statistical processing of digital material was performed using the Student's t-test.

RESULTS AND DISCUSSIONS

Essential in the cooling mode is the cooling rate in the critical temperature range, defined as the range in which the formation of ice crystals and subsequent dehydration of cells occurs. The cooling rate determines whether the cells remain in equilibrium with their extracellular environment or become increasingly super cooled with an increase in the possibility of intracellular ice formation (Kumar et al., 2003). The process of cell dehydration that accompanies slow freezing is potentially beneficial for cell survival, whereas fast freezing rates are thought to be more likely to cause cell death (Watson, 2000).

Freezing cells in medium induces ice formation in the extracellular space, creating an osmotic gradient across the plasma membrane between the initially isotonic intracellular solution and the frozen extracellular solution. Depending on whether the cooling rate is high or low, the intracellular water will either move through the cell membrane and join the extracellular ice phase, or freeze and form ice inside the cell, respectively. In most cases, cells undergoing the formation of intracellular ice become osmotically inactive or lysed due to the loss of the integrity of the cell membrane (Mazur, 1984). Likewise, cells that experience severe loss of intracellular water also become osmotically inactive due to prolonged exposure to high concentrations of solutes (Lovelock, 1953). Thus, too high or too low a cooling rate can be fatal to the cells. The optimal cooling rate exists between high and low speeds, which has been confirmed experimentally for a variety of celules. The cell survival curve, constructed as a function of the cooling rate, has a characteristic inverted U - shape (Mazur, 1972). Too low or too high a given cooling rate depends on the permeability of the cell membrane to water and on the likelihood that any water remaining in the cell at any given negative temperature will originate and turn into ice.

In the first series of experiments, the effect of the bull's semen cooling rate on the motility and longevity of thawed gametes was investigated. The cooling of the semen was carried out in the refrigerator at a rate of 0.52; 0.25 and 0.16°C/min to 4°C. As cryoprotectants were used: acrylamide, succinamide, glycerin, a mixture of acrylamide and polyacrylamide, a mixture of dextran, glycerol and globulin. The results are presented in Table 1.

Table 1. Influence of the rate of decrease in the temperature of bull semen from 30°C to 4°C on its quality when using various cryoprotectants

Cryoprotectants	Experimental variants	Gamete motility (points) after:			Longevity of gametes at 37°C (hour)
		Dilution	Cooling	Thawing	
		M ± m	M ± m	M ± m	M ± m
0,52°C/min					
Acrylamide	1	8.3 ± 0.13	7.7 ± 0.22	3.7 ± 0.37	4.0 ± 0.01
Succinamide	2	8.1 ± 0.21	7.6 ± 0.40	3.4 ± 0.21	4.0 ± 0.01
Acrylamide + polyacrylamide	3	8.1 ± 0.21	7.5 ± 0.35	3.3 ± 0.22	4.0 ± 0.01
Dextran + glycerol + globulin	4	8.2 ± 0.13	7.8 ± 0.13	4.0 ± 0.17	4.0 ± 0.17
Glycerol	5	8.1 ± 0.11	7.6 ± 0.07	3.8 ± 0.13	4.0 ± 0.01
0.25°C/min					
Acrylamide	1	8.3 ± 0.13	7.7 ± 0.40	4.0 ± 0.17	4.2 ± 0.22
Succinamide	2	8.1 ± 0.21	7.6 ± 0.40	3.4 ± 0.27	4.0 ± 0.01
Acrylamide + polyacrylamide	3	8.1 ± 0.21	7.3 ± 0.13	3.3 ± 0.13	4.0 ± 0.01
Dextran + glycerol + globulin	4	8.2 ± 0.13	7.6 ± 0.21	4.3 ± 0.13	5.4 ± 0.27*
Glycerol	5	8.1 ± 0.11	7.7 ± 0.13	4.2 ± 0.13	4.0 ± 0.01
0.16°C/min					
Acrylamide	1	8.3 ± 0.13	7.6 ± 0.2	3.7 ± 0.33	4.2 ± 0.22
Succinamide	2	8.1 ± 0.21	7.5 ± 0.17	3.2 ± 0.33	4.0 ± 0.01
Acrylamide + polyacrylamide	3	8.1 ± 0.21	7.6 ± 0.32	2.8 ± 0.22	4.0 ± 0.01
Dextran + glycerol + globulin	4	8.2 ± 0.12	7.7 ± 0.13	4.3 ± 0.13	5.2 ± 0.22*
Glycerol	5	8.1 ± 0.11	7.6 ± 0.11	4.2 ± 0.13	4.0 ± 0.01
Note: * The differences are statistically significant compared to the first cooling variant.					

It follows from the table that the change in the cooling rate within the limits of the speeds used by us does not significantly affect the motility and longevity of deconserved gametes (the exception is the 4th variant of the experiment).
A similar experiment was carried out on carp semen (Table 2). The cooling conditions are presented in the table.

Table 2. Influence of the cooling rate on the quality of deconserved carp semen

Experimental conditions	Temperature decrease rate, °C/min	Motility of thawed gametes, points
Cooling was carried out using pond water	0.56	4.7 ± 0.21
Cooling was carried out without using water	0.89	3.7 ± 0.18
Cooling was carried out using pond water and ice	1.07	4.2 ± 0.21

As can be seen from the table, cooling the carp semen at a rate of 0.56°C/min allows achieving the highest motility of thawed gametes. An increase of the cooling rate reduces its quality, which can be explained by the conditions of the experiment, under which the manifestation of a cold shock is possible (Ostachko et al., 2004).
Further studies were aimed at studying the effect of the surface temperature of the fluoroplast plate when freezing the sperm of breeding bulls in mediums containing various cryoprotectants on the motility and longevity of gametes after thawing (Figure 1). The data presented in figure 1 show that in the case of dilution of the semen with a medium containing acrylamide as a cryoprotectant, the protective properties of this substance are most fully manifested at a temperature of minus 100-120°C, while when using glycerin, the best results were obtained at a temperature of minus 120°C.
The next important issue that we have studied is the determination of the optimal duration of semen cooling at 4°C in the case of using various cryoprotectants (Figure 2). The physiological parameters of thawed semen were studied by cooling it for 0-7 hours with an interval of one hour. The figure shows the

optimal time for semen cooling in the case of using various cryoprotectants.

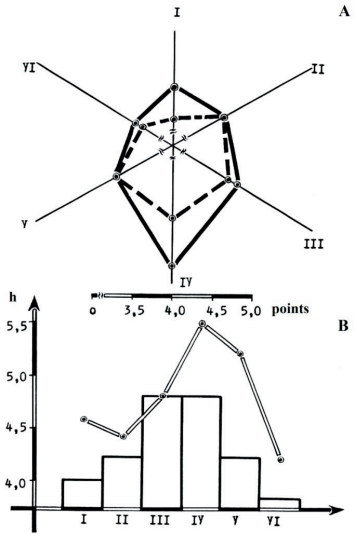

Figure 1. Influence of the surface temperature of the fluoroplastic plate on the quality of the bull semen:

A. Motility of gametes after frozen-thawing in a medium with: —— glycerin; - - - - acrylamide;
B. Longevity of gametes at 37°C, frozen in a medium with ═ glycerin, acrylamide;

I - 60°C; II - 80°C; III - 100°C; IV - 120°C; V - 140°C; VI - 160°C

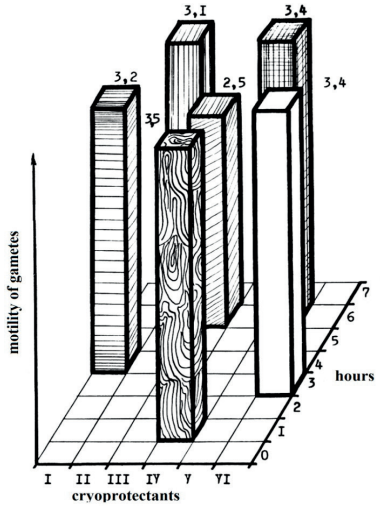

Figure 2. Duration of cooling of bull semen at 2-4ºC in case of using substances tested as cryoprotectants:

I - glycerin; II - polyacrylamide; III - polyethylene glycol 300; IV - acrylamide; V - acrylamide + polyethylene glycol 300; VI - acrylamide + polyacrylamide

The conducted studies have shown that the motility of thawed semen reaches its maximum value after 1 hour of cooling when using acrylic acid amide in the medium. In the case of using polyacrylamide and polyethylene glycol, the motility of gametes after thawing is higher when they are cooled for 7 and 6 hours, respectively. When using a cryoprotective mixture based on acrylamide and polyacrylamide, the best motility of the thawed semen was achieved when cooling lasted 3 hours, and when a cryoprotective mixture based on acrylamide and polyethylene glycol was used, the protective effect was most pronounced after 6 hours of cooling. The use of glycerin requires 3-4 hours of cooling.

In subsequent experiments was determined the optimal duration of exposure of bull and carp semen at 4°C, after reaching its specified temperature, in the case of using new cryoprotectants (Table 3).

Table 3. Optimal duration of exposure of bull and carp semen at 4°C in the case of freezing it with the use of various cryoprotectants

Cryoprotectant name	Exposure duration, min	Thawed semen indicators	
		Gamete motility, points	Longevity, hour
		M ± m	M ± m
Bull semen			
Acrylamide	0	4.1 ± 0.2	6.4 ± 0.76
Glycerol	60	4.5 ± 0.17	6.0 ± 0.61
Succinamide	40	3.8 ± 0.22	5.8 ± 0.65
Acrylamide + polyacrylamide	60	4.1 ± 0.21	6.2 ± 0.50
Dextran + glycerol + gamma globulin	25	4.7 ± 0.13	7.4 ± 0.80
Carp semen			
1,3-Butylene glycol	5	4.7 ± 0.21	-

It follows from the table that the optimal exposure for different cryoprotectants is not the same. Thus, the use of acrylamide as a cryoprotective agent in the composition of the medium for diluting and freezing bull semen makes it possible to exclude the period of its exposure at 4°C, while the use of glycerol, succinamide, mixtures of acrylamide with polyacrylamide and dextran, glycerol with globulin as a cryoprotectant requires holding the semen after cooling it to 4°C for 60, 40, 60

and 25 minutes, respectively. And when cryopreservation of carp semen under the protection of 1,3-butylene glycol, the exposure is only 5 minutes.

Thus, were determined the optimal rate of temperature reduction, semen exposure at 4°C, as well as the optimal duration of sperm cooling for the most promising cryoprotectants, as well as the optimal temperature of the fluoroplastic plate when freezing semen in the form of granules.

Summarizing the results of the studies presented in this article and previously published works (Борончук et al., 2003; Balan, 2013; Roşca, 2000), it can be concluded that the stabilization of the structural and functional parameters of gametes during cryopreservation of animal semen can be ensured by: maintaining the stability of bonds that determine intermolecular interactions by using in the composition of synthetic mediums of new cryoprotectants of both endo- and exocellular action; polar compounds capable of forming complexes of membrane components with elements of cryoprotective medium and membranotropes, maintaining the ionic potential and modifying biomembranes, as well as using specific parameters of technological methods of genome cryopreservation for each medium component and animal species.

CONCLUSIONS

Cryotechnologies should be developed taking into account the characteristics of the frozen object and the physicochemical properties of the components of protective mediums.

Cooling of frozen cells at 4°C is carried out for the purpose of penetration of protective agents into them, balancing the osmotic pressure in the cell-medium system, regulating biochemical processes aimed at preserving the functional state and implementing adaptive reactions.

The duration of exposure of the semen at 4°C depends on the physicochemical properties of the cryoprotectants used.

There is a corellative relationship between the duration of cooling of the semen at 4°C and the molecular weight of the tested substances.

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REPRODUCTIVE QUALITIES OF SOWS WITH DIFFERENT DURATION OF THE SERVICE PERIOD AND LACTATION

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Abstract

Reproduction is the main and most difficult element of the technological process of pork production. The most important signs of reproductive qualities are the live weight of piglets at birth, the safety of piglets, and the multiple pregnancy of sows. The studies were carried out in the conditions of a reproducer of an industrial pig-breeding complex, in which the influence of the duration of the service period and lactation of sows (suckling period) on their large-fruiting, fertility and safety of piglets was studied. The most significant increase in large-fruited was revealed depending on the duration of the service period when only one sow's heat was missed. In the group of sows weaned at 18, 21 and 24 days with a service period of 21-28 days, the large size was 0.054-0.197 kg more than when using sows with a service period of 1-7 days. Multiple pregnancies of the group of sows inseminated in the second heat, with a service period of 21-28 days, and a group of sows with a service period of 45 days or more, increased by 2.34 and 2.39 heads compared to the group of sows without missing heat (P <0.001).

Key words*: lactation period, large-fruited, multiple, safety of piglets, service-period.*

INTRODUCTION

In modern conditions of pork production for industrial purposes, one of the most important tasks is the intensification of pig breeding and an increase in meat production. One of the main ways to solve this problem is to increase the reproductive and productive qualities of the pig population through the full use of the production potential of the industry.

The basis for the flow technology of pork production is the reproduction of a herd of pigs therefore one of the main tasks of specialists in the pig industry is to improve the efficiency of using sows. To achieve these goals, the correct organization of herd reproduction is important, which ultimately determines the profitability of the entire industry (Narizhny, 2017).

The reproductive qualities that characterize the productivity of the brood of pigs are economically useful. These include multiple pregnancies, large-fruited, milk production of sows, the safety of piglets during the suckling period, the live weight of pigs at weaning. There are many factors that affect the reproduction quality of sows. Considering that reproductive qualities belong to low heritable indicators, the degree of manifestation of these qualities in the phenotype of sows is more influenced by different factors. Due to the rational use of environmental factors, it is possible to increase the rates of reproduction. Due to the low heritability of reproductive qualities, scientists have begun to develop more modern genetic methods that would quickly increase the economic profit of the pig industry (Kuiper et al., 1996).

In the conditions of industrial technology, a significant number of pigs do not show their potential capabilities (Mysik, 2016; Pokhodnya, 2002; Pokhodnya, 2015).

A possible condition for improving the reproductive qualities of the brood is a decrease in the number of stillborn piglets and an increase in their health status (Ponomarev, 2003). It is necessary to provide such conditions in which the sow would be able to maximize the possibilities of reproductive function (Bogdanovich et al., 2014).

A whole complex of factors affects the reproductive qualities of sows. Among the most important indicators of the reproductive qualities of sows, there are large-fruited, or live weight of piglets at birth, multiple births of sows, the safety of piglets, as well as the live weight of piglets at weaning.

According to some researchers, early weaning of piglets can reduce the loss of live weight of

sows during the suckling period carry out earlier insemination to obtain the next farrowing. It becomes possible to receive 2.5 farrowings and 25 piglets from each sow per year (Kabanov, 2007; Komlatsky, 2014). The live weight of piglets at birth is of great importance in the practice of pig breeding, since it is the initial value of the body weight, from which the growth of animals continues after their birth (Pokhodnya, 2013).

Piglets born with a live weight of more than 1.0 kg subsequently grow normally, develop and have a high safety. At the same time, piglets born with a live weight of less than 1 kg do not withstand competition for life in nests with larger animals, and 60-80% of them die in the first days of life, and the surviving ones lag far behind in growth, due to what are subject to rejection (Goldobina et al., 2015; Zatsarinin, 2015; Ivanova, 2008; Kuznetsov, 1996).

MATERIALS AND METHODS

The studies were carried out in the conditions of the reproducer of the pig-breeding complex of the Penza region in 2017-2020 with a capacity of 110 thousand heads of pigs. The experimental part of the work was organized in an industrial zone of a pig-breeding complex on hybrid pigs. In the reproducer of the pig-breeding complex for the production of pork, Topigs Norsvin hybrids were used.

Topigs Norsvin sows were represented by the TN70 line, obtained by crossing two breeds of large white (line Z) and Norsvin Landrace. Uterine pigs TN70 are characterized by good efficiency during the lactation period. The TN70 line combines high productivity and a positive effect on fattening indicators. TN70 has been in use on the farm since 2014. The pigs of this line combine high prolificacy, a large number of pigs grow at weaning, high viability and a positive heterotic effect in terms of fattening livestock indicators.

The aim of the study was to establish the influence of the duration of the service period and lactation in sows on their subsequent large-fruited, multiple births and the safety of suckling piglets. For the research, nine groups of hybrid sows were formed with different service periods and lactation periods (Table 1).

The weaning-to-insemination interval is one of the main indicators of the efficiency of the use of sows. This interval is defined as the time elapsing from weaning of piglets from a sow to her first, following this, fruitful insemination. Therefore, in order to have as few unproductive days as possible, there is always an interest in reducing this interval as much as possible.

It is known that this interval is associated not only with the unproductive period, but it is also capable of exerting a much greater influence on other indicators of reproduction than was previously thought.

The aim of the study was to establish the influence of the duration of the service period and lactation in sows on their subsequent large-fruited, multiple births and the safety of suckling piglets. The experiments were carried out in the conditions of the reproducer of the "Cherkizovo-Pig Breeding" complex in the Penza region. For the research, nine groups of hybrid sows were formed with different duration of the service period and lactation (Table 1). The livestock of sows was kept in a typical production facility with a high level of mechanization of production processes. The parameters of the keeping of pigs of different sex and age groups, the peculiarities of the microclimate of the pig-breeding premises corresponded to generally accepted standards. Sows were kept in individual pens with an area of 1.26-1.37 m^2.

Table 1. Scheme of the experiment

Group of sows	The duration of the suckling period (lactation of sows), days	Service period (days)
1st	Eighteen	1-7
2nd	Eighteen	21-28
3rd	Eighteen	45 and more
4th	21	1-7
5th	21	21-28
6th	21	45 and more
7th	24	1-7
8th	24	21-28
9th	24	45 and more

RESULTS AND DISCUSSIONS

Among all the reproductive qualities of sows, the large-fruited or live weight of piglets at birth is especially distinguished, which largely determines the growth rate of young animals in subsequent age periods. Piglets with low birth weights tend to have low live weights at weaning and are more likely to die and morbidity during the suckling period. Therefore, in order to prevent the birth rate of piglets with a low live weight, it is necessary to pay attention to the genetic aspects, conditions of keeping and feeding, the organization of mating, the state of health of sows and technological measures in the prenatal period (Bilkei, 1999; Trukhachev, 2008).

Diversity determines the number of young animals going to fattening and the total volume of meat produced. The safety of piglets ultimately determines how many young pigs will be fed and pork would be obtained.

Analysis of large off-sprigs of all groups of sows (Table 2) using weaning piglets for 18, 21 and 24 days showed that the average large off-springs were 1.408 kg. The lowest large-fruited yield of 1.355 kg was observed during insemination of sows in the first heat after weaning of piglets (service period 1-7 days). In the context of individual farrows, the lowest large-fruiting - 1.282 kg - was recorded in the first farrowing with a service period of 1-7 days. When a group of sows was inseminated in the second heat of the weaning field, a significant increase in large-fruited was observed, which was 1.480 kg, which is more than the indicator of a group of sows with a service period of 1-7 days by 0.106 kg ($P<0.001$). In the group of sows with a service period of 45 days or more, the fruit yield was 1.401 g, which is 46 g more than in the group of sows with a service period of 1-7 days.

Thus, the studies revealed the highest effect on large off-springs of skipping only one hunt after weaning piglets, the effect of missing two hunts was less pronounced. The most significant influence of the number of farrowing on large fertility was noted only in the second farrowing, and later on there was a gradual decrease in large fertility in the dynamics of farrowing.

Table 2. Large size of sow groups on average for all weaning dates of piglets, M ± m

Farrow	Sow service period						Average	
	1-7 days		21-28 days		45 days or more		n	$\overline{X} \pm m$
	n	$\overline{X} \pm m$	n	$\overline{X} \pm m$	n	$\overline{X} \pm m$		
1	210	1.282 ±0.035	189	1.487 ±0.038	200	1.390 ±0.027	604	1.393 ±0.026
2	204	1.344 ±0.017	200	1.493 ±0.027	203	1.427 ±0.019	607	1.421 ±0.018
3	207	1.380 ±0.028	194	1.443 ±0.019	204	1.418 ±0.023	605	1.414 ±0.019
4	205	1.380 ±0.021	195	1.415 ±0.025	206	1.398 ±0.028	606	1.398 ±0.020
5	206	1.373 ±0.018	202	1.447 ±0.029	206	1.385 ±0.021	614	1.401 ±0.021
6	205	1.373 ±0.025	208	1.480 ±0.028	203	1.387 ±0.020	616	1.413 ±0.022
Average	1237	1.355 ±0.020	1188	1.461 ±0.025	1222	1.401 ±0.020	3647	1.408 ±0.018

Analysis of the data on the multiple births in sows at weaning of piglets at 18 days, depending on the duration of the service period, showed a higher multiple birth rate with the longest service period. The highest number was recorded - 88 piglets received in a group of sows with a service period of 45 days or more. Only three piglets less were obtained in the group of sows with a service period of 21-28 days. And the smallest number of offspring- in a group of sows with a service period of 1-7 days - 75 heads.

Over the course of six farrowings with a service period of 1-7 days, abundance averaged 12.50 heads. With a service period of 21-28 days, the prolificacy was 14.17 heads. And with a service period of 45 days or more, the prolificacy reached 14.67 heads. The greatest increase in the number of obtained viable piglets with an increase in the service period was noted in the group of sows with a service period of 21-28 days (Table 3).

Based on the data on the multiple fertility of sows at weaning of piglets at 21 days of age, depending on the duration of the service period, a higher multiple fertility was also noted in animals with an increase in the service period. The largest number of piglets, 89 heads, was

obtained in the group of sows with a service period of 21-28 days. Five piglets were less in the group of sows with a service period of 45 days or more. And the smallest number of litters was obtained in the group of sows with a service period of one to seven days - 73 heads.

When analyzing the multiple fertility in sows when weaning piglets at 24 days of age, depending on the length of the service period, a higher multiple fertility was also noted with a longer service period, as well as when weaning piglets at 18 and 21 days of age. The largest number of piglets was obtained in the group of sows with a service period of 45 days or more - 89 heads. There were two piglets less in the group of sows with a service period of 21-28 days. And the least number of offspring- in the group of sows with a service period of one to seven days - 77 heads.

The preservation and the number of weaned piglets of sows in the experimental groups on average for all weaning periods are presented in Table 4.

Table 3. Multiple pregnancies in sows of experimental groups on average for all weaning periods (head) M ± m

Indicator	Sow service period			Average
	1-7 days	21-28 days	45 and more days	
1st farrowing	11.40± 0.39	12.69 ± 0.55	12.60 ± 0.65	12.23 ± 0.44
2nd farrowing	12.52 ± 0.42	16.03 ± 0.45	16.04 ± 0.57	14.86 ± 0.41
3rd farrowing	12.55 ± 0.33	14.81 ± 0.38	14.40 ± 0.58	13.92 ± 0.42
4th farrowing	12.51 ± 0.41	14.73 ± 0.44	15.31 ± 0.65	14.18 ± 0.47
5th farrowing	12.32 ± 0.44	14.12 ± 0.38	14.68 ± 0.71	13.71 ± 0.54
6th farrowing	11.40 ± 0.38	14.37 ± 0.47	14.04 ± 0.66	13.27 ± 0.44
Piglets received for 6 farrowings	225	261	261	747
Average	12.12 ± 0.30	14.46 ± 0.47	14.51 ± 0.54	13.70 ± 0.40

Table 4. Safety and number of weaned piglets of sows in experimental groups on average over all weaning period, M ± m

Indicator	Number of weaned pigs			
	Sow service period			Average
	1-7 days	21-28 days	45 days and more	
1st farrowing	12.43±0.30	11.44±0.31	11.29±0.34	11.72±0.29
2nd farrowing	10.04±0.30	14.27±0.27	14.86±0.33	13.06±0.28
3rd farrowing	11.22±0.33	13.41±0.27	13.31±0.29	12.65±0.28
4th farrowing	11.15±0.47	13.44±0.29	13.84±0.28	12.81±0.33
5th farrowing	10.40±0.54	12.59±0.43	13.80±0.34	12.26±0.41
6th farrowing	10.36±0.29	13.06±0.44	12.56±0.77	11.99±0.48
Total quantity after 6 farrowings	201	235	239	675
Average	10.94±0.35	13.04±0.31	13.28±0.39	12.42±0.35
Piglet safety during the weaning period, %	89.3	90.0	91.5	90.3

Table 4 shows that the average number of weaned pigs was 12.42 heads per nest. During insemination of sows in the first heat, the average number of weaned piglets was 10.94 heads per nest of sows. When one hunt was missed, this figure was 13.04 heads and when two hunts were missed - 13.28 heads. The increase in the number of weaned piglets when missing one heat in comparison with the group of sows inseminated in the first heat was 2.1 heads ($P<0.001$). The increase in the number of weaned piglets when missing two hunts in comparison with the group of sows inseminated in the first hunt was 2.34 heads ($P<0.001$). The difference between the indices of the groups of sows inseminated during the second and third estrus was only 0.24 heads.

The variability of this indicator, depending on the serial number of the farrowing, ranged from 11.72 to 13.06 heads. The largest number of weaned piglets was noted in the second survey, which amounted to 13.06 heads, which was 1.34 heads more than in the first farrowing ($P<0.01$). A sharp decrease in the number of weaned litters was observed in the sixth farrowing - 11.99 heads, which was less than the data of the second farrowing by 1.07 heads ($P <0.05$). Apparently, from the sixth farrowing, there was an age-related decrease in the functions of the reproductive system of sows, which was reflected in the number of weaned piglets.

Groups of sows with a service period of 21-28 days for six farrowings had 235 heads of weaned pigs, which is 34 heads more than groups of sows with a service period of 1-7 days. The difference in the number of weaned pigs between the groups of sows with service periods of 21-28 and 45 days or more was only 4 heads.

The survival rate of suckling piglets in the group of sows with a service period of one to seven days was 89.3%, and in the group of sows with a service period of 21-28 days it reached 90.0%, which is 0.7% more compared to a group of sows with a service period of one to seven days. In the group of sows with a service period of 45 days or more, the survival rate increased to 91.5%, which is 2.2% higher compared to the group of sows with a service period of one to seven days. Thus, with an increase in the service period of sows, an increase in the safety of piglets is observed.

CONCLUSIONS

Thus, the studies carried out indicate that some technological features of the use of sows in reproduction, namely the duration of the service period and lactation, have a significant impact on the final indicators of the reproductive qualities of sows. In the conditions of large pig breeding enterprises, the technologies being developed will help to significantly increase the yield of piglets from brood of pigs. In particular, skipping one sow hunt will significantly increase the large-fruited, multi-fertile sows and the safety of the piglets obtained from them.

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ORCHIDOMETRIC, SEMINAL AND SPERM DIMENSIONAL CHARACTERISTICS: A STUDY ON INTERRELATIONSHIPS IN NILI RAVI BUFFALO (*BUBALUS BUBALIS*) BULLS

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Abstract

Animal conservation and improvement programs for any species or breed require the basic knowledge of its reproductive anatomy, histology and physiology. The present study has been devised with an objective to determine interrelationships between body weight, various orchidometric, fresh seminal, and post thaw seminal attributes for Nili-Ravi buffalo bulls. Materials, Methods & Results: Adult Nili-Ravi buffalo bulls (n = 07) being reared for breeding at Semen Production Unit (SPU), Qadirabad, Sahiwal, Pakistan were used in the present study. Orchidometric attributes included scrotal circumference, testicular length, testicular width and paired testicular volume (PTV), whereas fresh seminal parameters included the assessment of ejaculatory volume, color, pH, mass motility and individual sperm motility, and sperm. The sperm dimensional characteristics included measurements of head length (HL), head breadth (HB), head shape (HS), midpiece length (MPL), tail length (TL) and total sperm length (TSL). Results revealed a significantly positive correlation ($P \leq 0.01$) between body weight and all the studied orchidometric attributes. Similarly, correlation coefficients between all the studied sperm dimensional characteristics were also statistically significant ($P \leq 0.01$). Semen color showed significantly positive correlation with sperm HL, HB, TL and TSL, being negative with HS and MPL. Semen pH showed significantly positive correlation with sperm HS and MPL and significantly negative correlation with other sperm dimensional characteristics. The individual sperm motility had significantly positive correlation with HL, TL and TSL being negatively correlated with MPL. The sperm count showed significantly positive correlation with HL, HB, TL and TSL. Various studies on males of livestock have reported a positive correlation between body weight and various testicular attributes especially SC and PTV. Similarly, semen parameters, both for fresh and post thaw, have also been shown to have interrelationships among each other. The present study has added sperm dimensional characteristics in the study. The perplexing diversity of sperm structure has always been an interest of research world-wide which has resulted in reports of its structure associated with its adaptive function i.e. appropriate fertility. The novelty of this study is that the work has been conducted on Nili-Ravi buffalo bulls, for which the results of this study endorse that body weight, orchidometric, seminal and sperm dimensional characteristics show positive correlation with each other and may be utilized for selection of breeding buffalo bulls as they are readily measurable and reliable indicators of their reproductive status. We recommend the incorporation of sperm morphological attributes in the Breeding Evaluation protocols of the SPUs of Pakistan for selection of reproductively sound breeding bulls.

Key words*: buffalo bulls, correlation coefficient, orchidometry, sperm morphology.*

INTRODUCTION

In Pakistan, livestock sector has a share of 60.54% in agriculture and 11.22% in GDP. This sector showed an annual growth of 4% as per the Economic Survey of Pakistan (ESP, 2019). With a population of 40.0 million buffalo (*Bubalus bubalis*) heads in Pakistan, its gross milk production is highest as compared to milk from other livestock species. From the total global buffalo population, Asia harbors about 98% with small landless farmers owing 3-4 animals per family (Hameed et al., 2016). The Nili, Ravi, Nili-Ravi and Kundi are four main buffalo breeds prevalent in Pakistan amongst which the Nili-Ravi is considered as the mainstay and the backbone of Pakistani rural livestock economy.

In order to attain maximum reproductive performance and fertility of breeding bulls, various readily measurable reproductive parameters such as body weight and orchidometric attributes *viz*. scrotal circumference (SC), testicular length, testicular width, paired testicular volume (PTV) are being utilized and validated for various tropical livestock breeds (Mahmood et al., 2014b; Shrestha et al., 1983; Gage and Freckleton, 2003; Lunstra and Cundiff; 2003; Siddiqui et al., 2008). The morphometric examination of testes provides a reliable estimate of spermatogenesis process of the testes which are dependable predictors of normal process of spermatogenesis (Gage & Freckleton, 2003). The SC is associated with body weight of animal (Devkota et al., 2008) and is highly correlated with weight of testes, while consistency of testes is correlated with bull fertility (Waldner et al., 2010). A significantly positive correlation between SC and spermatozoa concentration has been elucidated for Sahiwal cattle bulls (Ahmad et al., 2005).

Along with superior genetic potential, the selected bulls should also be superior in quality semen production which is ascertained through various seminal attributes assessed through fresh and post-thaw semen quality tests (Farooq et al., 2013a; Farooq et al., 2013b; Farooq et al., 2015). Similarly, sperm morphology has attained avid attention as main predictor of fertility in various species and breeds of livestock (Hameed et al., 2016; AL-Sahaf & Ibrahim, 2012; Pant & Mukherjee, 1972; García-Vázquez et al., 2016).

Animal conservation and improvement programs for any species or breed require the basic knowledge of its reproductive anatomy, histology and physiology. The studies on assessment and interrelationships between various orchidometric, seminal and sperm dimensional characteristics have been reported for various livestock species. Similarly, our group has earlier reported seasonal variations in seminal and sperm dimensional characteristics of buffalo bulls (Hameed et al., 2017; Hameed et al., 2016). However, there is dearth of literature regarding correlation between these attributes for Nili-Ravi buffalo bulls. The present study has, therefore, been devised with an objective to determine interrelationships among and between body weight, various orchidometric (SC, testicular length, testicular width and PTV), fresh seminal (ejaculatory volume, color, pH, mass motility and individual sperm motility), and sperm dimensional characteristics (head length, head breadth, head shape, midpiece length, tail length and total sperm length) for Nili-Ravi buffalo bulls.

MATERIALS AND METHODS

The **s**tudy was carried over 12-month tenure at Semen Production Unit (SPU), Qadirabad, Sahiwal, Pakistan located at latitudes 30º and 31.15º North and longitudes 73º and 74º East, and at an altitude of 564 feet above the sea level.

Nili-Ravi buffalo bulls (n = 07) donating acceptable quality semen were selected for the study. The age of breeding bulls was 5-8 years and all the bulls were ensured to have clinically normal reproductive tract before the start of the study. Throughout the study tenure, the bulls were observed for their normal health, standard management and feeding practices.

Body weight and orchidometric attributes *viz*. SC, testicular length, testicular width and testicular volume were recorded fortnightly during the study period. All the measurements were performed by only one person and with same method of restraint in order to minimize stress. A total of 24 observations were noted for each parameter per bull. The SC was measured by using a measuring tape (Ahmad et al.,

2005). Testicular length (proximal-distal) and width (medio-lateral) of both testes were measured using digital vernier caliper (Shrestha et al., 1983), and the averages were calculated. The PTV was deduced as per the formula given below (Lunstra & Cundiff, 2003):

$$PTV = 0.0396\ (ATL)\ (SC)^2$$

Semen from each bull was collected on weekly basis using artificial vagina (42°C). At each collection, two ejaculates per bull were attained. Resultantly, 48 collections per bull were carried out with a total of 670 ejaculates. After collection, each semen sample was processed for physical seminal attributes such as ejaculatory volume, color, pH, mass motility (score 0-5), individual sperm motility (40X, phase contrast microscope; Olympus BH-2, Tokyo, Japan) and sperm count (photometrically at 560 nm wavelength using Bovine Photometer n° 1119, IMV, France). Details of initial semen evaluation and relevant data have been presented elsewhere (Hameed et al., 2017).

The dimension al characteristics of spermatozoa *viz*. head length (HL), head breadth (HB), head shape (HS), midpiece length (MPL), tail length (TL) and total sperm length (TSL) were measured after staining with Eosin-Nigrosin stain (Ciftci & Zulkadir, 2010) using a software PixelPro. From each sample, 12 morphologically normal sperm were selected for micrometry. The detailed methodology and relevant data have been presented elsewhere (Hameed et al., 2016).

Statistical analyses were conducted through Statistical Package for Social Science (SPSS for Windows V 17.0, SPSS Inc., Chicago, IL, USA).Kolmogorov Smirnov test was employed to test the normal distribution of data. Interrelationships within and between various orchidometric, seminal and sperm dimensional characteristics were deduced through Pearson's correlation coefficient.

RESULTS

Significantly positive correlation (P≤0.01) between body weight and all the studied orchidometric attributes was noticed in the present study (Table 1).

Regarding the correlation among various seminal attributes, it was revealed that the ejaculatory volume of buffalo bulls had a negative correlation with semen color (r = -0.036), pH (r = -0.089), mass motility (r = -0.036) and sperm count (r = -0.035), and positive with individual sperm motility (r = 0.106), though statistically non-significant (P≥0.05).

Table 1. Pearson's correlation coefficients between body weight and various orchidometric attributes of Nili-Ravi buffalo bulls (n = 07)

Parameters	r value
Body weight × Scrotal circumference	0.870**
Body weight × Average testicular length	0.831**
Body weight × Average testicular width	0.868**
Body weight × Paired testicular volume	0.877**
Scrotal circumference × Average testicular length	0.895**
Scrotal circumference × Average testicular width	0.930**
Scrotal circumference × Paired testicular volume	0.990
Average testicular length × Average testicular width	0.954**
Average testicular length × Paired testicular volume	0.947**
Average testicular width × Paired testicular volume	0.957**

**Significant correlation (P ≤0.01, 2 tailed)

Semen color had significantly negative correlation with pH (r = -0.636; P≤0.01), and positive with mass motility (r = 0.957; P≤0.01), individual sperm motility (r = 0.740; P≤0.01) and sperm count (r = 0.679; P≤0.01). Similarly, semen pH showed significantly negative correlation with mass motility (r = -0.717; P≤0.01), individual sperm motility (r = -0.587; P≤0.01) and sperm count (r = -0.895; P≤0.01). The correlation between individual sperm motility and sperm count was also significantly positive (r = 0.633; P≤0.01) (Table 2).

Table 2. Pearson's correlation coefficients among various seminal attributes of Nili-Ravi buffalo bulls (n = 07)

Parameters	r value
Ejaculatory volume × Semen color	-0.036^{NS}
Ejaculatory volume × Semen pH	-0.089^{NS}
Ejaculatory volume × Mass motility	-0.036^{NS}
Ejaculatory volume × Individual sperm motility	0.106^{NS}
Ejaculatory volume × Sperm count	-0.035^{NS}
Semen color × Semen pH	-0.636**
Semen color × Mass motility	0.957**
Semen color × Individual sperm motility	0.740**
Semen color × Sperm count	0.679**
Semen pH × Mass motility	-0.717**
Semen pH × Individual sperm motility	-0.587**
Semen pH × Sperm count	-0.895**
Mass motility × Individual sperm motility	0.799**
Mass motility × Sperm count	0.767**
Individual sperm motility × Sperm count	0.633**

^{NS}Non-significant, **Significant correlation (P ≤0.01, 2 tailed)

Results regarding correlation coefficients between all the studied sperm dimensional

characteristics were statistically significant (P≤0.01) in the present study as given in Table 3. The sperm HL had positive correlation with HB (r = 0.935; P≤0.01), TL (r = 0.885; P≤0.01) and TSL (r = 0.883; P≤0.01), and negative with HS (r=-0.866; P≤0.01) and MPL (r = -0.835; P≤0.01). Similarly, sperm HB was negatively correlated with HS (r = -0.987; P≤0.01) and MPL (r = -0.769; P≤0.01), and positively correlated to TL (r = 0.916; P≤0.01) and TSL (r = 0.916; P≤0.01).

Table 3. Pearson's correlation coefficients among various sperm dimensional characteristics of Nili-Ravi buffalo bulls (n = 07)

Parameters	r value
Head length × Head breadth	0.935**
Head length × Head shape	-0.866**
Head length × Midpiece length	-0.835**
Head length × Tail length	0.885**
Head length × Total sperm length	0.883**
Head breadth × Head shape	-0.987**
Head breadth × Midpiece length	-0.769**
Head breadth × Tail length	0.916**
Head breadth × Total sperm length	0.916**
Head shape × Midpiece length	0.711**
Head shape × Tail length	-0.890**
Head shape × Total sperm length	-0.891**
Midpiece length × Tail length	-0.756**
Midpiece length × Total sperm length	-0.743**
Tail length × Total sperm length	1.000**

**Significant correlation (P ≤ 0.01, 2 tailed)

All the correlations between body weight, orchidometric, seminal and sperm dimensional characteristics were statistically non-significant (P≥0.05) (Table 4).

The results regarding correlation coefficients between various seminal and sperm dimensional characteristics are given in Table 5. Semen color showed significantly positive correlation with sperm HL (r = 0.616; P≤0.01), HB (r = 0.595; P≤0.01), TL (r = 0.594; P≤0.01) and TSL (r = 0.595; P≤0.01), being negative with HS (r = -0.561; P≤0.01) and MPL (r = -0.482; P≤0.01). Semen pH showed significantly positive correlation with sperm HS (r = 0.505; P≤0.01) and MPL (r = 0.344; P≤0.01) and significantly negative correlation with other sperm dimensional characteristics. The mass motility revealed correlation with sperm dimensional characteristics similar to those for semen color. The individual sperm motility had significantly positive correlation with HL (r = 0.376; P≤0.05), TL (r = 0.381; P≤0.05) and TSL (r = 0.375; P≤0.05) being negatively correlated with MPL (r = -0.446; P≤0.01). The sperm count showed significantly positive correlation with HL (r = 0.480; P≤0.01), HB (r = 0.536; P≤0.01), TL (r = 0.621; P≤0.01) and TSL (r = 0.625; P≤0.01).

DISCUSSIONS

The present study is a novel one being reported from Pakistan on interrelationships among and between various reproductive attributes (body weight, orchidometric, seminal and sperm dimensional characteristics) of Nili-Ravi buffalo bulls. In case of lack of prior studies on buffalo bulls, the comparisons of our results have been made with other livestock species.

In the present study, significantly positive correlation was noticed between body weight and all the studied orchidometric attributes (SC, testicular length, testicular width and testicular volume). Earlier studies have reported positive correlation between body weight and SC for Murrah buffalo bulls (da Silva SANTOS et al., 2013; Singh et al., 2014) and Nili-Ravi buffalo bulls (Javed, 1998). The positive correlations of the present study are also in agreement with other studies conducted on river buffalo (Viana, 2008), swamp buffalo (McCool et al., 1985) and Murrah buffalo (Pant et al., 2003). Findings of an earlier work (da Silva SANTOS et al., 2013) regarding the correlation between body weight, testicular length and testicular width are in line with the findings of the present study revealing the significantly positive correlations. Similar correlations have also been observed for *Bos taurus* cattle (Jain et al., 2008). A study on Cholistani breeding bulls (a Zebu indigenous cattle breed of Pakistan) has documented significantly positive correlations between body weight, SC and PTV (Mahmood et al., 2014b). Correlation results documented in Tho Tho bulls (Perumal, 2014) and in Cholistani bulls (Mahmood et al., 2014a) are also in agreement with present results. The PTV is gaining avid attention since last decade as a reliable indicator of reproductive status in breeding bulls (Unanian et al., 2000).

Regarding the correlation among various seminal attributes, the ejaculatory volume had non-significant correlation with all other studied seminal attributes *i.e.* color, pH, mass

motility, individual sperm motility and sperm count. These results are in close agreement with the earlier findings on Nili-Ravi buffalo bulls (Younis, 1996; Javed, 1998). This shows that in buffalo bulls, semen volume does not predict the semen quality (Javed et al., 2000). Similar correlations have also been documented for Cholistani bulls (Mahmood et al., 2014b). In contrary, significantly positive correlation of volume with mass motility and sperm count have been reported for Horro bulls (Galmessa et al., 2005). In present study, semen pH showed negative correlation with mass motility, individual sperm motility and sperm count which is supported by an earlier study on Nili-Ravi buffalo bulls (Javed et al., 2000). Semen color had a positive correlation with mass motility, individual sperm motility and sperm count which is supported another prior work (Javed, 1998).

Table 4. Pearson's correlation coefficients between body weight, orchidometric, seminal and sperm dimensional characteristics of Nili-Ravi buffalo bulls (n = 07)

Parameters	Seminal Attributes						Sperm Dimensional Characteristics					
	Ejaculatory Volume	Color	pH	Mass Motility	Individual Sperm Motility	Sperm Count	Head Length	Head Breadth	Head Shape	Midpeice Length	Tail Length	Total Sperm Length
Body weight	-0.172	0.120	-0.120	0.077	-0.027	0.199	0.119	0.206	-0.234	0.167	0.232	0.244
Scrotal circumference	-0.015	0.151	-0.214	0.121	0.183	0.234	0.195	0.254	-0.267	-0.070	0.303	0.308
Average testicular length	-0.029	0.157	-0.169	0.128	0.198	0.210	0.079	0.110	-0.120	0.034	0.136	0.141
Average testicular width	-0.075	0.127	-0.167	0.103	0.138	0.186	0.102	0.117	-0.120	0.059	0.160	0.166
Paired testicular volume	-0.010	0.154	-0.208	0.126	0.192	0.234	0.163	0.218	-0.231	-0.043	0.260	0.264

Table 5. Pearson's correlation coefficients between seminal and sperm dimensional characteristics of Nili-Ravi buffalo bulls (n = 07)

Parameters	Head length	Head breadth	Head shape	Midpiece length	Tail length	Total Sperm length
Ejaculatory volume	-0.263^{NS}	-0.230^{NS}	0.204^{NS}	0.045^{NS}	-0.244^{NS}	-0.251^{NS}
Color	0.616**	0.595**	-0.561**	-0.482**	0.594**	0.595**
pH	-0.480**	-0.512**	0.505**	0.344*	-0.536**	-0.539**
Mass motility	0.592**	0.566**	-0.530**	-0.480**	0.589**	0.589**
Individual sperm motility	0.376*	0.301^{NS}	-0.254^{NS}	-0.446**	0.381*	0.375*
Sperm count	0.480**	0.536**	-0.534**	-0.313^{NS}	0.621**	0.625**

**Significant correlation ($P \leq 0.01$, 2 tailed); *Significant correlation ($P \leq 0.05$, 2 tailed); ^{NS}Non-significant

Similarly, a positive correlation between mass motility and individual sperm motility in the present study is in line with other findings for Cholistani breed of cattle (Mahmood et al., 2013). A positive correlation between individual sperm motility and sperm count in the present study is also in agreement with prior studies (Galmessa et al., 2005).

The perplexing diversity of sperm structure has always been an interest of research world-wide which has resulted in reports of its structure associated with its adaptive function *i.e.* appropriate fertility. Regarding results of correlation coefficients between all the studied sperm dimensional characteristics, the sperm HL had positive correlation with HB, TL and TSL, and negative with HS and MPL in the present study. A negative correlation between MPL and HL has been reported earlier (Humphries et al., 2008) which is supporting the finding of present study. It has been previously reported that the correlation exists between MPL and sperm head (Piasecka & Kawiak, 2003; Cardullo & Baltz, 1991). No relationship between sperm head size and mid-piece in Iberian red deer has been reported (Malo et al., 2006). In contrary to the findings of present study, positive correlation of MPL with HL, HB, TL and TSL in cattle bulls have been reported (Sarder, 2005). It has been elucidated that the sperm dimensional characteristics are variable in different species, and in various breeds of specie. Furthermore, these attributes tend to vary as per general

health status of the animal and seasons (Hameed et al., 2016; Pant and Mukherjee, 1972; Sarder, 2005).

In the present study, the correlations were deduced between readily measurable reproductive traits (body weight and orchidometric attributes), and seminal and sperm dimensional characteristics. All the correlations were found to be non-significant. Body weight and orchidometric attributes were negatively correlated with ejaculatory volume and semen pH, and positively correlated with semen color, mass motility, individual sperm motility and sperm count. Similar results have been presented for crossbred rams (Moghaddam et al., 2012). According to another study on Cholistani breeding bulls (Mahmood et al., 2014a), there was a non-significant correlation between SC, testicular length, testicular width and PTV with mass motility and individual sperm motility as observed in the present study for Nili-Ravi buffalo bulls. In present study, body weight and orchidometric parameters showed non-significantly negative correlation with ejaculatory volume which has been reported positive in earlier study on Cholistani bulls (Mahmood et al., 2014a). A plausible justification for these variations in results of correlation may be the difference in species, breed, and season of study. Regarding sperm dimensional characteristics, it has earlier been demonstrated that the morphological features of sperm lose their relationship with body mass after phylogenetic controls.

The seminal and sperm dimensional characteristics of the presented study were studied for correlation coefficients in the present study. Semen color showed significantly positive correlation with sperm HL, HB, TL and TSL, being negative with HS and MPL. Semen pH showed significantly positive correlation with sperm HS and MPL and significantly negative correlation with other sperm dimensional characteristics. The individual sperm motility had significantly positive correlation with HL, TL and TSL being negatively correlated with MPL. The sperm count showed significantly positive correlation with HL, HB, TL and TSL. Comparing these results with prior studies, a wide variability has been reported while studying importance of sperm morphology for its transport in female reproductive system and its fertilizing ability (García-Vázquez et al., 2016). The TSL and MPL have been demonstrated to be positively correlated to fertilizing ability in various primates (Humphries et al., 2008). Swimming velocity of sperm enhances sperm competitiveness and ultimate fertilizing ability. Extensive studies have reported a positive correlation between sperm length and its swimming velocity for many mammals as in line with results of present study (Humphries et al., 2008; Gomendio and Roldan, 2004).

CONCLUSIONS

In a nutshell, the present study demonstrates that body weight, orchidometric, seminal and sperm dimensional characteristics show positive correlation with each other for Nili-Ravi buffalo bulls. These attributes may be utilized for selection of breeding buffalo bulls as alternate to expensive methods of bull selection as they are readily measurable and reliable indicators of their reproductive status. The sperm dimensional characteristics are not a part of evaluation in Pakistani SPUs as yet. We recommend that these sperm morphological parameters may be incorporated in Breeding Soundness Evaluation protocols of breeding buffalo bulls for attaining an optimal fertility rate.

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PROVENTRICULAR PATHOLOGIES WITH GENERALISED TUBERCULOSIS IN PEAFOWL (*PAVO CRISTATUS*): PATHOMORPHOLOGICAL ANALYSIS

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Abstract

The results of pathological anatomical changes in the proventriculus of a decorative peacock that died from tuberculosis were described in this article. Mixed chronic destructive multifocal ulcerative panproventriculitis with a predominance of ulcerative component without signs of healing was diagnosed. Absence of epithelialization of large ulcer and relief in this area is a sign of malignancy. Complicating pathologies were classified: rupture of proventriculus wall, hemorrhage, atrophy of glands of mucous membranes, cicatrization, deformity. Proventricular component in the pathomorphosis of ornamental peacock tuberculosis is an accessible and convenient target for the diagnostician and can significantly supplement the pathomorphological criteria used to decipher the variants of avian tuberculosis course. The severity of damage to the glandular stomach in peacocks is due to the tropism of tuberculosis pathogens to the lymphoid structures located here.

Key words*: avian tuberculosis, peafowl, proventriculus, pathomorphological analysis.*

INTRODUCTION

Ecological parks where birds of the so-called tactile group are kept have become popular in Ukraine. Visitors are invited to feed and pet birds. Sudden deaths from tuberculosis are often reported among peacocks and pheasants. This disease remains a global problem, despite all the efforts of the world community to overcome it. (Kennedy, 2017; Nitu et al., 2017). As for avian tuberculosis, it threatens the extinction of the entire collections and populations of individual bird species. In particular, because this disease is characterized by a decrease in egg productivity. Bird population management programs have been developed in different regions of Ukraine. Their goal is to improve the ecological environment and increase the ecological sustainability of the anthropogenic ecosystem. Lack of environmental education of people remains the problem. Workers and visitors to eco-parks are unaware of their own risk of likely infection with *Mycobacterium avium* complex (MAC) by contact with ornamental poultry. Particularly dangerous is the effect of MAC on the health of people with immunodeficiency, in particular, infection with viruses of the respiratory group (Bazzi et al., 2020; Crisan-Dabija et al., 2020; Azar et al., 2019; Auguste et al., 2018). Often such a contingent of people choose to rest in ecoparks. The possibility of infection of poultry from people with tuberculosis is not excluded.

Sectional examination of the corpses of ecopark peacocks and pheasants revealed both classic lesions with localization in the liver, intestines and spleen, and a number of destructive forms of pathology (Lyakhovich et al., 2020; Liakhovych et al., 2019; Liakhovych et al., 2018). During an outbreak of tuberculosis in peacocks on a private farm in Romania, researchers found damage typical of avian tuberculosis - granulomas in the lungs, liver and spleen (Iancu et al., 2017).

Lymphotropic mycobacteria, in particular, their influence on the development of pathologies in the lymph nodes and ulcers of the oral mucosa of patients with tuberculosis is known (Popescu et al., 2015; Popescu et al., 2014). This indicates a potential pattern of development in tuberculosis of birds of the corresponding lesions in organs rich in lymphoid structures, in particular, in the wall of the glandular stomach. Because physiological norm is characterized by the saturation of the wall with lymphocytes and

lymphoid nodules (Kovtun & Harchenko, 2005). Romanian researchers Ciobotaru et al. (2012), studying changes in tuberculosis in a peacock, found damage at the border of the esophagus and glandular stomach.
Specific pathologies in the wall of the glandular stomach in pigeons with tuberculosis, have also been described (Mayahi et al., 2013). At the same time, information on the proventricular localization of pathologies caused by MAC is not covered in available information sources and is not associated with probable avian tuberculosis. Therefore, each case of detection of pathologies of the proventriculus in a bird diagnosed with tuberculosis complements the data on the pathomorphosis of this disease.

MATERIALS AND METHODS

Pathologies of proventriculus of an adult (5 years old) ornamental peacock (male), an Indian breed, which died of generalized tuberculosis, was objected to this study. In summer, the bird and peacocks were kept in an aviary of a mini-ecopark. In winter, birds of different species were kept in an adapted room in crowded conditions.
The incidents of deaths from avian tuberculosis among peacocks and pheasants in the ecopark have increased recently. The aim of the study was to identify and classify the pathomorphological changes in the proventriculus of the peacock with tuberculosis. The work was done at the Department of Normal and Pathological Morphology of the Kharkiv State Zooveterinary Academy. Pathoanatomical section and its analysis, macro-microscopic examination of the peacock's proventriculus using weakly multiple optical lenses methods were used.
The diagnosis of "Avian tuberculosis" was established on the basis of comprehensive studies. Pathoanatomical examination were done in the section hall.
The corpse of the peacock was dissected at dorsal position by the method of partial evisceration according to the generally accepted rules (Dobin & Cocurichev, 1963) (Figure 1).

Figure 1. The corps of a dissected peacock that died of generalized tuberculosis

Macroscopic examination of the proventriculus was performed according to existing rules. We determined the integrity and thickness of its wall, the degree of blood supply and filling of the cavity, the state of the serous membrane (color, surface character, humidity); the presence and nature of the content (quantity, consistency, color), patency, condition of the mucous membrane (color, thickness, relief, integrity, humidity, layering), condition of muscular membrane.
Macro-microscopic examination of the organ was performed on a native planar preparation, which was studied visually using weakly multiple optical lenses under special artificial lighting.
Available parts of the body (apex, body and isthmus) were evaluated to identify possible pathological changes: inflammatory processes, changes in glandular structures (ectasia, necrosis, abscess), ulcers, mucosal erosions, hyperplasia, dysplasia, neoplasia, vascular disorders.

RESULTS AND DISCUSSIONS

Classic for tuberculosis of birds, granulomas with intestinal (Figure 2) and splenic localization were found during the pathoanatomical section of the dead peacock (Figure 3).

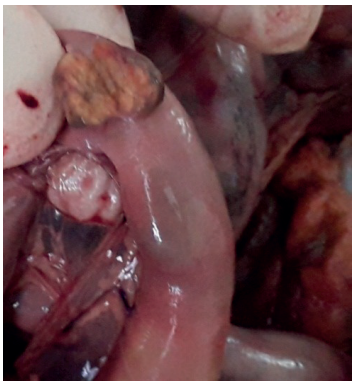

Figure 2. Mature granuloma with subserous localization in the wall of the jejunum in peacock with generalized tuberculosis

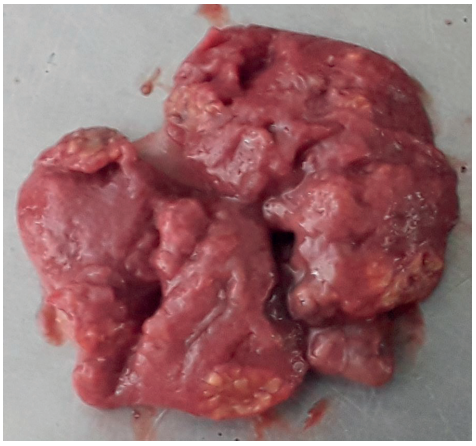

Figure 3. Spleen of Peafowl *(Pavo cristatus)*. Multiple coalescent whitish to yellow granulomas visible on cut surface

Peacock's proventriculus on macroscopic examination had a characteristic spindle-shaped, uneven wall thickness with rigidity in various parts of it. The anterior lobe (apex), posterior lobe (body) and isthmus were distinguished in the organ. The apex was located more dorsally from the heart (between the air sacs); the body of the proventriculus was located dorsal to the left lobe of the liver. Multiple ruptures of the structures connecting the dorsal edge of the liver and the proventriculus occurred due to massive hemorrhage into the thoracic-abdominal cavity from the hepatic vessels. Examination of the outer wall of proventriculus body in its front part revealed a single gap in the shape of a crescent moon, the size of 5 mm (Figure 4).

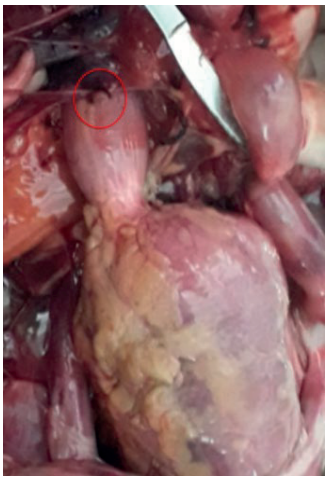

Figure 4. General view of the proventriculus and gizzard of the peacock that died from generalized tuberculosis. Area with a rupture of the outer wall of proventriculus marked with ellipse

Destruction of ventral proventricular veins *(Vv. Proventriculares ventralis*) was also estabished. The right side of the proventriculus touched the spleen and ileum, the left side - the cecum. At the border with the gizzard, the proventriculus was narrowed due to the annular sphincter located there. On the surface of the mucous membrane were visible 22 cone-shaped papillae of different heights (glands that opened with special holes) with an uneven degree of protrusion and filling with secretions after dissection of the proventriculus along its axis. The proventriculus did not contain feed masses (Figure 5). Its mucous membrane had uneven pigmentation; in the area of the body on its surface there was no specific relief (detected defect in the form of a large ulcer - d 1.5 cm). In one area, the ulcer due to the spread in the thickness of the wall, corroded the relevant vessels that passed there, which caused bleeding - a natural complication of ulcerative pathology. However, the main source of bleeding with a high degree of its activity was in the liver. The incurability of a large ulcer of the gastric mucosa was indicated by areas with its hyperemia, the presence of necrotized tissues, the depth of the defect (the wall of the gastric gland was thinned due to the destruction of mucosal structures, submucosal base/glandular components).

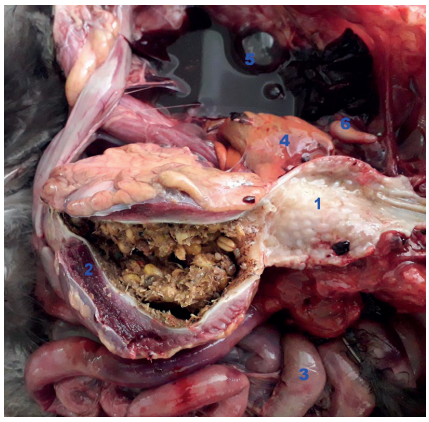

Figure 5. Visceral organs of a peacock that died of generalized tuberculosis: 1 - proventriculus in section; 2 - gizzard in section; 3 - intestinal loops; 4 - a fragment of the liver; 5 - mass of blood in the thoracic-abdominal cavity due to rupture of hepatic vessels; 6 - testis

The absence of mature granulation tissue at the edges of the ulcer defect indicates the inability to protect them and the development of regeneration of the epithelium and other components of the wall. This is due to severe damage to many systems of the bird during generalized tuberculosis. Large proventricular ulcer was complicated by scarring deformation of the organ, atrophy of the mucous glands.

New superficial ulcers in the form of peculiar niches (defects) were found on the mucous membrane in the area of transition of the proventriculus to the muscular one (Figure 5 - 2). Some of them were healed. Signs of edema and petechial hemorrhage were detected using a weakly multiple optical lens. On macroscopic examination in the externally areas, the mucous membrane of the proventriculus was painted white, had a locally swollen and edematous surface; in a large area it was excessively covered with masses of clear viscous mucus (starting from the border with the esophagus and in the body part of the proventriculus) (Figure 6). Excess mucus substances should be considered a criterion for suspicion of neoplastic pathology of mucus-producing cells.

On the background of chronic destructive pathologies of peacock proventriculus wall, which contributed to microcirculation disorders (increased blood viscosity with a tendency to thrombosis due to exicosis as a result of intestinal disorders), proteolysis of the structures within the damaged mucous membranes and other layers of gastric enzymes (with a regular seasonal and/or post-dietary increase in activity), the size of the ulcer increased due to its marginal (periulcerous) segments.

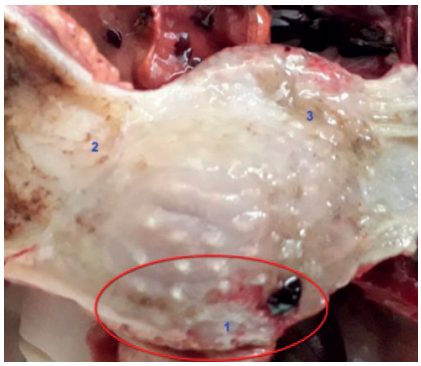

Figure 6. Proventriculus of a peacock which died of generalized tuberculosis (view from the mucous membrane): 1 - large ulcer with hemorrhage in the body of the organ; 2 - small ulcers in the isthmus; 3 - mass of mucus

Combination of randomly located areas of sclerosis and fibrosis, atrophy and hypertrophy in the periulcerous zone in the mucous membrane of the organ wall indicated the presence of persistent chronic proventriculitis in the peacock. Potentially MAC, by penetrating the body of the bird's alimentary way, can infect the epithelium of the proventriculus and submucosal layer, where the lymphoid structures are located. As a result, the proventriculus should be considered a target for mycobacteria! Following facts indicated tuberculous genesis of chronic ulcers in proventriculus of peacock in this case: generalized tuberculosis was diagnosed in this bird; with alimentary inflow of MAC to the proventriculus there were conditions of delay of the forage on the background of malabsorbtion syndrome with partial obstruction of the intestine affected by tuberculosis (this created conditions of longer than normal contact of MAC with the gastric mucosa); the wall of the proventriculus in peacocks contains lymphoid elements to which MAC exhibits tropism; actually, the topography of a large unhealed ulcer with localization inside the body of the organ, and not near the gizzard (where, on the contrary, if ulcers occur, their origin is due to anaerobic

microorganisms, because there are appropriate conditions for their reproduction); massive involvement of the structures of mucous membrane and in some areas - muscular - indicated chronicity of the ulcer. Chronic inflammation of the gastric mucosa should and other alike reasons needs to be examined as factor which provokes malignancy.

Proventriculitis was classified by type, location, etiological factors, pathomorphological picture of wall structures. Therefore, mixed chronic destructive multifocal ulcerative panproventriculitis with a predominance of ulcerative component without signs of healing was diagnosed in studied peacock.

According to such an indicator as ulcer healing (in the studied case there was no complete healing of ulcers), it is necessary to assess the actual immune status of the bird. The predominance of the destructive form of proventriculitis indicates a stable in time duration of damage to the structures of the organ wall. In the case of poultry tuberculosis, it is not possible to observe all the dynamics of damage to body systems, in particular, the gastrointestinal tract, because its life is limited to reaching the slaughter age. Corresponding changes have time to develop to a fuller picture in decorative bird. Actually, that's why it's more informative.

CONCLUSIONS

Mixed chronic destructive multifocal ulcerative panproventriculitis with a predominance of ulcerative component without signs of healing was diagnosed in peacock that died from generalized tuberculosis. Absence of epithelialization of the ulcer and relief in this area is considered a sign of malignancy. Rupture of the gastric wall, hemorrhage, atrophy of glands of mucous membranes were classified as complicating pathologies. The proventricular component in the pathomorphosis of ornamental peacock tuberculosis is an accessible and convenient target for the diagnostician and can significantly supplement the pathomorphological criteria used to decipher the variants of the course of avian tuberculosis. A combination of the following forms of pathology of glandular stomach: ulcer, which is a variant of necrosis; hemorrhage; cicatrization deformity - it is advisable to consider as pathomorphological markers of possible tuberculous damage to its mucous membrane and other structures. The severity of damage to the proventriculus in peacocks is due to the tropism of tuberculosis pathogens to the lymphoid structures located there. Absence of classic nodular forms of tuberculosis in the proventriculus of birds is probably due to anaerobic conditions. According to the physiological norm, the environment there does not promote the growth of bacteria. Also in the cavity of the proventriculus there is an objectively small space for the formation of granuloma.

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EFFECT OF LACTOSE EGG YOLK GLYCEROL EXTENDER SUPPLEMENTED WITH TREHALOSE ON POST-THAW CHARACTERISTICS AND FERTILITY OF BUFFALO BULL SPERMATOZOA

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Abstract

The present study was designed to: 1) study the influence of various concentrations of trehalose on buffalo bull spermatozoa extended in lactose- egg yolk-glycerol extender (LEYGE) and 2) compare the fertility rate between LEYGE supplemented with an optimal level of trehalose and traditionally used tris-citric-acid-fructose extender. Semen of Nili-Ravi buffalo bulls (n = 04) routinely used for artificial insemination, was collected using an AV at SPU once a week, for eight weeks. Semen samples with ≥70 % spermatozoa motility were pooled and diluted at 37°C in LEYGE extender containing trehalose at 0.0, 30.0, 50.0 and 70.0 mM. The sperm motility, viability, acrosomal integrity, plasma membrane integrity, and DNA integrity were significantly (P≤0.05) higher in LEYGE supplemented with 70.0 mM trehalose as compared to other groups. The fertility rate was significantly (P≤0.05) higher when semen doses extended in LEYGE and supplemented with 70.0 mM trehalose were used (n = 19; 38%) as compared to semen doses extended in the conventional tris-citric-acid-fructose extender (n = 27; 54%). In conclusion, 70 mM is an optimal inclusion level in buffalo bull semen for trehalose in LEYGE in respect to seminal attributes. Furthermore, LEYGE supplemented with 70 mM trehalose presented higher fertility rate in buffaloes as compared to traditionally used tris-citric-acid-fructose extender under field conditions.

Key words*: buffalo bull, spermatozoa, trehalose, lactose, cryopreservation.*

INTRODUCTION

Artificial insemination (AI) has been playing a vital role in improving the production of milk, meat, wool, leather, and hair. The success of AI relies on semen collection, preservation, storage and its utilization (Leboeuf et al., 2000). Various protocols have been developed and are being used for semen cryopreservation in cattle and buffalo at the moment. In most of the species, cryo-survival rate of spermtoza is still not optimum. Previously, irreversible damages to spermatozoa caused by cryopreservation have been appraised (Medeiros et al., 2002). The factors which are liable for sperm damage during semen dilution, freezing, and thawing are mainly the temperature change, ice formation, toxicity of cryoprotectants, alterations in sperm membrane and osmotic stress (Watson, 2000). Additionally, lipid peroxidation (LPO) and reactive oxygen species also contribute to reduced sperm motility and fertility rate (Leboeuf et al., 2000) through disrupting the plasma membrane of spermatzoa. The addition of sugars in semen extenders provides energy to spermatozoa and maintain osmotic pressure of the diluents (Aboagla and Terada, 2003). During cryopreservation, about 50% of the spermatozoa are damaged due to exertion of chemical and mechanical stresses, which is reflected in poor quality of post-thaw sperm characteristics. Besides, loss of viable spermatozoa leads to poor fertility rate (45%) as compared to fresh semen (Akhter et al., 2010).

Trehalose is a non-permeable, non-reducing disaccharide, consisting of two glucose moie-

ties joined by an alpha-1, 1 glucosidic bond which prevents spermatozoa from the deleterious effects of dehydration (Aboagla and Terada, 2003). Several studies have been conducted on trehalose supplementation in semen extenders of cattle (El-Sheshtawy et al., 2015), goat (Atessahin et al., 2008), ram (Bucak et al., 2007), and buffalo (Reddy et al., 2010) to improve post-thaw semen characteristics. Different concentrations of trehalose (25-400 mM) have been used in several species to assess its effect on post-thaw semen characteristics such as in goat (Khalili et al., 2009), ram (Jafaroghli et al., 2011) and bulls (Hu et al., 2010; Ahmad and Aksoy, 2012). Results revealed an improved plasma membrane integrity (PMI) of spermatozoa while in buffalo bull semen, it reduced cryo-capacitation and maintained acrosomal integrity (Reddy et al., 2010). In buffaloes, various studies on trehalose have been conducted using tris-based extender (Badr et al., 2014; Iqbal et al., 2016), its use in lactose extender has not been reported yet. The present study was, hence, designed to: 1) study the influence of various concentrations of trehalose on buffalo bull spermatozoa extended in lactose-egg yolk- glycerol extender (LEYGE) and 2) compare the fertility rate between LEYGE supplemented with trehalose and traditionally used tris-citric-acid-fructose extender.

MATERIALS AND METHODS

This study was conducted in two phases. In the first phase, semen was extended in lactose egg yolk glycerol extender supplemented with trehalose at 0.0, 30.0, 50.0, and 70.0 mM concentrations and evaluated for post-thaw sperm characteristics (motility, viability, acrosomal integrity, PMI, DNA integrity and LPO). In the second phase, semen doses of LEYGE (supplemented with 70.0 mM trehalose) and traditionally used tris-citric-acid-fructose extender were used to inseminate multiparous buffaloes (n = 50/group) in heat for fertility trial in September/October. Semen collection, evaluation, processing, and cryo-preservation were carried out at Semen Production Unit (SPU), Qadirabad, District Sahiwal, Pakistan. Post-thaw semen evaluation was performed in post-graduate laboratory of the Department of Physiology, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. The fertility trial was executed in Sheikhupura District at artificial insemination (AI) Centers of Livestock and Dairy Development Department, Government of the Punjab, Pakistan. The study was approved in full by the Office of Research, Innovation, and Commercialization (ORIC) of UVAS, Lahore, Pakistan vide 20-133.

Extenders preparation

The LEYGE was prepared as described by Mughal et al. (2013). Briefly, a 100 gm of D-Lactose monohydrate was dissolved in distilled water to attain a final volume of 1000 mL. After vortexing, the osmotic pressure was maintained at ≥300 mOsm/kg. At the time of semen collection, benzyl penicillin and streptomycin sulphate (1000 IU/mL and 1000 µg/mL, respectively.) At each collection, LEYGE was divided into four parts, maintained at 37°C, and trehalose was added in three extenders at 30.0, 50.0 and 70.0 Mm, respectively, whereas the fourth extender was kept as the control group having no trehalose (0.0) in it.

Tris-citric-acid-fructose extender was prepared as: 24.2 g of Tris (Hydroxymethyl) aminomethane, (Research Organics Inc, Ohio, U.S.A.), 13.4 g of citric acid monohydrate (Riedel-de Haën, Germany) and 10.0 g of D (-) Fructose (Riedel-de Haën, Germany) were mixed in bi-distilled water to achieve 730 mL final volume. This solution was pasteurized at 65ºC for 30 minutes and then cooled down to 38ºC to add 200 mL egg yolk and 70 mL glycerol (Riedel-de Haën, Germany) and 1.0 g benzyl penicillin (Sinobiotic, Shanxi Shuguang Pharmaceutical Co, China). Extender was well mixed at 37ºC using a magnetic stirrer and stored overnight in a refrigerator at 4ºC and kept at 37°C before use.

Semen collection and processing

The semen of Nili Ravi buffalo bull (n = 4) was collected at a weekly interval for 7 weeks. Two ejaculates were collected from each bull at each collection. Ejaculates with >70% motile spermatozoa were chosen for further processing. Semen collection, evaluation,

processing, and storage were carried out as described earlier (Mughal et al., 2013). At each collection, equal volumes of ejaculates were pooled to eliminate individual differences and divided into five equal aliquots. Three aliquots were diluted with LEYGE supplemented with trehalose at 30.0, 50.0, 70.0 mM concentration, the fourth aliquot was diluted in LEYGE without trehalose as a control, whereas, the fifth aliquot was used to process semen doses extended in tris-citric-acid-fructose extender for fertility trial. A total spermatozoa concentration of 20×10^6 was maintained in each of the 0.5 mL straws.

Post thaw spermatozoa characteristics

The semen doses with trehalose in LEYGE, and control group were evaluated for various post-thaw attributes (motility, viability, acrosomal integrity, PMI, DNA integrity, and LPO) after thawing at 37°C for 30 seconds. motility rate of spermatozoa was assessed under a phase-contrast microscope as described, whereas viability rate was evaluated through vital staining (Khan and Ijaz, 2008). Assessment of acrosomal integrity and PMI was carried out using by counting spermatozoa having normal acrosomal ridge and hypo-osmotic swelling test, repectively to assess spermatozoa (Adeel et al., 2009).

Acridine orange staining technique was used to evaluate DNA integrity of spermatozoa under a fluorescent microscope (Labomed Lx 400, USA) as explained by Tejada et al., (1984). Heads of spermatozoa with green fluorescence were counted as having an intact DNA, whereas, heads with red fluorescence were counted as having a damaged DNA. The LPO was determined using thiobarbituric acid assay as described (Wadood et al., 2015). The absorbance of organic layer was estimated at 532 nm and results were expressed as nanomole of malondialdehyde.

Fertility trial

Frozen thawed semn samples were utilized for coductng in vivo fertility trial. Semen doses extended in LEYGE (supplemented with 70.0 mM trehalose) and traditionally used tris-citric-acid-fructose extender were used as treatment and control groups (n = 50 per group), respectively to inseminate the buffaloes in heat under field conditions to assess the fertility rate. Fifty buffaloes in each group were inseminated with semen doses, prepared under the same conditions by the same AI technicians to avoid any variation in the and were kept under the same environment throughout the trial. All the buffaloes were in their 2nd to 4th lactation ad were pluriparus. Thawing of semen straws was performed for 30 seconds at 37°C. The AI was done using an AI gun and the pregnancy test was carried out at day 50 ± 10 post-insemination through rectal palpation. Fertility rate attained at single insemination was recorded.

Statistical analysis

It was executed by using Statistical Package for Social Science (Version 13, SPSS Inc., USA). The results are presented as mean ±SE. The data were analyzed using a one-way analysis of variance (ANOVA). The difference in groups was compared by Duncan's Multiple Range Test. The difference in fertility rate was analyzed using chi-square. The fertility rate has been given along with a 95% confidence interval *i.e.*, $P \leq 0.05$.

RESULTS AND DISCUSSIONS

The results on post-thaw seminal attributes revealed that sperm motility, viability, acrosomal integrity, PMI and DNA integrity were significantly ($P \leq 0.05$) greater in LEYGE supplemented with 70.0 mM trehalose as compared to other groups (Table 1).

Table 1. Post thaw spermatozoa characteristics after supplementing lactose egg yolk extender with various concentrations of trehalose

Trehalose Concentration (mM)	Spermatozoa characteristic (%)					LPO (nm)
	Motility	Viability	Acrosomal Integrity	PMI	DNA Integrity	
0.0	49.3±2.4b	58.0±3.3b	69.9±2.1c	59.7±3.3c	96.0±0.2b	66.2±8.8b
30.0	48.3± 1.7b	59.8±2.9b	74.6±2.3a	60.3±2.3b	97.6±0.1b	66.7±11.0b
50.0	48.3± 1.5b	65.8±3.3a	73.8±1.9b	58.0±2.1c	98.0±0.3a	81.2±12.3a
70.0	51.0± 2.5a	63.2±2.3a	75.1±2.4a	62.0±2.5a	98.4±0.1a	57.33±9.1c

Values are represented as Mean ± S.E

Dfferent letters (a-c) within a column indicate indicate significan at $P \leq 0.05$.

Trehalose is a non-permeant disaccharide, found in various animals and plant tissues. It protects spermatozoa during osmotic changes and creates specific interaction with plasma membrane phospholipids to minimize the cell injuries caused by ice crystals before freezing (Bucak et al., 2007). The spermatozoa motility rate in 70.0 mM trehalose supplemented group (51.0 ± 2.54%) is comparable to those for buffalo bull semen supplemented with 50.0 mM of trehalose (51.25 ± 1.25%) in tris-based extender (Badr et al., 2010). However, other studies conducted by Uysal and Bucak (Uysal and Bucak, 2009) and Bucak et al. (Bucak et al., 2007) on ram semen supplemented with 50.0 mM of trehalose have reported a greater motility rate of 68.0 ± 2.9 and 59.0 ± 2.9%, respectively. The nature of extender used during dilution of semen has vital effects on post-thaw motility of spermatozoa. Another report supports this statement, as a significant drop in post-thaw motility rate was observed at 0 hr for buffalo semen extended in Andromed supplemented with 25.0 mM and 75.0 mM of trehalose (Piri et al., 2014). After using various concentrations of trehalose in this study, it is found that 70 mM trehalose concentration seems an optimal inclusion level of trehalose in LEYGE in terms of sperm post-thaw motility.

Spermatozoa viability also improved significantly (P>0.05) with the supplementation of trehalose at supplementation level of 70.0 mM (63.2 ± 2.3%). Our findings are in accordance with another study that reports 66.0 ± 4.4% viability of ram spermatozoa with the same concentration of trehalose while decreased viability rate compared to that of control group using tris-based extender (Bucak et al., 2007). These results are also in agreement with earlier findings in cattle bull semen using the same concentration of trehalose, which declined significantly as the concentration of trehalose was increased up to 100.0 mM and 200.0 mM (El-Sheshtawy et al., 2015). It is speculated that higher concentrations of trehalose may exert deleterious effects on spermatozoa viability rate. However, the chemical nature of extender, presence of antioxidants in seminal plasma, specie difference, and methods of handling semen might be some of the key factors responsible for these changes.

Spermatozoa with normal acrosome are of prime importance for successful acrosome reaction and fertilization (Bailey et al., 2000). In the present study, the addition of trehalose improved acrosomal integrity, and an increase (P≤0.05) was noticed for 70.0 mM trehalose being 75.1 ± 2.4%. As compared to our results, lower values in rams supplemented with 50.0 and 100.0mM of trehalose using tris extender have been reported being 50.4±0.68 and 38.5 ± 3.0% of spermatozoa with damaged acrosomes, respectively (Bucak et al., 2007; Tonieto et al., 2010). Another study (Iqbal et al., 2016) has revealed a 10.0% improved acrosomal integrity by using 30.0 mM trehalose supplementation in buffalo bull semen extended in tris-citric-acid-fructose extender compared to control group. However, further increase in trehalose concentration in groups supplemented with 45.0 or 60.0 mM of trehalose reduced acrosomal integrity rate. The results of the present study using trehalose supplementation support the finding of work conducted in rams also (Panyaboriban et al., 2015). Trehalose supplemented group showing improved acrosome integrity (5.52%) compared to the control group in the present study is a feature that needs to be assessed using lactose egg yolk glycerol extender in other species.

The plasma membrane is the exterior structure and protective barrier of spermatozoa. if damaged during semen cryopreservation, it exerts detrimental effects on spermatozoa capacitation, acrosome reaction, and sperm-oocyte fusion (Giraud et al., 2000). Structural changes in plasma membranes disturb spermatozoa viability, longevity, and fertility rate (Iqbal et al., 2016). Spermatozoa PMI being higher at 70 mM (62.0 ± 2.5%; P≤0.05)) in LEYGE supplemented by trehalose also depicted significant effects These findings are higher from those reported in rams which were 22.9 ± 1.8 and 49.0 ± 8.9% by supplementing 100.0 mM and 50.0 mM of trehalose, respectively (Bucak et al., 2007; Tonieto et al., 2010). Results of the present study are comparable to those in rams which had 66.1 ± 2.8% PMI of spermatozoa when supplemented with 50.0 mM of trehalose (Uysal and Bucak, 2009). However, while working on different Iberian red deer and stallions, other researchers have reported no effect of trehalose addition

(Fernández - Santos et al., 2007; Squires et al., 2004). On the other hand, a substantial improvement in PMI of buffalo bull semen has been reported elsewhere, though, their reported integrity rate was lower compared to our study (Iqbal et al., 2016). This variation in results may be due to the extender difference in both studies. It is speculated that trehalose renders the plasma membrane less prone to changes during water efflux by, by forming a hydrogen bond between the sugar hydroxyl and phospholipid polar group to substitute the water molecules under cryopreservation (Giraud et al., 2000).

Embryo development is negatively affected by spermatozoa DNA damage caused by cryopreservation. The damage exceeding 8%, is irreparable and might result in impaired development of the embryo and early pregnancy loss. Additionally, oxidative stress on spermatozoa during cryopreservation also supports DNA damage and alters spermatozoa head DNA (Anzar et al., 2002). In bulls, DNA integrity rate of 97% to 99% with high fertility has been reported (Bochenek et al., 2001). In present study, spermatozoa head DNA integrity rate significantly ($P \leq 0.05$) increased with trehalose supplementation at 70mM concentration (98.4 ± 0.1). Results of this study are comparable to another in which supplementing 25.0, 50.0 and 100.0 mM of trehalose and documented 2.64 ± 0.69, 2.56 ± 0.56 and 1.83 ± 0.71% spermatozoa with damaged DNA in buffalo bull semen, respectively (Badr et al., 2010). Similar results in buffalo spermatozoa extended in tris-citric-acid-fructose extender with 30.0 mM trehalose concentration have been also reported (Iqbal et al., 2016). Our results are also in line with another report that adding of trehalose in freezing extender reduced cryodamage of the buffalo sperm (Reddy et al., 2010). Exact mechanism liable for DNA damage is not understood. However, high ROS production as a result of antioxidant imbalance in seminal plasma and high contents of unsaturated fatty acids in sperm plasma membrane are believed to affect nuclear membrane and sperm DNA (Aitken & Krausz, 2001).

Cryopreservation exerts cold shock and oxidative stress, trehalose addition in semen extender can improve antioxidant action to protect spermatozoa plasma membrane and decrease LPO (Aisen et al., 2005). The spermatozoan cryoprotective capacity varies with the concentration of trehalose supplementation in the extenders (Naing et al., 2010). In our study the LPO was significantly ($P \leq 0.05$) lower at 70 mM supplementation of trehalose being 57.33 ± 9.1 nM. This indicates that minimum ROS production took place at this concentration and, hence, stands for good semen quality (Chaudhari et al., 2008).

Based on the influence of various concentrations of trehalose on buffalo bull spermatozoa extended in LEYGE, 70mM was considered most optimal and was carried forward for fertility trial in the present study. In all the SPUs of Pakistan, tris-citric-acid-fructose extender is being used for AI purposes. Hence, a comparison of fertility rate was made between semen doses extended in LEYGE supplemented with 70.0 mM trehalose and traditionally used tris-citric-acid-fructose extender. The fertility rate of inseminated animals through rectal palpation at day 50 ± 10 post-insemination was 38% with routinely used tris-citric-acid-fructose extender and 54% with LEYGE supplemented with 70.0 mM of trehalose (Table 2) being significantly ($P \leq 0.05$) different.

Table 2. Comparison of pregnancy rate in groups inseminated with semen extended in tris citric acid fructose extender and LEYGE supplemented with trehalose

Group	Number of Inseminations	Pregnant (%)	Non-pregnant (%)	95% CI	Chi Square Value	P-value
1*	50	19 (38%)	31 (62%)	24.55-51.45	2.576	0.005
2**	50	27 (54%)	23 (46%)	40.19-67.81		

*Tris citric acid fructose extender
**LEYGE (supplemented with 70.0 mM trehalose)

Microscopic evaluation of cryopreserved semen using different protocols or advanced computer software is not an alternative for estimating fertility through AI. Fertility is dependent on various factors including accuracy of heat detection, time of insemination, and inseminators skill. During the present study, the fertility rate of the trehalose-supplemented group (70.0 mM) was statistically ($P \leq 0.05$) higher as compared to the traditionally used tris-citric-acid-fructose

extender group. The fertility rate of this study is comparatively lower than that of 69.2% in buffalo with tris-citric-acid-fructose extender (Anwar et al., 2008). However, current results for the trehalose-supplemented group are in accordance with the fertility rate (58.55%) reported in buffaloes extended in skimmed milk and better (45.8%) (Akhter et al., 2007). Many factors including milk production, species difference, body condition of the animal, heat detection, lactation state, semen quality, inseminator skills and time of insemination are critical to improving the pregnancy rate of buffalo under field conditions through artificial insemination (Anzar et al., 2003).

CONCLUSIONS

An optimal inclusion level for trehalose in LEYGE in respect to seminal attributes is 70mM. Furthermore, LEYGE supplemented with 70 mM trehalose presented higher fertility rate as compared to traditionally used tris-citric-acid-fructose extender under field conditions. The additive effect of trehalose and fructose needs to be studied in future with a larger sample size and over a longer period of time.

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STUDY OF REPRODUCTION INDICES IN COW POPULATIONS BELONGING TO THE TWO SPECIALIZED MILK BREEDS: HOLSTEIN AND SPOTTED WITH ROMANIAN BLACK FROM AGRO-ZOOTECHNICAL FARMS IN NEAMȚ COUNTY

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Abstract

This paper presents the results obtained with reference to: average age at first calving (AFC), calving interval (CI), service period (SP) and mammary repose (RM). They were studied 3 farms with populations of cows of the breeds: Holstein and Spotted with Romanian Black (BNR). The studied reproduction indicators recorded the following average values: in the farm "Î.I. Dascălu Sînzîiana", in the Holstein breed, AFC - was 31.03 months, SP - 89.24 days, RM - 55.82 days and CI - 458.27 days, in the farm "Nacu Gheorghe" in the Holstein breed, AFC- was 25.72 months, SP - 87.96 days, RM - 57.58 days and CI - 459.51 days, and in the Spotted Romanian Black breed, AFC - was 27.54 months, SP - 97.22 days, RM - 56.92 days and CI - 401.64 days. The best results regarding the breeding activity were obtained in the farm "Î.I. Râtan Gheorghe", in Spotted with Romanian Black cattle, where AFC - was 27.52 months, SP - 80.04 days, RM -59.43 days, and CI - 393 days. From the analysis of the obtained data, it is revealed the need to reduce the service period, in the farms "Î.I Dascălu Sînzîiana" and "Nacu Gheorghe", at the optimal level of 80 days, because it is the only way to improve the reproduction indices.

Key words*: cows, Neamt, reproduction indicators, reproduction management.*

INTRODUCTION

In the world economy, cattle breeding accounts for over 96% of the total milk production. The average annual global consumption of milk is: 98.37 kg/inhabitant, of which 96.31 kg are cattle milk. It is therefore necessary to genetically improve and develop technologies, especially in cattle breeding, which can lead to an increase in milk production to a level where it can meet, in many countries of the world, a large part of human food requirements (Coman et al., 2019). The numerical increase of cattle herds and the continuous improvement of its productive potential, expressed mainly by the increase of milk and meat production, must be a permanent concern for cattle breeders. Along with the other farm factors (differentiated feed on productive levels, milking of lactating cows, hygiene conditions, appropriate microclimate), the reproductive function is specific and dominant (Maciuc, 2012). The gradual return to the traditional growth system, with small and medium farms, a conclusive expression of the privatization process of agriculture, at the same time with the introduction and expansion of appropriate growth and exploitation technologies, must ensure in our country the increase of reproduction indicators (Otiman, 1995). The reproduction process can be conducted and monitored on a farm by estimating the values of reproduction indices. Depending on their level, we can intervene to remove deficiencies during the technological flow (Acatincăi, 2004). Cattle have some reproductive features, namely: they are introduced later for breeding, the first calving takes place between 25-27 months, they produce only one calf per year, the twin calvings represent only 2-4% and the reproductive life is not too long, requiring a high replacement rate (15-25%) (Georgescu et al., 1998).

MATERIALS AND METHODS

In order to make an analysis of the reproductive performances, three farms were studied, with populations of cows specialized for milk production, from the Holstein and Spotted with Romanian Black (BNR) breed, during

01.10.2017 - 30.09.2020, registered in the COP (Official Control of Production). In the agro-zootechnical farm "Î.I. Dascălu Sînzîiana", the performances regarding the breeding function in cattle, from the Holstein breed (29 heads) were analyzed. In the "Nacu Gheorghe" farm, the breeding indicators were estimated for Holstein cattle (29 heads) and Spotted with Romanian Black (25 heads). In the farm "Î.I. Râtan Gheorghe", the reproductive performances were analyzed for BNR cattle (32 heads). A series of indicators were studied such as: age at first calving (AFC), period of service (SP), mammary repose (RM) and calving interval (CI). The primary data were extracted from the records of the holdings, but also from the records of the accredited Associations for performing the control of their own individual performances in the cattle species. They have been systematized, statistically processed and interpreted by methods specific to such research. The statistics, respectively the parameters, which characterize a normal distribution, are on the one hand the average or median, and on the other hand, the dispersion indices, represented by variance, standard deviation, variability coefficient (V%) of the followed character. Statistics are noted in Latin letters: arithmetic mean (X), variance (s^2), standard deviation (s), coefficient of variability (V%), and parameters in Greek letters: theoretical mean (media), variance ($ó^2$) and deviation standard (ó). It should be mentioned that the data analysis was performed in terms of merging and correlating with the numerous observations made directly on farms and with the reporting of the results obtained to the requirements and rules of the European Union (EU).

RESULTS AND DISCUSSIONS

Tables 1 and 2. shows the average and variability of reproduction indices in the populations of cows of the breeds BNR and Holstein. The analysis of the main reproduction indicators showed the following:

- Age of first calving (AFC). This indicator is closely related to the precocity of the breed and is calculated from the zootechnical records, which record the date of birth and the date of first calving. Both breeds are early breeds, so in the Holstein breed the first calving is done at 25 months, and in the Spotted with Romanian Black breed at the age of 27 months (Alexoiu & Roșca, 1988). In the Holstein breed, AFC - had an average value (X = 25.72 months with limits between 20-38 months), in the farm "Nacu Gheorghe", a higher value, respectively (X = 31.03 months with variability between 24-39 months) was registered in the farm "Î.I Dascălu Sînzîiana", which indicates that in this farm, the calves in heat were not detected and sown at the optimal time. The average age at the first calving was close to the data from the specialized literature, in the Spotted with Romanian Black and breed, for the 2 farms (X = 27.54 with variability between 24-39 months) in the "Nacu Gheorghe" farm and, respectively (X = 27.52, with variability between 22- 40 months) in the farm "Î.I Râtan Gheorghe".
- Service-period (SP). For cows that have calved before, it represents the interval (in days) from parturition to fertilization. Individually, after each parturition, the service-period is calculated, using the formula, SP = Zg - Zf in which Zg = the day of fertile sowing, Zf = the day of the last calving. The optimal duration of gestational rest in cows is 80 days, when it ensures a lactation of 305 days (Baul, 2009). This indicator had normal average values for the BNR breed from the farm "Î.I. Râtan Gheorghe" (X = 80.04 with variables between 28-228 and higher values X = 97.22, with variables between 19 and 441 days) in the farm "Nacu Gheorghe" also in the Romanian Black and White breed. For the Holstein breed, this indicator had approximately equal values in the 2 farms, but higher than the optimal value (X = 89.24, with variables between 26 and 228 days) in the farm "Î.I. Dascălu Sînzîiana" and, respectively (X = 87.96, with variables between 24 and 244 days) in the farm "Nacu Gheorghe". The gestation was performed in the first 3-4 cycles, but the individual variability for this index was extreme, the gestation was performed after 228, 244 days and in some cases even after 441 days.
- Mammary repose (RM). The duration of breast rest is, on average, 60 days, being in a

positive correlation with the productive level (Georgescu et al., 1998). This indicator was within normal limits, in terms of average value, of the Holstein breed (X = 55.82, with variables between 10 and 145 days) on the farm „Î.I. If Sînzîiana and, respectively (X = 57.58 with variables between 20 and 125 days) in the farm "Nacu Gheorghe". The average value of this indicator was also in the case of the Spotted with Romanian Black breed, within normal limits (X = 56.92 with variables between 15 and 135 days) in the "Nacu Gheorghe" farm and, respectively (X = 59.43 with limits between 30 and 115 days) on the farm "Î.I. Râtan Gheorghe". The individual variability for this index was in some cases very high, both in the Holstein breed and in the BNR.

- Calving interval (CI). It can be calculated individually, representing the interval between two successive calvings, for cows that have calved before, and results from the formula CI = SP + DG where: SP = gestational rest, DG = gestational duration (Virginia et al., 2003). It is estimated that the interval between calvings is good if its duration is between 365-395 days (Cassandro & Marusi, 2000). The interval between calves is a synthetic index, which best highlights the reproductive activity of a farm (Gîlcă & Doliș, 2006). The average value of this reproduction index, in the Holstein breed populations of the two farms, was well above the normal limits (X = 458.27 with variables between 405 and 516 days) in the farm "Î.I. Dascălu Sînzîiana" and, respectively (X = 459.51 with variables between 392 and 516 days) in the "Nacu Gheorghe" farm. Slightly better results regarding this indicator were obtained for the BNR breed, in the "Nacu Gheorghe" holding having the value (X = 401.64, with variables between 325 and 483 days and, respectively (X = 393, with variables between 329 and 474 days) in the farm "Î.I. Râtan Gheorghe". In farms that raise and farm Holstein cows, the calving interval exceeded the average value of 400 days, which proves that the management of the breeding function was not a basic concern (Figure 1.). Dispersion indices also highlight some particular situations with a calving at an interval of 516 days in the Holstein breed, in both farms.

Table 1. The average and variability of reproduction indexes at Holstein breed cow population registered in the COP (official production register) for 2017-2020

Specification	Samples statistics	AFC (months)	SP (days)	RM (days)	CI (days)
Î.I. Dascălu Sînzîiana - Holstein breed	n	29	29	29	29
	X	31.03	89.24	55.82	458.27
	s	4.57	56.16	30.28	23.98
	s^2	20.96	3154.90	917.29	575.33
	v%	14.72	62.93	54.24	5.23
	Min	24	26	10	405
	Max	39	228	145	516
Nacu Gheorghe - Holstein breed	n	29	29	29	29
	X	25.72	87.96	57.58	459.51
	s	3.68	59.49	26.09	26.57
	s^2	13.56	3539.46	680.75	706.49
	v%	14.30	67.63	45.31	5.78
	Min	20	24	20	392
	Max	38	244	125	516

Table 2. The average and variability of reproduction indexes at Spotted with Romanian Black breed cow population

Specification	Samples statistics	AFC (months)	SP (days)	RM (days)	CI (days)
Nacu Gheorghe Spotted With Romanian Black breed	n	25	25	25	25
	X	27.54	97.22	56.92	401.64
	s	4.8	89.10	26.87	32.95
	s^2	23.11	7940	722.32	1086.24
	v%	17.42	91.58	47.20	8.20
	Min	24	19	15	325
	Max	39	441	135	483

Specification	Samples statistics	AFC (months)	SP (days)	RM (days)	CI (days)
Î.I. Râtan Gheorghe Spotted With Romanian Black breed	n	32	32	32	32
	X	27.52	80.04	59.43	393
	s	4.74	44.60	22.26	37.48
	s^2	22.51	1989.87	495,93	1404.92
	v%	17.22	55.72	37.45	9.53
	Min	22	28	30	329
	Max	40	228	115	474

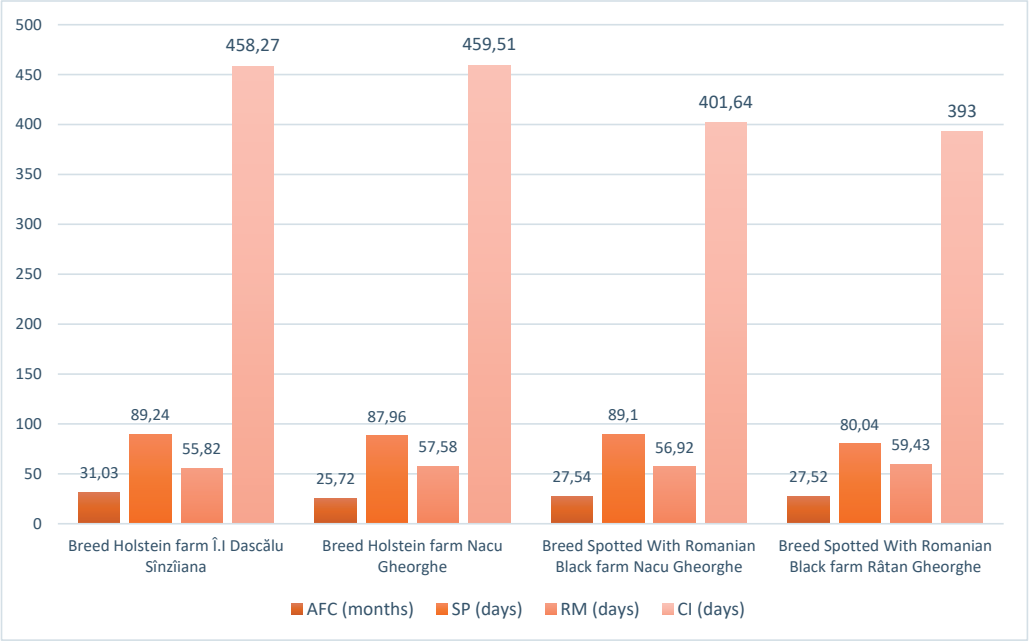

Figure 1. The average of reproduction indexes at Spotted with Romanian Black and Holstein cow population registered in the COP (Official Production Register) for 2017-2020

CONCLUSIONS

From the analysis of the systematized data, statistically processed and interpreted in this study, the following conclusions can be drawn:

1. Age of first calving (AFC). This index had average values close to those indicated in the literature for the 2 breeds analyzed in this study, except for the exploitation of "Î.I. Dascălu Sînzîiana", where this indicator exceeded 30 months, recording the average number of 31.03 months with variables between 24 and 39 months. The age at which cattle should be introduced for breeding has a special importance (correlated with a normal weight and body development) on the economic future of the herd. It should be noted that in cattle, the young are suitable for breeding or artificial insemination, when they reach 70% of adult weight. Late introduction to reproduction can lead to the occurrence of fatty degeneration of the ovaries, and repeated heat causes ovarian cysts, which affect the reproductive function of the calf, reduced calf count and milk production per life time, higher costs per animal and per unit. of product. At the same time, it is possible to observe the premature age of introduction of calves for reproduction, in the “Nacu Gheorghe” farm, at only 10.5 months. Animals do not sow artificially immediately after the onset of sexual maturity, but only at the optimal age for reproduction (in the case of the Holstein breed the optimal age is 15-16 months). Sowing too early can lead to, interruption of the growth process, animals remain at a stage of incomplete body development, below the standard weight of the breed, after calving give totally inadequate milk production, well below

the potential of the breed, the products obtained are also, poorly developed.

2. Service-period (SP). This indicator had the normal average value, for the BNR breed from the farm "Î.I. Râtan Gheorghe" (X = 80.04 with variables between 28-228 and higher values and, respectively (X = 97.29, with variables between 19 and 441 days) in the farm "Nacu Gheorghe", also in the BNR breed. It should be noted in this case as well the great variability between the individuals of the population, the maximum amplitude having high values, of 228 days in the farm "Î.I Râtan Gheorghe" and, very high of 441 days in the farm "Nacu Gheorghe". We also notice that cows were sown in the first heat cycle, but in cows that have more than one calving, the sowing is done in the third heat cycle, especially in high production cows. In the second heat cycle, cows with lower milk production are sown, and under 60 days they are not sown. This reproductive parameter is of particular importance because it influences both the reproductive capacity and the age structure of the cattle herd on the farm, milk production and farm efficiency, so it is necessary to track and detect cows in heat with great responsibility in dairy cows.

3. Mammary repose (RM). This indicator was within normal limits, in terms of average value, both in the Holstein breed and in the Spotted with Romanian Black Breed. Note, however, the individual variability for this index, the minimum value being 10 days in the farm "Î.I. Dascălu Sînzîiana", for the Holstein breed, for 15 days for the BNR breed in the "Nacu Gheorghe" farm and, respectively, for 20 days for the Holstein breed in the "Nacu Gheorghe" farm. Breast rest cannot be reduced below 30 days, regardless of the productive level and even if the cows are in a very good state of maintenance, as the milk production decreases in the next lactation.

4. Calving interval (CI). It is a breeding indicator that can only be calculated in cows that have calved at least twice. Satisfactory results regarding the average value of this indicator were obtained in the BNR breed, in the holding "Î.I. Râtan Gheorghe" having the value (X = 393, with variables between 329 and 474 days) and, respectively in the farm "Nacu Gheorghe" (X = 401.64, with variables between 325 and 483 days), also for the BNR breed. It is also revealed in the case of this indicator, which defines the reproductive activity, the accentuated individual variability, registering high values, well above the optimal value, which is up to 400 days. Calving interval is a weak heritable character, the value of heritability being 0.1, therefore obtaining superior results in improving the management of cattle breeding activity, from the farms studied, will be possible if a series of measures are taken, such as: improving the breeding and exploitation environment of cattle, optimizing the structure of the herd, directing the breeding activity (retention and judicious scheduling of all calves, elaboration of the mating plan, daily detection of females in heat, insemination at the age and optimal moment of the females, monitoring their "return"), establishing the diagnosis of pregnancy, following the involution of the uterus after calving, 7-14 days, practicing the monthly gynecological examination in cows and calves with reproductive disorders, correct and timely recording of all reproductive events. In the cow farms studied, the system of staggered inseminations and calvings is practiced, this involves planning for insemination and, therefore, for calving 20-30% of cows in each quarter of the year, thus ensuring the maintenance of the optimal structure of the herd by physiological conditions respectively throughout the year: 80% of the cows should be lactating and 20% at breast rest. The decrease in the percentage of cows in lactation indicates deficiencies in reproductive activity.

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EVOLUTION OF BLOOD METABOLIC PROFILE AND ANTIOXIDANT ENZYMES ACTIVITIES IN EWES DURING DIFFERENT PHYSIOLOGICAL STATUS

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Abstract

Blood metabolic profiles are widely used to monitor health, reproductive and nutritional status. In the last few years, the evaluation of oxidative stress level of the organism has contributed increasingly as a complementary tool in the evaluation of metabolic status. The aim of this paper was to investigate the possible influence of age and physiological status on blood metabolic profile: Hb, PCV, TP, Creatinine, Glucose, TL, Cholesterol, Triglycerides, ALT and AST in ewes. We also measured two antioxidant enzymes, GPx and SOD to evaluated the oxidative stress level and establish the relationship between those and variation of biochemical parameters. The results showed that all the blood parameters determined in serum, were within or close to normal range value for healthy ewes and were significantly ($P<0.05$) affected by the age and variations of physiological status. A strong correlation ($P<0.01$) was observed in lactation period between antioxidant enzymes and lipid profile. Taking the results together suggests that age and physiological status have to be taken into consideration for a correct interpretation of the serum chemistry values of sheep.

Key words*: age, blood metabolic profile, ewes, oxidative stress, physiological status.*

INTRODUCTION

Sheep keeping was, is and will continue to be part of Romanian agriculture because there is still a great tradition of and experience with sheep production with adapted local and multipurpose breeds (Ilișiu et al., 2013; Wojtas et al., 2014). Among Merino breeds exploited in Romania, Merino of Cluj represents about 0.23% of the total sheep population of the country and had mixed characteristics: fine wool-meat-milk. This breed presents the following morpho-productive traits and it is the one adapted to agro-pedo-climatic conditions of Transylvania, especially to hill zones with a more accentuated raining level, unsuitable for other breeds with fine wool (Dărăban et al., 2009). The availability of information on haematological and biochemical parameters is essentials to research conducted with an aim to increase yields in sheep production (Dönmez et al., 2016). Blood metabolic profile is a set of diagnostic procedures that are based on determining the various indicators in the blood of animals and is affected by the internal and external environment (Oramari et al., 2014; Sharma et al., 2015). For example, age, nutrition, sex, genetics, physiological status, housing, environmental factors, starvation and stress are known to affect haemato-biochemical parameters (Opara et al., 2010; Elzein et al., 2016). One of the important factors is physiological/reproductive status which affect on concentration of indicators in blood that are involved in the development of the blood metabolic profile (Antunovic et al., 2011; Alkudsi et al., 2015). In sheep, the peripartum and early lactation periods are especially critical and present considerable physiological challenges to homeostasis by imposing significant metabolic stressors that may contribute to the onset of diverse disorders (Castillo et al., 2005; Celi, 2010). Also, pregnancy is a period when oxidative stress can be expected due to a high energy demand and increased oxygen requirement (Mohammadi et al., 2016). In the last few years, the detection of free radical damage and protection against it has become increasingly important in clinical medicine as a complementary toll in the evaluation of the metabolic status (Castillo et al., 2005). There are numerous studies on the

effects of oxidative stress during different phases of the reproduction cycle on biochemical parameters in domestic animal species (Roubies et al., 2006). Although, sufficient literature is available on the haemato-biochemical profiles of sheep breeds like Merino (Wojtas et al., 2014; Alhidary et al., 2015; Chauhan et al., 2014) during different reproductive/physiological phases but literature on Merino of Cluj sheep is scarce.

The aim of this study was to investigate the possible influence of age and physiological status on the blood metabolic profile in ewes. We also were evaluated two antioxidant enzymes, Glutathione peroxidase (GPx) and Superoxide dismutase (SOD), with a view to establishing oxidative stress level in ewes during different physiological status.

The parameters were measured in different age groups, to evaluate the effect of gestation, parturition and lactation as a form of stress on Merino of Cluj ewes. Relationship between antioxidant status markers and other biochemical parameters were also investigated. Establishment of reference value was also maintained as an object of this study. For that reason only age and physiological status were evaluated as variables, maintaining all other parameters unchanged.

MATERIALS AND METHODS

This study was conducted during one year at the Research and Development Station for Sheep of the University of Agricultural Science and Veterinary Medicine from Cluj-Napoca (Figure 1).

Figure 1. Research and Development Station for Sheep USAMV Cluj-Napoca (46°46'30.5"N 23°32'43.6"E)

From the flock at the station, 60 estrus synchronized clinically healthy 2-6 years old Merino of Cluj ewes, with a body weight of 45.0 ± 2.30 kg (ranging from 39.0 to 47.0 kg) were used in the trial (Figure 2).

Figure 2. Merino of Cluj ewes

To determine the effect of age of the animals on the normal ranges of the serum biochemical parameters, the sheep were assigned to one of three groups. The first group (PP) was consisted of 20 primiparous ewes aged less than 2 years old, the second group (MP_1) of 20 multiparous ewes aged 2-4 years old and the third group (MP_2) of 20 multiparous ewes aged more than 4 years old. To determine the effect of physiological status of the animals on the blood metabolic profile blood samples were taken in every 3 months over one year and the experiment started at the beginning of autumn. The periods were as follows: early gestation (EG), Post-partum (*Pp*), mid of lactation (ML) and end of lactation (EL). To understand the possible variations on the serum chemistry, sheep raised under the uniform pasture conditions.

To facilitate the restraint and limit variations related to food intake and stress, the blood samples were taken by puncture of the jugular vein with a 21G needle in the early morning before feeding. 4 ml of blood was collected using sterilized needles and plastic syringe in vacutainer plastic blood collection tubes with Li-Heparin (Figure 3). Blood samples were stored at 0-4°C and transported to the university's biochemical analysis laboratory.

From the blood samples we evaluated 12 parameters: Haemoglobin (Hb), Packed Cell Volume (PCV), Total Protein (TP), Glucose, Creatinine, Total Lipids (TL), Cholesterol, Triglycerides, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), GPx and Superoxide dismutase (SOD).

Figure 3. Blood sampling

Hematological parameters, Hb and PCV, were analyzed on the same day as the outrage of the blood samples, and the biochemical parameters were analyzed in the following days. For this, it was necessary to divide the blood samples into two aliquots microcentrifuge tubes and kept frozen at -20°C till further analysis. One aliquot was used for estimation of Hb concentration and hematocrit (PCV) while the other was used for plasma separation. Plasma was separated from the blood by centrifugation at 1200 rpm at room temperature for 5 min. Plasma samples were used to estimate biochemical parameters.

The haematological parameters were determined as follows: Hb was determined by the colorimetric method and the hematocrit value was determined by the Janetzki capillary microhaematocrit method (Kádár, 2002). Haemoglobin is dosed at room temperature by an End-Point colorimetric reaction, and the extinction is read in the visible range at a wavelength equal to 546 nm. The intensity of the colour is directly proportional to the amount of haemoglobin in the sample. The PCV is measured after centrifugation by determining the fraction of total blood volume in a microhematocrit tube that is occupied by erythrocytes (Figure 4).

All biochemical parameters were detected using the commercial available kits. The analyses were carried out according to the manufacturer's instructions. Data were measured on UV/VIS spectrophotometer Screen-Master Touch (λ=340 - 620 nm), (Figure 5). Glucose, PT, cholesterol and triglyceride were dosed by an End-Point colorimetric reaction in the visible range (λ 450-620 nm). Creatinine was dosed by an enzymatic reaction using the Fixed-time method in the UV at 340 nm. The enzymes, ALT, AST and also antioxidant enzymes, GPx and SOD, were dosed at a wavelength of 340 nm in UV by the kinetic method involving the reading of the enzymatic reaction at 37ºC (Kádár, 2002).

Figure 4. The Janetzki capillary microhaematocrit method

Figure 5. Spectrophotometer Screen-Master Touch

All statistical analysis was performed using Graph Pad Prism 7 and Microsoft Office Excel. Mean values, standard errors, minimum and maximum value were calculated for each parameter. To compare the variables according to physiological status in each group was performed by using the one way ANOVA test. In order to explain the probable interactions between various the physiological status Tukey-honest significantly different (HSD test) was used for multiple comparisons of the variables. Statistical significance was established at $P<0.05$. To determine the significance of interactions between antioxidant

status and other biochemical parameters, each period, Pearson correlation was performed.

RESULTS AND DISCUSSIONS

Haematological diagnostic techniques have become an essential part of the minimum data base to monitor and evaluate health and nutritional status of animals. Blood composition is not static, but rapid changes may occur as a response to various physiological events triggered by stress (Polizopoulou, 2010; Badawi & AL-Hadithy, 2014). The influence of the physiological status and the age of the sheep on the haematological parameters in the blood were presented at Figures 6 and 7. The analyses performed demonstrated that, in the Merino of Cluj ewes included in the study, Hb level and PCV value were within the physiological ranges determined in general for sheep (Etim, 2015; Ghergariu et al., 1985) with one exception.

The Hb value for all three groups was lower than the references values during the gestation period (Figure 6). The same things was observed by other authors when they studied the influence of temperature, altitude and landform on some hetological parameters in sheep (Wojtas et al., 2014; Titaouine & Meziane, 2015).

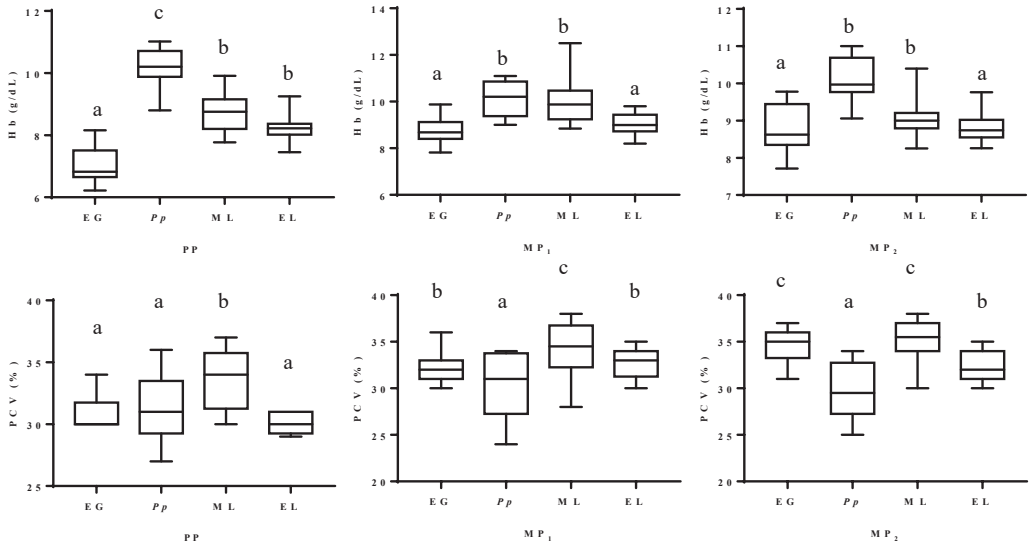

Figure 6. The influence of physiological status on hematological parameters in blood of the Merino of Cluj ewes during the study

Hb=Haemoglobin; PCV=Packed Cell Volume, PP=primiparous ewes aged <2 years old; MP_1=multiparous ewes aged >4 years old; MP_2=multiparous ewes aged 2-4 years old; EG=Early gestation; *Pp*=Post-partum; ML=Mid of lactation; EL=End of lactation.
All values are given as mean and min and max value (n = 20).
a, b, c Means with the same letter do not differ significantly at the 5% level (Tukey's HSD test).

The results also indicated that a significant age difference (P<0.05) for Hb and PCV (Figure 7). The values of Hb (7.02 ± 0.13 g/dl) and PCV (29.75 ± 0.67%) were significantly lower (P<0.05) in primiparous than multiparous sheep, in contrast other researchers who reported a significantly higher haematological values in young than adult sheep (Oramari et al., 2014). Effects of the seasonal variations or age on the hematological parameters were observed on the other ruminant, like buffalo (Enculescu at al., 2017), goats (Piccione et al., 2014; Guzmán & Callacná, 2013) and cattle (Yokus et al., 2006). The Hb values of Merino of Cluj sheep revealed significantly higher (P<0.05) during *Post-partum* period than other reproductive phases and the PCV values were significantly higher (P<0.05) in mid of lactation than other periods (Figure 6).

These results are in agreement with the earlier reports (Antunovic et al., 2011; Sharma et al., 2015). Significantly higher (P<0.05) Hb and PCV concentration in the pregnant ewes and *Post-partum* period are probably due to

increased demand for oxygen and the requirements of higher metabolic rate for pregnancy. According to other study, PCV is involved in the transport of oxygen and absorbed nutrients, and increased PCV shows a better transportation (Erisir et al., 2009). The Hb and PCV subsequently decreased during lactation, which might be attributed to the hemodilution effect resulting from an increase in plasma volume and/or increasing water mobilization to mammary gland through the vascular system (Sharma et al., 2015).

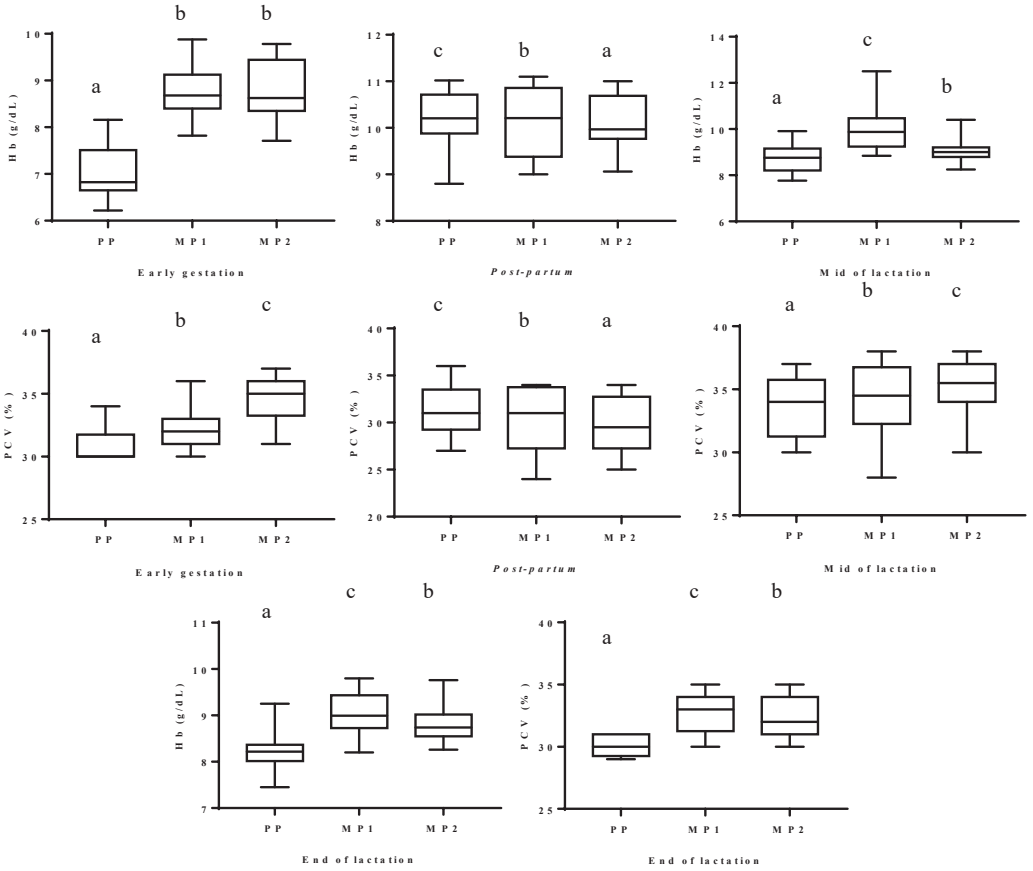

Figure 7. The influence of age on haematological parameters in blood of the Merino of Cluj ewes during the study
Hb=Haemoglobin; PCV=Packed Cell Volume; PP=primiparous ewes aged <2 years old; MP_1=multiparous ewes aged >4 years old; MP_2=multiparous ewes aged 2-4 years old;
All values are given as mean and min. and max. value (n = 20).
a, b, c Means with different letter differs significant (P<0.05) (Tukey's HSD test)

The blood metabolic profile of the Merino of Cluj sheep during different physiological status for each age group was summarized at Table 1. In the current study all the biochemical parameters were within or close to normal range values for healthy ewes (Ghergariu et al., 1985; Kádár, 2002) except TL, cholesterol and triglyceride, who were slightly higher. Hyperlipidemia is found in ketosis of ruminants or other liver disorders and hypercholesterolemia accompanied by increased triglyceride is observed under stressful conditions to which the animal is subjected (Safsaf et al., 2014). Animal reproduction is also negatively affected by the lack of lipids in the body, as it damages the vital cellular phenomena and negatively affects the cell permeability (Kádár, 2002). In our study this increase of total lipids, cholesterol and triglyceride was small and certainly not pathological and was due to the good

maintenance of ewes in the gestation period. Significantly higher (P<0.05) levels of cholesterol were recorded during gestation in present study which might be due to its role in ovarian steroidogenesis. Similar results were also reported by other researcher (Alkudsi et al., 2015; Safsaf et al., 2014; Piccione et al., 2009). The data of serum triglyceride are globally in accordance with total lipids and cholesterol level. Decline in the triglyceride in the blood of ewes in the *Post-partum* period are similar to those of other researcher in goats (Alkudsi et al., 2015; Safsaf et al., 2014; Piccione et al., 2009). Pregnancy and lactation are physiological states considered to modify metabolism in animals and induce stress (Mohammadi et al., 2016).

Major adaptations in maternal physiology and metabolism are required for successful pregnancy outcome (Gürgöze et al., 2009). The values of glycemia were significantly lower (P<0.05) in *Post-partum* (63.67 ± 0.37 mg/dl) than other periods four all three groups. Increased blood glucose concentrations in lactating ewes have to be considered as a result of good maintenance.

Table 1. Mean and standard error of blood metabolic profile of the Merino of Cluj ewes during different physiological status for each age group

Biochemical parameter	Group n=20	Physiological status			
		Early gestation	*Post-partum*	Mid of lactation	End of lactation
Total Protein (g/dL)	PP	$6.31 \pm 0.05^{a,A}$	$7.02 \pm 0.07^{b,A}$	$6.38 \pm 0.07^{a,A}$	$6.47 \pm 0.06^{a,A}$
	MP_1	$6.40 \pm 0.07^{a,B}$	$7.07 \pm 0.05^{b,A}$	$6.40 \pm 0.07^{b,A}$	$6.93 \pm 0.07^{a,C}$
	MP_2	$6.29 \pm 0.03^{a,A}$	$7.21 \pm 0.03^{c,B}$	$6.62 \pm 0.08^{b,B}$	$6.82 \pm 0.09^{b,B}$
References value		6.0 – 7.9 (Fielder, 2015); 6.0 – 6.5 (Ghergariu et al., 1985; Kádár, 2002)			
Creatinine (mg/dL)	PP	$0.73 \pm 0.02^{a,A}$	$0.87 \pm 0.01^{b,B}$	$0.89 \pm 0.01^{bc,A}$	$0.91 \pm 0.01^{c,C}$
	MP_1	$0.75 \pm 0.01^{a,B}$	$0.87 \pm 0.01^{b,B}$	$0.90 \pm 0.01^{b,B}$	$0.87 \pm 0.01^{b,A}$
	MP_2	$0.77 \pm 0.01^{a,C}$	$0.84 \pm 0.01^{b,A}$	$0.90 \pm 0.01^{c,B}$	$0.88 \pm 0.01^{c,B}$
References value		1.2 – 1.9 (Fielder, 2015; Ghergariu et al., 1985) 0.5 – 2.0 (Kádár, 2002)			
Glucose (mg/dL)	PP	$84.06 \pm 0.48^{b,B}$	$63.67 \pm 0.37^{a,A}$	$82.37 \pm 1.79^{b,C}$	$83.12 \pm 3.02^{b,A}$
	MP_1	$79.18 \pm 0.61^{b,A}$	$68.40 \pm 1.66^{a,C}$	$79.51 \pm 1.36^{b,B}$	$82.04 \pm 2.61^{b,A}$
	MP_2	$78.69 \pm 0.15^{b,A}$	$64.67 \pm 0.48^{a,B}$	$74.94 \pm 1.11^{b,A}$	$88.25 \pm 2.34^{c,B}$
References value		50.0 – 80.0 (Fielder, 2015, Kaneko et al., 2008]); 30.0 – 60.0 (Ghergariu et al., 1985, Kádár, 2002)			
Total Lipids (mg/dL)	PP	$497.1 \pm 0.47^{c,A}$	$182.7 \pm 1.46^{a,A}$	$382.7 \pm 5.48^{b,A}$	$342.4 \pm 10.5^{b,A}$
	MP_1	$499.3 \pm 0.52^{c,B}$	$209.0 \pm 5.27^{a,B}$	$384.1 \pm 8.43^{b,A}$	$366.5 \pm 7.20^{b,C}$
	MP_2	$499.2 \pm 0.35^{c,B}$	$203.5 \pm 4.09^{a,B}$	$406.2 \pm 8.64^{bc,B}$	$356.9 \pm 8.09^{b,B}$
References value		382.0 (Ghergariu et al., 1985, Kádár, 2002)			
Cholesterol (mg/dL)	PP	$196.1 \pm 0.35^{c,B}$	$62.45 \pm 1.57^{a,A}$	$92.21 \pm 2.02^{ab,A}$	$128.4 \pm 3.63^{b,C}$
	MP_1	$196.6 \pm 0.91^{c,B}$	$65.39 \pm 1.90^{a,C}$	$99.15 \pm 2.46^{ab,B}$	$126.5 \pm 3.22^{b,B}$
	MP_2	$195.7 \pm 0.37^{c,A}$	$63.19 \pm 0.56^{a,B}$	$99.27 \pm 1.55^{ab,B}$	$122.7 \pm 2.35^{b,A}$
References value		52.0 – 76.0 (Fielder, 2015, Kaneko et al., 2008); 64.0 – 108.0 (Ghergariu et al., 1985; Kádár, 2002)			
Triglyceride (mg/dL	PP	$241.5 \pm 0.70^{c,A}$	$34.69 \pm 1.37^{a,A}$	$125.1 \pm 6.79^{b,A}$	$146.2 \pm 9.33^{b,A}$
	MP_1	$245.1 \pm 0.18^{c,B}$	$36.21 \pm 1.60^{a,C}$	$124.3 \pm 6.92^{b,A}$	$147.2 \pm 6.71^{b,A}$
	MP_2	$245.3 \pm 0.13^{c,B}$	$35.45 \pm 0.50^{a,B}$	$127.9 \pm 5.93^{b,B}$	$150.8 \pm 4.57^{b,B}$
References value		NA			
ALT (U/L)	PP	$21.01 \pm 0.46^{c,A}$	$13.17 \pm 0.11^{a,B}$	$16.48 \pm 0.39^{b,A}$	$22.29 \pm 0.34^{c,B}$
	MP_1	$21.05 \pm 0.59^{c,A}$	$11.98 \pm 0.22^{a,A}$	$16.64 \pm 1.01^{b,A}$	$21.96 \pm 0.92^{c,A}$
	MP_2	$21.95 \pm 0.59^{b,B}$	$12.01 \pm 0.17^{a,A}$	$19.21 \pm 0.65^{b,B}$	$22.11 \pm 0.62^{b,B}$
References value		6.0 – 20.0 (Kaneko et al., 2008); 26.0 – 34.0 (Fielder, 2015); 20.0 – 40.0 (Kádár, 2002)			
AST (U/L)	PP	$95.35 \pm 0.86^{b,A}$	$70.95 \pm 0.75^{a,A}$	$75.57 \pm 3.26^{a,C}$	$101.6 \pm 3.65^{b,C}$
	MP_1	$99.73 \pm 3.16^{b,B}$	$73.67 \pm 0.96^{a,C}$	$69.28 \pm 2.06^{a,A}$	$97.72 \pm 2.69^{b,A}$
	MP_2	$95.09 \pm 1.55^{c,A}$	$72.30 \pm 0.85^{a,B}$	$82.09 \pm 3.32^{b,B}$	$98.34 \pm 1.94^{c,B}$
References value		60.0 – 280.0 (Fielder, 2015; Kaneko et al., 2008); 25.0 – 510.0 (Kádár, 2002)			

ALT=Alanine aminotransferase; AST=Aspartate aminotransferase; PP=primiparous ewes aged <2 years old; MP_1=multiparous ewes aged >4 years old; MP_2=multiparous ewes aged 2–4 years old;; NA=Not available.

All values are given as mean ± standard error. In this study, measured variables was compared with both according to the periods in same group (Tukey's HSD test) and between the groups.

^{a,b,c} Means with different superscripts within a row differs significant (P<0.05)

^{A,B,C} Means with different superscripts within a column differs significant (P<0.05)

Specifically, these changes suggest that the combination of increased utilization of glucose for milk lactose synthesis and the high intake of nutrients during investigation was sufficient to maintain blood glucose homeostasis (Antunovic et al., 2011). Current results are consistent with earlier report in lactating ewes (Mohammadi et al., 2016; Roubies et al., 2006) and lactating goats (Elzein et al., 2016).

A highly significant difference was noted between the groups (P<0.05). Total plasma proteins showed a decreasing trend from lactation to gestation period, which might be due to the preparation of reproductive system during pregnancy (growth of uterus) which requires large quantity of protein during pregnancy (Mohammadi et al., 2016; Mireșan et al., 2003). The highest value of total protein (7.21 ± 0.03 g/dl) was registered in the second group (MP_2) in *Post-partum* period, and the lowest value was observed in early gestation for all three groups. The decrease of protein concentration after parturition is due to using protein for colostrum production.

Similar results were also reported in sheep (Antunovic et al., 2011; Gürgöze et al., 2009) in goats (Opara et al., 2010) and cattle (Yokus et al., 2006). The values of TP were significantly lower (P<0.05) in primiparous then multiparous sheep. The physiological status, in present study, had significant effect (P<0.05) on the serum concentration of TP and creatinine.

Creatinine content of serum was statistically significant higher (P<0.05) in multiparous ewes in mid of lactation (0.90 ± 0.01 mg/dl) than primiparous in early gestation (0.73 ± 0.02 mg/dl). The quantity of creatinine formed each day depends on the total body content of creatinine, which turn depends on dietary intake, rate of synthesis of creatinine and muscle mass (Piccione et al., 2009) In other study the serum creatinine concentration decreased during lactation and increased during post-weaning (Gürgöze et al., 2009).

In these investigation activities of enzymes in the blood of the Merino of Cluj sheep were in physiological limits any gestation and lactation dependent changes. The effects of pregnancy in serum AST and ALT activity levels are somewhat controversial. In a few studies, an increase in AST and ALT activities have been found at *Post-partum* (Elzein et al., 2016; Antunovic et al., 2011) while some researchers (Opara et al., 2010; Wojtas et al., 2014) determined a decreased in that Gürgöze et al. (2009) observed an increase in AST and ALT levels during pregnancy. However, in some published studies, serum AST activity levels do not change during pregnancy and the *Post-partum* period (Yokus et al., 2006; Wojtas et al., 2014). The lowest value (11.98±0.22 U/L) were statistically significant (P<0.05) in *Post-partum* period in MP_1 and the highest significant (P<0.05) value (22.11±0.62 U/L) was noted in MP_2 in end of lactation. Similar results were also reported (Gürgöze et al., 2009; Elzein et al., 2016). Activity of ALT and AST can be recommended as a reliable liver status criterion. The increase in ALT activity and AST in the blood of ewes in lactation indicated an increase in hepatic metabolism. Current results in blood of lactating ewes are consistent with earlier reports (Antunovic et al., 2011).

The organisms are well-equipped with a network of substances capable of counteracting oxidative attack, enzymatic antioxidants, including SOD and GPx, and are the main form of intracellular antioxidant defense (Celi, 2011). Figures 8 and 9 shows the influence of physiological status and age on the oxidative status markers during the study.

SOD and GPx are high molecular weight enzymatic antioxidants that work together in preventing oxidative damage. SOD is responsible for dismutation of superoxide radicals into hydrogen peroxide, whereas GPx is responsible for the removal of hydrogen peroxide (Chauhan et al., 2015; Yuksel et al., 2015). Also, GPx has been used as an indicator of selenium status in animals (Celi, 2010; Chauhan et al., 2014).

It is well known that reactive oxygen metabolites are produced continuously by normal metabolic processes, but the rate of production may be increased markedly under diverse conditions of increased metabolic demand. The metabolic requirements imposed on colostrum production sheep and the onset of lactation far exceed the requirements of the fetus (Castillo et al., 2005).

These individual adaptations, especially after lambing, lead to inter-individual variation in metabolic activities, with variable tissue

consumption of O_2, and hence variable lipoperoxide production (Erisir et al., 2009). The marked individual variations observed in GPx and SOD around lambing may explain the lack of statistical significance. In fact, these individual variations probably reflect not only individual metabolic adaptations but also variations in the physical effort of lambing, which is likely to generate free radicals and thus lipid peroxidation, since increased demand for energy activities mitochondrial respiration in the skeletal musculature and increases oxygen uptake by muscles (Castillo et al., 2005; Erisir et al., 2009; Chauhan et al., 2015). In fact, we found GPx concentrations peaked after lambing, in parallel with SOD concentrations suggesting that the antioxidant system, can cope efficiently with lipoperoxide production, during this critical period and thus protect against oxidative stress, the cause of several reproductive diseases (Castillo et al., 2005). Moreover, the placental environment is one of enhanced oxidative stress that induces protective mechanisms against free radicals as gestation progresses. The plasma free radical trapping and antioxidant potential are able to counteract oxidative stress in normal pregnancy. GPx pregnant sheep protection systems adapted very early to maintain a stable balance (Erisir et al., 2009).

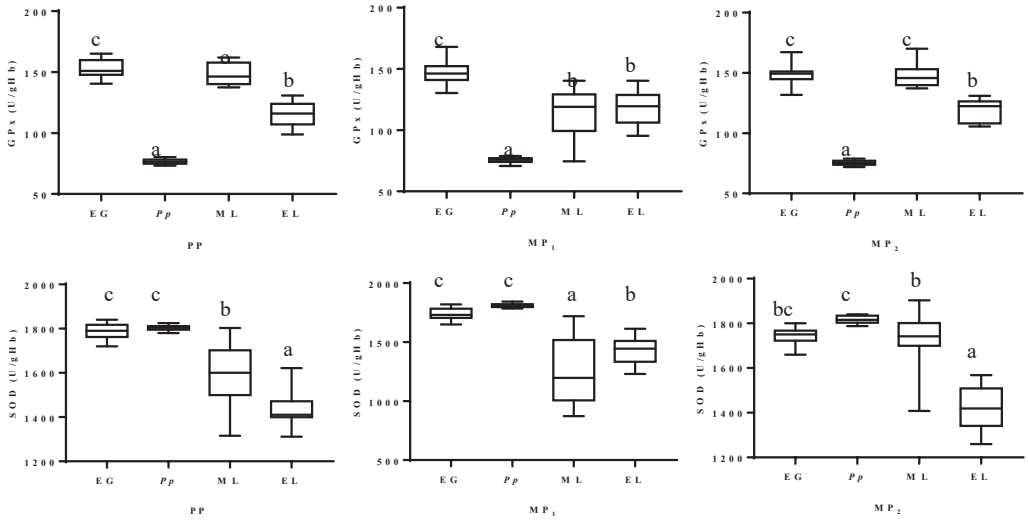

Figure 8. The influence of physiological status on antioxidant enzymes in the blood serum of the Merino of Cluj ewes during the study

GPx=Glutathione peroxidase; SOD=Superoxide dismutase; PP=primiparous ewes aged <2 years old; MP_1=multiparous ewes aged >4 years old; MP_2=multiparous ewes aged 2-4 years old; EG=Early gestation; *Pp*=Post-partum; ML=Mid of lactation; EL= End of lactation.
All values are given as mean and min and max value (n = 20).
a, b, c Means with different letter differs significant (P<0.05) (Tukey's HSD test

During milking period, we observed a gradual increase of oxidative processes, which is probably related to the larger amount of thyroid hormones during the first month of lactation sheep.

These hormones induce lipolysis and intensify the basal metabolism, increasing O_2 consumption, with subsequent excess of free radicals. The high values of GPx and SOD at the end of milking period testify the compensative response of the organism to oxidative stress (Piccione et al., 2006; Piccione et al., 2008). Mean GPx and SOD levels obtained in multiparous and primiparous ewes at all stages were higher than in previous reports of lactating ewes (Erisir et al., 2009) but were in agreement with other researchers (Piccione et al., 2008), who likewise observed a progressive decline in antioxidant activity as lactation progresses, probably due to the depletion of fat-soluble antioxidants by milk (Castillo et al., 2005).The value of GPx and SOD obtained in all three groups can be considered a reflection of the physiological

balance, especially during reproductive cycle. Considering that these metabolic changes are in agreement with previous studies (Sgorlon et al., 2006; Chauhan et al., 2014). However, our mean values of GPx and SOD cannot be compared with previous studies for several reasons: most earlier work was undertaken in sick animals, different management and environmental conditions (Sgorlon et al., 2006; Alhidary et al., 2015; Chauhan et al., 2014), or used different protocols during investigations and the different methods employed (Gaál et al., 2008), so that direct comparison are difficult and previous studies generally considered tissues other than plasma (Piccione et al., 2006; Lipko-Przybylska & Kankofer, 2012; Yuksel et al., 2015).

Our results must therefore be considered in the light of the available literature on the effects on antioxidant status of the homeostasis changes that appear in reproductive cycle of ewes, and the relationships between antioxidant status and other metabolic parameters during the study (Castillo et al., 2005; Erisir et al., 2009).

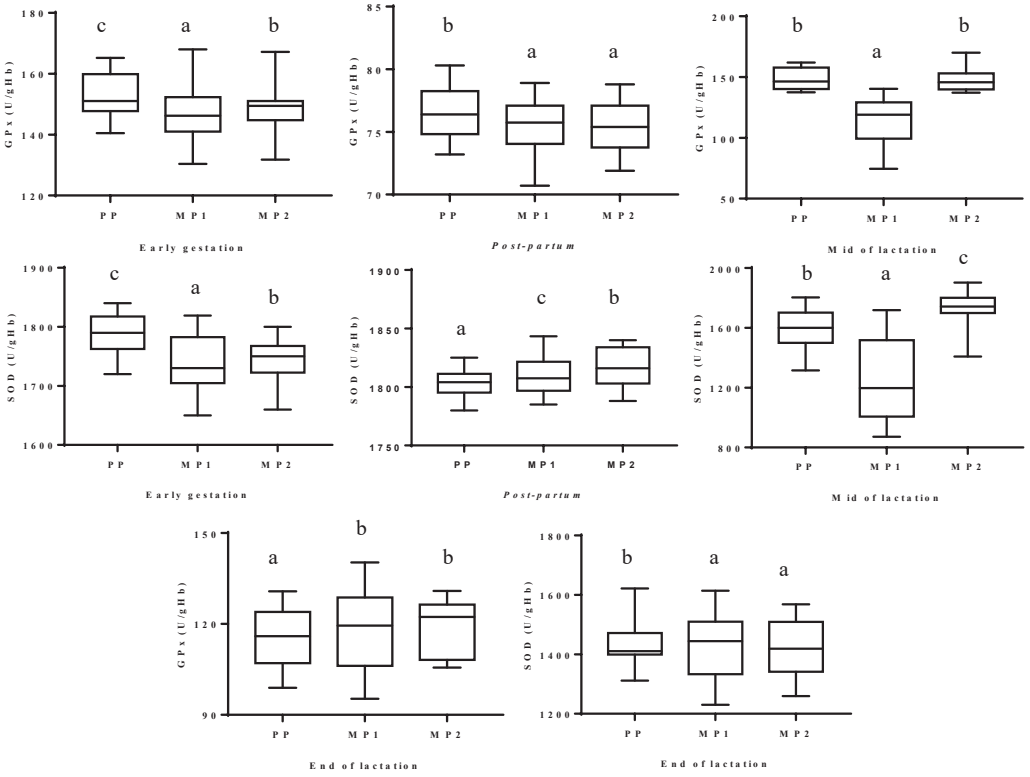

Figure 9. The influence of age on antioxidant enzymes in the blood serum of the Merino of Cluj ewes during the study
GPx=Glutathione peroxidase; SOD=Superoxide dismutase; MP_1=multiparous ewes aged >4 years old; MP_2=multiparous ewes aged 2-4 years old; PP=primiparous ewes aged <2 years old;
All values are given as mean and min and max value (n = 20).
a, b, c Means with different letter differs significant (P<0.05) (Tukey's HSD test)

Table 2 summarizes the correlations observed between GPx and SOD and metabolic parameters in primparous and multiparous ewes for each period. In the primiparous ewes SOD showed a positive correlation with creatinine (P<0.05), TL (P<0.01) and triglyceride (P<0.01) in lactation period. Multiparous ewes in end of lactation showed a negative correlation with Hb (P<0.05), TP (P<0.01), creatinine (P<0.01) and cholesterol (P<0.05). The possibility that metabolic activity may determine oxidant status is supported by various correlations detected depending on the physiological condition.

Table 2. Pearson correlation between anti-oxidant status indicators and other metabolic indicators in each group during different physiological status

		Physiological status							
		Early gestation		*Post-partum*		Mid lactation		End of lactation	
		SOD	GPx	SOD	GPx	SOD	GPx	SOD	GPx
Hb	PP	0.24	-0.16	0.37	0.06	-0.17	0.16	-0.38	**-0.52***
	MP_1	-0.15	-0.10	0.45	0.07	0.32	**0.48***	**-0.46***	0.00
	MP_2	-0.18	0.07	0.38	0.24	-0.28	-0.13	-0.02	0.26
PCV	PP	0.18	-0.13	0.12	**-0.49***	-0.33	0.10	-0.10	-0.32
	MP_1	-0.41	-0.17	0.13	-0.24	0.24	0.22	-0.44	-0.03
	MP_2	0.23	0.06	0.43	0.07	-0.36	**-0.53***	-0.14	0.19
Total Protein	PP	0.26	0.30	-0.17	-0.03	0.14	0.26	-0.03	0.22
	MP_1	0.43	0.11	0.07	-0.34	-0.35	-0.29	**-0.59****	0.02
	MP_2	-0.21	-0.14	-0.14	0.07	0.26	0.13	0.16	-0.14
Creatinine	PP	0.15	0.15	**0.54***	**0.46***	0.08	-0.19	0.00	-0.29
	MP_1	-0.16	-0.26	0.00	0.09	0.09	-0.17	**-0.63****	-0.12
	MP_2	-0.08	0.00	0.26	0.16	0.05	0.16	0.18	**-0.54***
Glucose	PP	-0.40	-0.34	0.35	0.11	0.43	0.00	0.12	-0.34
	MP_1	-0.22	**0.54***	-0.03	-0.03	-0.01	0.30	0.00	-0.44
	MP_2	0.44	-0.36	0.30	0.01	-0.05	-0.12	0.23	0.05
Total Lipids	PP	-0.25	-0.07	0.02	0.14	-0.29	-0.01	**0.64****	0.09
	MP_1	0.09	0.05	0.20	0.14	-0.16	0.01	**0.55***	**-0.63****
	MP_2	0.17	-0.14	-0.13	**0.46***	-0.17	-0.18	0.27	-0.09
Cholesterol	PP	0.21	-0.17	0.34	-0.20	-0.07	-0.23	-0.25	-0.25
	MP_1	0.09	0.32	**0.52***	0.14	**-0.50***	-0.43	0.32	-0.42
	MP_2	0.32	-0.14	-0.16	0.01	-0.25	-0.02	**-0.46***	0.00
Triglycerides	PP	0.21	-0.29	0.29	-0.29	-0.33	0.39	**0.59****	0.39
	MP_1	-0.22	-0.19	**0.55***	-0.14	**0.46***	0.36	0.35	-0.02
	MP_2	0.26	-0.18	-0.04	-0.32	-0.28	0.25	0.05	0.36
ALT	PP	0.30	0.24	0.28	0.17	**-0.59****	0.14	-0.09	-0.38
	MP_1	0.07	-0.09	0.00	**0.47***	0.03	**-0.46***	-0.22	**0.54***
	MP_2	**-0.47***	-0.39	0.19	0.00	0.32	-0.23	-0.21	0.04
AST	PP	0.38	0.00	-0.12	0.16	0.19	-0.21	**-0.47***	-0.22
	MP_1	0.21	-0.19	0.37	-0.41	0.01	-0.11	0.03	-0.08
	MP_2	0.21	-0.35	-0.42	-0.17	**0.52***	-0.26	-0.09	0.23

ALT=Alanine aminotransferase; AST=Aspartate aminotransferase; GPx=Glutathione peroxidase; SOD=Superoxide dismutase; PP=primiparous ewes aged<2 years old; MP_1=multiparous ewes aged>4 years old; MP_2=multiparous ewes aged 2–4 years old;
*P<0.05; **P<0.01.

It is evident from above, that all correlation coefficients among different hematological and serum biochemical which were calculated are expected. Such results are in agreement with those reported earlier by Oramari et al. (2014).

A different pattern was observed in the lactation period, lipoperoxide production showed negative correlations with serum glucose and AST levels, while after lambing lipoperoxide concentrations showed strong positive correlations with lipid metabolism indicators. The relationship between blood lipid and triglyceride in the peripartum period has been well documented and the increase in the oxidative metabolism implies peroxidation of fatty acids leading to formation of lipid peroxides. This antioxidant role acquires great importance if we consider the negative correlation observed between GPx and cholesterol. In fact, cholesterol metabolism requires cytochrome P-450, which is an important source of reactive oxygen metabolites that consume antioxidants (Castillo et al., 2005).

CONCLUSIONS

In conclusions effect of physiological status and age were significantly manifested on the haematological parameters, biochemical indicators and antioxidant status in the blood serum of the Merino of Cluj ewes during the study. Increased metabolic activities due to lactation significantly affected certain biochemical parameters and correlation between the antioxidant enzymes and blood metabolic profile. Our data on GPx and SOD

concentrations suggest that several unknown factors, not only physiological status or age, determine oxidative stress risk around parturition, and these must be taken into account in pursuing research in this area. Taking the results together suggests that age and physiological status have to be taken in to consideration for a correct interpretation of the serum chemistry values of sheep. Therefore, it is recommended development of the blood metabolic profile of ewes and antioxidant enzymes in assessing the nutritional status and ensuring good health states in very demanding physiological conditions, pregnancy and lactation. Hence, the haematological and biochemical parameter values from this research can be used as normal reference to assess the health status of the Merino of Cluj ewes.

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AN OVERVIEW ABOUT GUT MICROBIOTA OF PIGS IN FEED EFFICIENCY

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Abstract

The aim of this study was to investigate the relationship between the gut microbiota and feed efficiency, which is an important parameter in pig production, with economic and environmental impact. The gut microbiota has a fundamental responsibility in nutrient digestibility and influences feed efficacy and metabolism. It provides several functions: supply of enzymes which improve the feed value, metabolism of feed to produce nutrients and synthesis of vitamins useful to the animal body. The animals develop a gut microbiota over time and space. For pigs, the scientists use 16S rRNA amplicon metagenomic sequencing for identify bacteria species and 18S rRNA for fungi. The gut microbiota is an important component of the growth variability in all living organisms, and microbiota knowledge could change the actions to obtain a sustainable and efficient lean meat production.

Key words*: FE, feed, meat, metabolism, pig.*

INTRODUCTION

The attention paid to the intestinal flora in humans, but also in pigs, significantly increased. In the last 15 years more than 20,000 papers have been published on this topic. Due to the diversification of the methodology for identifying microbial species, scientists have discovered new methods to treat various pathologies of the digestive tract, respiratory tract, and ways to manipulate the microbiota, the communities of microorganisms in the gut, for nutritional purposes (McCormack et al., 2017).

The complexity of the microscopic world in the intestine with its involvement in the metabolic and immunological functions of the macroorganism created the title of "new organ" (Ramayo-Caldas et al., 2016).

Volatile fatty acids resulting from microbial metabolism have been shown to interact with intestinal mucosal cells by increasing the absorption of nutrients and thus increasing feed efficiency (FE) (Shirkey et al., 2006; Willing & Van Kessel, 2010).

After birth the animal body is populated with an enormous variety of bacteria, protozoa, archaea, fungi and viruses, whose number varies between 10 and 100 times the total number of cells in the body. The proportion between species of microorganisms changes with increasing and changing diet, for example: weaning, but also by genetic factors such as the host (Thursby & Juge, 2017).

With the improvement of genome segmentation technology, tens of thousands of entities with different functions that regulate the homeostasis of the organism as a whole have been identified and can be considered as an additional organ. To better characterize the phenomenon, two different terms have been introduced: the microbiome, which defines the collection of genomes from all microorganisms in the environment, and the microbiota, which identifies specific microorganisms that are found in a specific environment (Bergamaschi et al., 2020).

MATERIALS AND METHODS

Metagenome is based on diversity and functional prediction. The methodology is realized on 16S ribosomal RNA gene sequencing. 16S/18S/ITS amplicon metagenomic sequencing is frequently used to identify and differentiate microbial species. Short (<500 bp) hypervariable regions of conserved genes or intergenic regions, such as 16S of bacteria and archaea or 18S/ITS of fungi, are amplified by PCR and analyzed

using next generation sequencing (NGS) technology (Novogene - High Quality Gene Sequencing).

The resulting sequences are compared against microbial databases. Applications range from identifying a single species in pure culture and characterizing the microbiota of animals or plants, to comparing species diversity and population structure from various environmental sources or geographic regions.

Metagenomic biomarker is a test of biological consistency and effect size estimation. This addresses the challenge of finding organisms, genes, or pathways that consistently explain the differences between two or more microbial communities, which is a central problem to the study of metagenomics. The method was validated on several microbiomes and a convenient online interface for the method is provided at http://huttenhower.sph.harvard.edu/lefse/ (The Huttenhower Lab, 2021).

Such samples can be analyzed by high-speed DNA sequencing methods, known as metagenomics, metabarcoding, and single-species detection, for rapid monitoring and measurement of biodiversity. To better differentiate between organisms in a sample, DNA metabarcoding in which the sample is analyzed is used and previously studied DNA libraries, such as Basic Local Alignment Search Tool, are used to determine which organisms are present.

The resulting biological observation matrix files were normalized according to known/predicted 16S rRNA gene copy numbers, and the metagenomes were predicted using precalculated Kyoto encyclopedia of genes and genomes (KEGG) orthologs.

Profiling phylogenetic marker genes, such as the 16S rRNA gene, is a key tool for studies of microbial communities but does not provide direct evidence of a community's functional capabilities. It was used the PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states), a computational approach to predict the functional composition of a metagenome using marker gene data and a database of reference genomes. PICRUSt uses an extended ancestral-state reconstruction algorithm to predict which gene families are present and then combines gene families to estimate the composite metagenome. Using 16S information, PICRUSt recaptures key findings from the Human Microbiome Project and accurately predicts the abundance of gene families in host-associated and environmental communities, with quantifiable uncertainty. The results demonstrate that phylogeny and function are sufficiently linked that this 'predictive metagenomic' approach should provide useful insights into the thousands of uncultivated microbial communities for which only marker gene surveys are currently available (Langille et al., 2013).

PCR based on deep pyrosequencing of the 454 platform has revealed extensive microbial diversity that was previously undetected with culture-dependent methods.

DNA sequencing allows the use of data to identify and classify the Bacteria and Archaea microorganisms. EzBioCloud is an integrated database, where the taxonomic hierarchy of Bacteria and Archaea is located. At the genome level are important information's, which contributes to the species taxonomy description. Taxonomically significant information about species can be extracted and statistically compiled using species multiple genomes (Yoon et al., 2017).

The codes for metagenomic analyses are publicly available at https://github.com/strowig-lab/PIBAC, referenced under https://doi.org/10.5281/zenodo.4075065.

The latest bacterial collection from pig intestine can be verified in the article published in Nature Communication by Wylensek et al. (2020).

For the microbial functional prediction, a more accurate evaluation of the biological values is made by correlating the microbiotic profile with the feed efficiency (FE). A series of tests are used such as: salivary cortisol, serum haematological and biochemical tests, immunological tests, serum haptoglobin, lipopolysaccharides in cecal digestion, microbiota profile, concentrations of volatile fatty acids in faeces and digestion (McCormack et al., 2019b).

RESULTS AND DISCUSSIONS

In the past, the relationship between the host and the intestinal microbiota was known as a commensalism or a parasitism; however, recent

researches revealed their relationship as mutualism. It is important to choose the proper feed additives for the growth stage, and therefore, an understanding of the alteration of the intestinal microbiota with the growth of pigs is required (Ramayo-Caldas et al., 2016).

The practical application for correcting intestinal microbial imbalances, but especially for improving the nutritional performance of feed, is the fecal microbiota transplant (FMT). The use of (FMT) showed that growth performance increased significantly without changing the overall microbiome of the subjects (Wang et al., 2019).

From genera co-occurrence network analysis, we revealed several relationships within the swine intestinal microbiota at various growth stages. Overall, a positive correlation was observed between the genera within the same phylum, while a negative correlation was observed between the genera belonging to the different phylum, with some exceptions. The enzymatic equipment of the microbiota contributes to the destructuring of the feed producing metabolites with direct influence on the physiology of the host organism.

The resulting biological observation matrix files was normalized according to known/predicted 16S rRNA gene copy numbers, and the metagenomes were predicted using precalculated KEGG orthologs.

In conclusion, the FE-associated bacterial taxa consistently found across rearing environments may have a role to play in improving FE in pigs, mainly because of their importance in relation to carbohydrate metabolism. In addition, methanogenic members of the Archaea (*Methanobrevibacter*) are also likely to shape FE in pigs. In the future, these FE-associated taxa could potentially be used as probiotics or targeted by dietary means as a strategy for improving FE in pigs. Alternatively, they could be exploited as potential predictive biomarkers for porcine FE (McCormack et al., 2019b).

Xiao et al., in 2016, identified 7.7 million non-redundant genes, representing 719 metagenomic species, by deep sequencing the fecal DNA metagenome from 287 pigs. The study showed that the sex, age and genetics of the host influence the intestinal microbiome of the pig. Analysis of the prevalence of antibiotic resistance genes has demonstrated the effect of eliminating antibiotics from animal diets and therefore reducing the risk of spreading antibiotic resistance associated with agricultural systems. *Firmicutes, Bacteroidetes, Actinobacteria, Spirochaetes* and *Proteobacteria* were the five dominant threads found in the specimens. The microbial diversity of females was significantly higher compared to males; castration increased the intestinal microbial diversity of males. The functional prediction showed that the metabolism of cofactors and vitamins were also rich in the female group; fecal microorganisms of castrated males influenced membrane transport in enterocytes. The genera *Prevotella* and *Ruminococcus* were consistent with the two enterotype groups identified in the pig microbiota (Xiao et al., 2016).

Recently, studies have demonstrated associations of microbial profiles with nutrition and productivity parameters. Notably, the gut microbiota metabolizes various food components, providing nutrients to the host in the form of fermentation end-products and other by-products, amino acids, vitamins, and indole derivatives. In the context of swine FE, the gut microbiota plays important roles in nutrient uptake, energy harvest, and carbohydrate metabolism, particularly in processing indigestible polysaccharides (Yang et al., 2017). Recent studies have reported that the composition of the pig gut microbiota are correlated with nutrient digestibility, average daily gain, and body weight. Variation in the gut microbiome has also been associated with life stage. However, to the best of our knowledge, only a few studies have reported the effect of different microbial populations on feeding efficiency of different breeds. Singh et al., in 2014, reported a correlation between gut microbiota diversity and FE, while Tan et al., in 2017, identified differences in the microbiomes of pigs with high and low feed efficiencies.

The genera *Bacteroides*, *Cellulosilyticum*, and *Prevotella,* were more abundant in low FE pigs, and *Oscillibacter* and *Rhodococcus* were found in animals that were more feed efficient. It is expected that host genetics has the potential to meaningfully influence the gut microbiota, and consequently FE, by favouring

or disfavouring microbes that significantly contribute to nutrient digestion and energy harvest. Therefore, the gut microbiome composition could be associated with intestinal morphology and physiology that can impact the production traits such as growth and feed intake (Tan et al., 2017).

Characterizing the relationship between gut microbial composition and FE revealed a positive association between four genera (*Lactobacillus*, *Blautia*, *Dorea*, and *Eubacterium*) and FE. Moreover, previous studies in swine demonstrated that an increase in the production of short-chain fatty acids could improve the absorptive capacity of the intestine, promoting the growth of beneficial bacteria, thereby increasing FE (Yang et al., 2017; Bergamaschi et al., 2020).

The functional prediction of the microbiome shows that bacterial community interactions in the gut is very complex and the overall functions of the microbiome as a community outweighs the contribution of a single member of that community (Umu et al., 2020).

Microbial diversity varied by geographic location and intestinal sampling site but not by Residual Feed Intake (RFI) rank, except in one geografical location, where more-feed-efficient pigs had greater ileal and cecal diversity. Although none of the 188 RFI-associated taxonomic differences found were common to all locations/batches, *Lentisphaerae*, *Ruminococcaceae*, F16, *Mucispirillum*, *Methanobrevibacter*, and two uncultured genera were more abundant within the fecal or cecal microbiota of low-RFI pigs in two geographic locations and/or in both other geographic location batches. These are major contributors to carbohydrate metabolism, which was reflected in functional predictions. Fecal volatile fatty acids and salivary cortisol were the only physiological parameters that differed between RFI ranks (McCormack et al., 2017).

The gut microbiota is an essential requirement for host health and it performs many functions. These include: driving intestinal development, strengthening intestinal barrier function and controlling epithelial cell proliferation; the provision of enzymes which increase the value of food; metabolism of non-digestible foods to produce nutrients useful to the host; and synthesis of vitamins which cannot be consumed or generated by the host (Lewis, 2013; Pajarillo et al., 2014).

The major ingredients of formula diet provided to the experimental pigs in this study included fibber-enriched corn and high-protein soybean. Therefore, we hypothesized that gut microbiome of the high feed-efficiency pigs might have a greater ability to utilize the dietary indigestible cellulose. The Short Chain Fatty Acids (SCFAs) produced by fermenting dietary polysaccharide are the preferred energy source rather than glucose and lactose for colonic mucosa (Pryde et al., 2002). Moreover, SCFAs could reduce intestinal inflammation, which improves the absorptive capacity of intestine, and increases porcine FE. Interestingly, *L. casei* was also identified to enrich in the high feed-efficiency pigs. As a probiotic, *Lactobacillus* can promote intestinal development and metabolism. The study in chicken showed that *Lactobacillus johnsonii BS15* promotes growth performance and lowers fat deposition (Wang et al., 2017).

These results suggested that the gut microbiome of pigs with the high FE have a greater ability to utilize the dietary polysaccharides and protein. It was inferred that gut microbiota might improve porcine FE through promoting intestinal health by the SCFAs produced by fermenting dietary polysaccharides. However, the functional capacities of gut microbiome inducing fatness might reduce porcine FE. These results provide important insights into how gut microbiome influences porcine FE, and gave the basic knowledge for improving porcine FE through modulating the gut microbiota in pig industry (Yang et al., 2017).

CONCLUSIONS

Studies have shown the direct involvement of the intestinal microbiome in the physiological processes of enterocyte levels by altering membrane transport mechanisms, carbohydrate metabolism and glycocalyx formation.

Intestinal microbiom ecosystems influence the growth rate of pigs. In the future, their handling may lead to an increase in feed efficiency, with implications for production costs and environmental protection

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INFLUENCE OF SEA-BUCKTHORN FRUIT EXTRACT ON THE QUALITY OF MALE RABBIT SEMEN

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Abstract

Obtaining high-quality reproductive material from different species of animals is one of the most important problems of purposefully solving and preserving the animal species, improving the quantitative and qualitative indicators of the resulting products of different animal species, and it can also be predetermined as an important factor in the conservation of biodiversity. This article presents an analysis of the results of the review of specialized literature and experimental data obtained in our laboratory. As a result of the impact of external negative factors on the functioning of the reproductive system of males and the quality of reproductive material, it encourages specialists and researchers in this area to conduct additional comprehensive studies of the reproductive potential of males and to prevent the occurrence of such disorders at any level of organization of the functioning of the reproductive system and the quality of spermatogenesis. All these disorders lead to a lack of antioxidants, which in turn lead to the formation and accumulation of reactive oxygen species (ROS).

Key words*: ecological disturbances, males, morphological changes, reproductive material, reproductive system.*

INTRODUCTION

In order to maintain the constancy of the flow of processes to prevent the formation of ROS as a result of oxidative stress, any living organism needs to receive huge amounts of antioxidants from the outside. One of the richest in such substances are the sea-buckthorn fruits. It contains a lot of caratenoids, flavonoids and many other active substances.

Sea-buckthorn carotenoids include zeastaxanthin, lycopene, and carotenes, but are mainly represented mainly by β-carotene and exhibit antioxidant properties due to its content (Макаркина et al., 2011).

Sea-buckthorn berries contain a complex of flavonoid biologically active substances, such as catechins, leukoanthocyanidins, flavonols, flavonol glycosides. In particular, this complex is represented by such flavonoids as rutin, quercetin, hyperoside and astragalin (Корулькин et al., 2007). Flavonoids in sea-buckthorn fruits have anti-inflammatory, choleretic, antitumor, immunomodulatory and antimicrobial properties (Тринеева et al., 2012).

Flavonoids, in contrast to phenolic antioxidants (tocopherols), in addition to direct antiradical action, are able to bind metal ions with variable valence (transition metals), forming stable chelate complexes. It is known that the formation of such complexes of flavonoids with transition metal ions leads to the inhibition of free radical processes (Afanas'ev et al., 1989). Due to their chelating properties, flavonoids entering the body with food are able to affect the ion (metal) balance and the oxidative status of cells and tissues.

Metal complexes of flavonoids are significantly more effective interceptors of the oxygen anion radical than the initial complexones. In this case, the ligands in the complex are oxidized much more slowly than the free ligands. One of the features of the biological action of flavonoids is an extremely wide range of potential targets that they can act on in the body. On the one hand, this is due to the wide variety of plant pigments themselves, both in terms of their structure and redox properties. At the same time, each specific flavonoid is able to influence many structural and functional

systems of the cell and the body as a whole. As an example, we can point to quercetin, one of the most widespread and studied flavonoids, which is contained in a rather impressive amount in sea-buckthorn fruits. Quercetin from numerous positive effects on different systems and organs of living organisms, through different mechanisms, has an anti-inflammatory effect, namely, it help to stabilize collagen production, inhibit platelet aggregation and stimulate the production of prostaglandins by the endothelium, which in turn lead to vasodilation (Rossi et al., 2003).

MATERIALS AND METHODS

In the study used male rabbits of the New Zealand breed at the age of six months, which were injected with an aqueous extract of sea-buckthorn fruit. For obtain a water extract, we used frozen sea-buckthorn fruits at -50°C, collected from the central part of the Republic of Moldova, the harvest of 2019. The fruits were ground well in a porcelain mortar. The resulting mass was weighed and transferred to a conical flask, where distilled water was added at the rate of 1 g of the resulting mass and 5 ml of distilled water. It mixed well until a homogeneous suspension was obtained and kept in a water bath at 60°C for 40 minutes. The resulting extract was passed through five layers of gauze (for filtration) and, using a disposable syringe, 15 ml of the obtained extract was injected into male rabbits through the oral cavity for 60 days.

RESULTS AND DISCUSSIONS

To date, special attention is paid to the mechanisms of ROS formation and their impact on the reproductive system and the process of spermatogenesis. A number of authors (Tremellen et al., 2008) have proved that as a result of any stress, endogenous initiators of inflammation are first released into the intercellular environment. This is followed by stimulation of phagocytosis, activation of neutrophil NADPH oxidase and, ultimately, the formation of reactive oxygen species - ozone, free radicals, hydrogen peroxide, etc. The imbalance between the production of free radicals and the weakening of antioxidant protection in various parts of the male reproductive system, regardless of the etiological factor, is the main indicator of oxidative stress, which has a positive correlation with the degree of infertility of males of different animal species. Excessive production of ROS, which causes damage of membranes, can lead to a decrease in the motility and fertilizing ability of spermatozoa (Tremellen et al., 2008). ROS can have a direct damaging effect on the DNA of chromosomes, and in addition, they are able to initiate endonuclease-mediated sperm apoptosis, which in the vast majority of cases can cause infertility. ROS are universal limiters of sperm count and regulators of ejaculate quality from the point of view of evolution (Tremellen et al., 2008). Since the main substrate for free radical oxidation are phospholipids, the intensity of lipid peroxidation processes will directly depend on their composition and structural organization, the violation of which can lead to a decrease of motility and quality of spermatozoa and, as a consequence, to infertility (Хышиктуев et al., 2010). Assessment of the level of oxygen free radical generation in the ejaculate is one of the important methods that allow us to characterize the fertility of sperm in the conditions of normospermia and pathospermia (Aitken et al., 2012).

The internal component of the antioxidant system is also very important. Glutathione peroxidase has a unique position in the mammalian reproductive system, as it is directly related to the acquisition and maintenance of sperm integrity. Unlike superoxide dismutase, which is rather a pro-oxidant, forming aggressive and stable H_2O_2 from short-lived superoxide, and the catalase, which is active only at high substrate concentrations, glutathione peroxidase destroys, in addition to hydrogen peroxide, other organic peroxides, even with a slight increase in their concentration, maintaining cell homeostasis (Miao et al., 2009; Sharma et al., 2006; Колесникова et al., 2013). Glutathione peroxidase forms the first response to oxidative stress and acts as a scavenger during ROS leakage and the development of chain uncontrolled processes. Glutathione-S-transferase is the most important polyfunctional protein of ejaculate, since it not only protects

against xenobiotics and ROS, but also, being localized on the surface of spermatozoa, plays the role of a trigger that triggers their interaction with the ligands of the zona pellucida at the stage of initiation of the acrosomal reaction. That is why the determination of the content of glutathione-S-transferase can be used not only to check the antioxidant activity of drugs, but also to establish the fertilizing ability of spermatozoa (Wu et al., 2008).

The production of excessive amounts or accumulation of active substances that interfere with redox reactions can adversely affect macromolecules, cell membranes, and DNA (Valko et al., 2008).

All of the above can change the biological properties of membranes, enzymes and receptors, disrupt the functioning of the cell and lead to its death (Dalle-Donne et al., 2006).

The identification of free radicals as promoters of inhibition of the processes of cell activity and metabolic disorders in it, allowed us to come to the idea that their inactivation or complete blockade (correction of excessive oxidation reactions, or oxidative stress) can be a pathogenetic basis for effective prevention of the manifestation of most dysfunctions in the body of animals, including the state of the reproductive system and the quality of reproductive material. Redox reactions occur with a change in the degrees of oxidation of the atoms that are part of the reacting substances. The change is realized through the redistribution of electrons between the oxidizing atom (acceptor) and the reducing atom (donor), which are an integral attribute of the normal biochemistry of any healthy cell. Since living organisms are an aerobic system, it is oxygen that acts as a key oxidant in the course of cellular redox reactions. About 95% of all consumed oxygen in the cell is reduced in mitochondria to water during respiration and energy synthesis by the cell (in the form of adenosine triphosphate). The remaining 5% as a result of various redox reactions inevitably transform into reactive oxygen and nitrogen forms, which are designated by the general term "free radicals" (Bartz et al., 2010). Thus, the theory of oxidative stress, substantiating the key role of oxidative stress in the pathogenesis of most dysfunctions and necessitating the inclusion in the diet of plant-based foods rich in antioxidants. Increased oxidative stress leads to an imbalance between the production of free radicals and the antioxidant defense of the body (Sastre et al., 2000). At the same time, oxidative stress affects almost all structures of the body, including DNA, proteins and lipids (Weinert et al., 2003). There is evidence that oxidative stress can be an important factor leading to the development of new somatic mutations, which made it possible in 1998 to formulate another daughter theory of aging - the theory of somatic mutations. Thus, normally, the vast majority of biochemical processes in cells occurring under aerobic conditions are associated with the natural formation of free radicals in the course of physiological redox reactions (Rahal et al., 2014). However, some of the free radicals (primary free radicals) are constantly formed in the process of vital activity of the organism and are "useful" for it, since they participate in the whole spectrum of physiological reactions necessary for normal life: a) regulation of cellular processes (cell division, respiration) through signaling dependent on reactive oxygen species; b) providing a bactericidal and oncostatic effect; c) activation of immune reactions of leukocytes; d) providing an anti-inflammatory systemic and local response, etc. Among the most important "physiological" primary free radicals for the body are superoxide anion radical (O_2–), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hypochloric acid (HOCl), nitric oxide (NO), peroxynitrite (ONOO-). Secondary free radicals, unlike primary ones, do not perform physiologically useful functions. On the contrary, they have a destructive effect on cellular structures, seeking to take away electrons from "full-fledged" molecules, as a result of which the "affected" molecule itself becomes a weak (tertiary) free radical. To protect against free radicals (endogenous) formed in the process of vital activity and free radicals coming from outside (exogenous), cells have a special system that inactivates the synthesis and negative effects of free radicals – an antioxidant system. This system is formed by low molecular weight antioxidants and specialized antioxidant enzymes. Key antioxidant enzymes include enzymes of a specialized enzyme system,

which includes superoxide dismutase, catalase, and glutathione peroxidase, which are found in large quantities in the mitochondria. These enzymes are the first to begin to catalyze the biochemical reactions of free radical inactivation, as a result of which free radicals and peroxides are converted into inactive compounds (Меньщикова et al., 2006). In addition, there are numerous non-specialized cellular enzymatic systems for inactivation of free radicals, represented by low molecular weight antioxidants - vitamins A, E, K, C, D, steroid hormones, flavonoids, polyphenols (vitamin P, coenzyme Q10, or ubiquinone), thiol-disulfide system on based on glutathione (in particular alpha-lipoic or thioctic acid - ALA), aromatic compounds, uric acid, taurine, carnosine, acetyl-L-carnitine, L-acetylcysteine, natural chelators of iron, zinc, selenium, manganese, chromium ions and others (Jones, 2008; Костюк et al., 2004). Normally, in a living organism, the principle of the "golden triangle of oxidative balance" is always observed, according to which only a dynamic balance between the level of free radical production, the activity of the antioxidant defense system and the normal functioning of transmitter (transmitting biological signals) biomolecules can ensure the biological safety of the cell and the whole organism as a whole. In case of violation of the "golden triangle of oxidative balance" (hyperproduction and/or excessive intake of free radicals in the body in combination with an insufficient rate of their inactivetion due to a deficiency or depletion of the protective mechanisms of the antioxidant defense system, or a combination of all the above pathological processes), the physiological behavior of the course of redox reactions characteristic of a healthy cell disappears. This is accompanied by a loss of control over the metabolism of free radicals, which leads to a cascading and uncontrolled increase in them in the cell and the body, which is called "pathological oxidative stress" (Carmeli et al., 2002). Excessive oxidative stress affects almost all body structures, including DNA, proteins and membrane lipids. At the cellular and tissue level, it is manifested by various disorders of homeostasis:

1) an imbalance between anti-inflammatory cytokines;
2) endothelial dysfunction;
3) membranopathies due to activation of lipid peroxidation of cell membranes (hypoxia);
4) violation of cellular reception and perception;
5) disorders of the metabolism of biogenic amines;
6) energy and metabolic disorders;
7) disorders of telomerase activity of cell chromosomes.

For effective management of pathological cellular reactions underlying oxidative stress, it is proposed to use biological active substances of plant origin - anthocyanins, polyphenols, flavonoids etc., combined under the general name "antioxidants". Antioxidants can be numerous chemicals, including natural products of the body's activity, and nutrients from food, which neutralize the oxidative effect of free radicals and other harmful substances (Тюзиков et al., 2018). Antioxidants are divided into two large subclasses, depending on whether they are soluble in water (hydrophilic) or lipids (lipophilic). Water-soluble antioxidants are oxidized in the cell cytosol and blood plasma, while fat-soluble antioxidants protect cell membranes from lipid peroxidation on the surface (Костюк et al., 2004). Various antioxidants are present in a wide range of concentrations in body fluids and tissues, while some (glutathione or ubiquinone) are mainly localized within cells, while others (uric acid) are more evenly distributed (Меньщикова et al., 2006; Костюк et al., 2004). Antioxidants are a specific group of chemicals of various chemical structures that have a common property - the ability to bind free radicals and slow down pathologically excessive redox reactions. However, based on the results of well-designed experimental biomedical studies there is still insufficient evidence of the effectiveness of antioxidants in these processes. The choice of specific drugs and their use are not yet fully developed and require further experimental studies.

The scientific staff of our laboratory conducted experiments to study the effect of biological active substances obtained from sea-buckthorn on the reproductive system and the quality of reproductive material.

The data obtained are presented in Table 1.

Table 1. Functional parameters of sperm of male breeder rabbits that were given sea-buckthorn extract

Sperm indicators	Interval, days						
	I	10	20	30	40	50	60
Volum, ml	0.42±0.097	0.72±0.096	0.82±0.097	0.92±0.52	1.0±0.141	1.12±0.21	1.14±0.19
Mobility, %	49.66±1.196	50.92±1.93	55.44±0.88	76.36±0.74	82.6±0.56	88.86±0.79	94.08±0.58
Spermatozoa speed, μm⁄s	18.8±0.83	20.2±0.83	22.6±0.54	22.4±0.54	23.4±0.59	22.0±0.70	22.8±0.44
Spermatozoa concentration, million/ml	121.8±0.83	180.6±1.14	221.2±1.71	285.78±0.148	286.38±0.311	300.28±0.589	340.3±0.524

From the data of the table 1, it can be seen that the studied indicators of spermogram change significantly with a pronounced trend throughout the entire period of the study. The volume of the ejaculate increases on average from 0.42 ± 0.097 to 1.14 ± 0.197 ml, the motility from 49.66 ± 1.196 to 94.08 ± 0.58%, and the concentration of reproductive cells from 121.8 ± 0.83 to 340.3 ± 0.524 million/ml on the 60th day. The speed of movement of reproductive cells is maintained in the range of 18.8 ± 0.83 - 22.8 ± 0.44 μm/sec.

All these changes in the functioning and maintenance of the male reproductive system are caused by polyphenolic compounds that are contained in the composition of sea-buckthorn fruits, due to their antioxidant effect and possessing the main protective properties of the cell membrane, maintaining the stable functioning of cellular components due to the activation and deactivation of many enzymes of the antioxidant system, these substances support the homeostasis of the body as a whole.

CONCLUSIONS

The body's antioxidant defense system are an important role in protecting cells from excess ROS and consists of an endogenous component (uric acid, superoxide dismutase, catalase, glutathione peroxidase, etc.) and an exogenous component (bioflavonoids, carotenoids, tocopherols, ascorbate, etc.).

Changes caused by free radicals affect lipids in the structure of cell membranes, cell organelles (mitochondria, lysosomes) and components of blood vessel walls.

The obtained data indicate a sufficiently high content of antioxidants of the flavonoid structure and indicate the prospects of using extracts from sea-buckthorn fruits to regulate and stabilize the functional activity of the reproductive system, the process of spermatogenesis in male rabbits and further to preserve biodiversity.

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DYNAMICS OF IMMUNOLOGICAL PARAMETERS OF BLOOD SERUM OF CALVES IN THE TREATMENT OF KERATOCONJUNCTIVITIS USING THE DRUG LIGFOL

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Abstract

Ophthalmopathology in young cattle is one of the reasons for the decline in the growth and development of calves. The cause of keratoconjunctivitis is very often a different microflora. Economic losses in keratoconjunctivitis are formed due to a slowdown in the growth and development of young animals, a decrease in productivity, and a loss of live weight. 25-30% of recovered animals remain blind, the same number lose sight by 50%. Determination of the protein spectrum and immunoglobulins in the blood of calves is of great diagnostic and prognostic value, which reflects the degree of intensity of metabolic processes and the level of nonspecific resistance of the organism. Serum proteins are components of a dynamic circulating system and reflect the physiological and biochemical characteristics of the body as a whole. They take part in tissue nutrition, the formation of immunity in infections and invasions (γ-globulin is a fraction of serum globulin, which consists mainly of immunoglobulin antibodies), maintaining pH and osmotic pressure. In ophthalmic diseases, there is a violation of the ratio of plasma protein fractions (dysproteinemia).

Key words*: albumin, blood serum, globulins, keratoconjunctivitis, Ligfol.*

INTRODUCTION

Ophthalmic pathology in young cattle is one of the reasons for the decline in the growth and development of calves. The cause of conjunctival keratitis is very often different microflora (Bezruk, 2019).

Keratoconjunctivitis causes serious economic damage due to the high frequency of manifestation and widespread prevalence in dairy farming (Zagumennov, 2019). Economic losses in keratoconjunctivitis are formed due to a slowdown in the growth and development of young animals, a decrease in productivity, and a loss of live weight. 25-30% of recovered animals remain blind, the same amount lose sight by 50% (Zagumennov et al., 2019).

Determination of the protein spectrum and immunoglobulins in the blood of calves is of great diagnostic and prognostic value, which reflects the degree of intensity of metabolic processes and the level of nonspecific resistance of the organism. Serum proteins are components of a dynamic circulating system and reflect the physiological and biochemical characteristics of the body as a whole. They take part in tissue nutrition, the formation of immunity in infections and invasions (γ-globulin is a fraction of serum globulin, which consists mainly of immunoglobulin antibodies), maintaining pH and osmotic pressure (Ermolaev et al., 2015).

The purpose of our research is to study the dynamics of the immunological parameters of the blood serum of young cattle with keratoconjunctivitis, using various treatment regimens.

Recently, in the Russian Federation, the number of farms unfavorable for infectious keratoconjunctivitis (KCS) has increased, especially among the calves of the current year of birth and fattening animals. Bacterial pathogens are taken into account as a concomitant factor. But there is also a specific bacterial pathogen of KCS - *Moraxella bovis*. This pathogen is not rare (Kondrakhin et al., 2004).

Rather, it is poorly understood due to the lack of diagnostic schemes both in livestock enterprises and in diagnostic laboratories.

Moraxellosis is a highly contagious eye disease in cattle, characterized by conjunctival

hyperemia, lacrimation, photophobia, opacity and ulceration of the cornea, deformation of the eyeball, loss of vision of the affected eye of the animal (Figure 1).
The incubation period lasts from 2 to 18 days, depending on the season of the year and the external ambient temperature. The infection affects one or both eyes of the animal. During clinical examination of sick animals, swelling of the eyelids, conjunctivitis and lacrimation are given: at first, serous-mucous, and a little later, the outflow of purulent exudate.

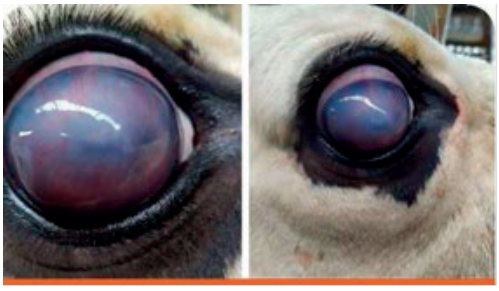

Figure 1. A characteristic symptom of initial stages of keratoconjunctivitis caused by *Moraxella* spp.

The eyesight of animals is weakened, they look for a dark and cool place. Due to the reduced consumption of feed and water, animals lose weight. Palpation reveals soreness of the eyelids, an increase in local temperature. After 24-72 hours, a milky-white cloudiness forms on the cornea, leading to loss of vision.
The causative agent of the disease *Moraxella bovis* belongs to the Neisseriaceae family. Short coccobacilli, located more often in pairs, can form a capsule. Optional aerobic. It grows on media in the form of small, rough, milky-white or yellowish colonies, slightly growing into the medium (Figures 2-3). They exhibit hemolytic properties of varying intensity, directly related to the degree of their pathogenicity. Oxidase- and catalase-positive (Daricheva et al., 2009).
The pathogenicity and virulence factors of moraxella are the surface structures of the bacterial cell - fimbria, which allow bacteria to attach to the epithelial cells of the cornea of the eye. Additionally, the causative agent releases endotoxins and hemolysins, which cause irreversible changes typical for keratoconjunctivitis. Moraxellosis is more often recorded in countries with warm climates and, accordingly, with a long grazing season. In the Russian Federation it is registered everywhere.
The predisposing factors of the disease are:
• crowded content in poorly ventilated rooms,
• grazing animals in hot, dry weather on pastures with high herbage,
• increasing the density of the population of flies,
• hypovitaminosis A.
The seasonality of the disease is seen. In the republic, it is more often registered from mid-summer to late autumn, in isolated cases - in the winter stall period. In a severe form, the disease manifests itself in farms where it was not previously registered.
The source of the disease is infected, sick or ill carriers of bacteria (within several months after recovery), which excrete the pathogen with exudate from the eyes and nose. On the body of mechanical carriers - *Moraxella* flies can survive up to three days. Calves are most susceptible to diseases. After recovering from the disease, animals develop immunity, which restrains repeated infections or causes a mild form of the disease (Kopenkin et al., 2008).
In the pathogenesis of the disease, infectious rhinotracheitis (IRT) often acts as a triggering mechanism. Solar ultraviolet light irritates the cornea of the eye, exacerbating the inflammatory process.

MATERIALS AND METHODS

The diagnosis of moraxellosis is made taking into account clinical data and laboratory research methods. Swabs from affected eyes can be sent to the laboratory on a transport medium.
For the differential diagnosis, viral infections are often taken into account rickettsiosis, mycoplasmosis, chlamydia, and thelaziosis.
Treatment.
One of the most important stages in the treatment of animals is the elimination of accompanying factors: flying insects (mice and flies), mechanical damage, trauma.
As modern means of fighting flies, the use of Larvenol GR and Kelion KE is relevant. Fly control significantly reduces the spread of infection.
As a specific prophylaxis, you can vaccinate animals. However, experience shows that the *Moraxella bovis* vaccine can reduce morbidity

but cannot completely prevent morbidity. Do not forget that the main method of prophylaxis is the quarantine of all newly admitted animals, which are subjected to mandatory clinical examination and diagnostic testing, vaccination according to indications.

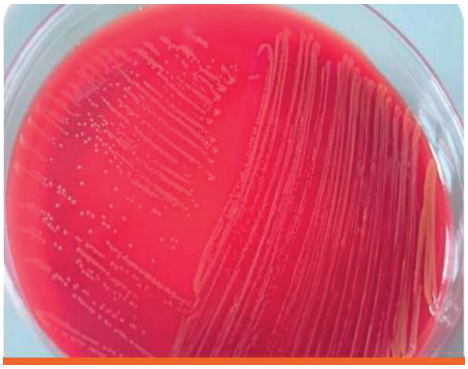

Figure 2. Growth of *Moraxella bovis* on blood agar

Treatment is carried out with broad-spectrum antibacterial agents (gentamicin, ceftiofur, cephalexin, levofloxacin, amoxicillin with clavulanic acid). Treatment is started as soon as the first signs of the disease are identified. An additional agent that alleviates the course of the disease is novocaine blockade. Animals should be placed in a shaded, isolated room. The use of vitamin A is shown.

Disinfection of premises should be carried out with Nanocide, the most effective agent, which includes both detergent components and active substances in the form of glutaraldehyde and quaternary ammonium compounds (QAC).

Animals are provided with free access to clean drinking water, since the secretion of the lacrimal glands provides effective washing of the eyes (Kopenkin et al., 2008).

Based on the analysis of the literature review, it can be concluded that today veterinary ophthalmology has a large number of veterinary medicines to combat infectious keratoconjunctivitis (Shcherbakova, 2013).

According to many scientists, there is a great need for complex treatment of ophthalmopathologies, in which it is necessary to take into account drugs and therapy intended for pathogenetic, symptomatic and etiological methods of treatment.

For the use of etiotropic therapy, much attention is paid to antimicrobial drugs, but these drugs have a large number of disadvantages: resistance of microorganisms, the presence of an antibiotic in milk and meat products, which significantly reduces the veterinary and sanitary quality of the products obtained and, as a result, causes a large number of undesirable consequences in humans with the use of such products (Stekolnikov et al., 2017).

Food allergies in animals often have the etiology of the use of low-quality products, because antibiotics are chemotherapy drugs and their presence in meat and dairy products is unacceptable (Shcherbakova, 2013).

The clinical picture is the most important criterion in the early diagnosis of viral keratitis. However, its inherent polymorphism makes it difficult timely diagnosis of this disease, however, it is possible to identify a group of common signs characteristic of various clinical forms of the disease: frequent connection of viral keratitis with a general infectious disease; the presence of concomitant herpetic eruptions on the skin of the face and the mucous membrane of the lips; neurotrophic nature of the lesion; neurological pain along the branches of the trigeminal nerve; a tendency to relapse.

In the agro-industrial development of the Ulyanovsk region in cattle, data on clinical and etiological signs have not been studied, differential diagnosis and prevention of keratoconjunctivitis is an urgent issue when conducting planned preventive medical examinations.

All the existing pharmaceutical and diagnostic arsenals for the treatment of animal ophthalmopathologies cannot reach the required level for a cost-effective method of these pathologies.

Therefore, the introduction, study and development of new comparative treatment methods available for mass use is of great relevance for veterinary medicine.

Ligfol is a safe new generation stress corrector adaptogen. The drug is of natural origin and is recognized as environmentally friendly. Ligfol contains humic substances obtained by hydrolysis of natural lignin, sodium pyrophosphate decahydrate, sodium chloride and pyrogen-free water.

The drug is a sterile dark brown liquid for intramuscular injection. Ligfol is packed in glass bottles from 1.0 to 100.0 ml. It is recommended to store it in a dry, dark place at a temperature of 10 to 25°C.

Pharmacotoxicological studies have proved that the drug is not toxic, does not exhibit mutagenic and embryotoxic effects.

Recently, more and more interest has been attracted by preparations of natural origin, characterized by harmlessness and environmental safety.

The main advantages of Ligfol are adaptogenic, antioxidant, antitumor and regenerative properties.

The existing effects of using the drug are directly related to its chemical structure, namely the presence of modified humic substances in its composition. The main products of lignin hydrolysis are the so-called humic substances (from the Latin humus - earth, soil).

The formation of humic substances in natural conditions occurs in the soil and peatlands in the process of enzymatic degradation of plant residues.

The study was carried out on the basis of LLC Megaferma - Oktyabrsky, Cherdaklinsky district, Ulyanovsk region. Four groups of Holstein calves were formed at the age of 5-6 months.

Each group consisted of eight heads with characteristic signs of keratoconjunctivitis. All animals had a similar constitution, weight and exercise, were kept in the same microclimatic conditions, their diet was the same. For each group of calves, a specific treatment regimen was determined.

A feature of our chosen treatment was the daily irrigation of the conjunctiva with a 0.5% Dioxidine solution and intramuscular administration of Ligfol in an amount of 5 ml (Table 1).

Table 1. Treatment regimens for calves

Room group	Amount heads	Dioxidin solution 0.5% + Ligfol 5 ml/m + "additional drug"	Status
1	8	Tetracycline ointment 10,000 units	Background/control
2	8	Levomycetin 0.25%, 2-3 drops	Experience
3	8	Ciprofloxacin 0.3%, 2-3 drops	Experience
4	8	Gentamicin sulfate 3%, 2-3 drops	Experience

In the blood serum, the following parameters were determined: Albumin globulins α, β, γ and immunoglobulins, A, M and G.

Blood for the study was taken from the jugular vein into disposable tubes with coagulation activator (SiO2).

The calves were treated daily for 10 days. Blood samples were taken for research on days 1, 3, 7, 10, and 14.

Calf blood serum investigated in a clinical laboratory, the interdepartmental center of veterinary medicine of the Ulyanovsk State Agrarian University using an acoustic, reagent-free, computerized analyzer of protein and protein fractions - AKBa-01- "BIOM®".

The data obtained by us were subjected to statistical processing in the computer program "Statistika 12".

RESULTS AND DISCUSSIONS

Results of the research are reflected in Table 2.

During the period of application of the immunomodulator Ligfol in the first experimental group, the albumin indicator on the third day decreased by 19.9%, in the second group it decreased by 11.72%, in the third group it decreased by 2.2%, and in the fourth group it increased by 1.6%.

On the 14th day of the experiment, it was found that in the first group the albumin level was reduced in all groups, in the first group by 17.8%, in the second group by 9.2%, in the third group by 26.9%, in the fourth group by 16.6%.

The cornea consists of 80% water, 18% definitive collagen of mesenchymal origin, as well as mucopolysaccharides, proteins (albumin, globulin), lipids, vitamins C, B2, etc.

A decrease in the level of albumin is one of the factors in the occurrence of keratitis.

Level α1-globulins on the 3rd day increased by 8%, in group 2 by 8.1%, in group 3 by 11%, in group 4 decreased by 10.8%.

On the 10th day, according to the experimental scheme, the use of the immunomodulatory drug was suspended; in the study of the modified serum on the 14th day of the experiment, it was revealed that in the first group the level α1-globulins decreased by 11%, in the second group by 8%, in the third group increased by 8%, in the fourth group decreased by 6%.

Table 2. Dynamics of immunological parameters of blood serum

Gr.	Day	Albumen	Globulins				Immunoglobulins		
			α1	α2	β	γ	A	M	G
1st group	1	46.17 ± 1.19	4.42 ± 0.22	7.17 ± 0.33	16.20 ± 0.61	20.23 ± 1.04	8.31 ± 0.63	1.19 ± 0.07	1.83 ± 0.02
	3	36.97 ± 3.63 *	4.78 ± 0.19	10.04 ± 2.41	7.94 ± 0.54 ***	22.01 ± 3.19	8.51 ± 1.18	1.50 ± 0.08 *	2.95 ± 0.28 **
	7	37.42 ± 2.25 **	4.34 ± 0.15	6.58 ± 0.60 *	10.30 ± 1.55 **	21.28 ± 1.70	9.58 ± 0.62	1.82 ± 0.07 ***	2.07 ± 0.14
	10	34.05 ± 1.40 ***	4.10 ± 0.20	6.84 ± 0.64	8.53 ± 0.67 ***	20.86 ± 2.38	8.12 ± 0.77	2.06 ± 0.02 ***	2.52 ± 0.31 *
	14	37.92 ± 1.41 ***	3.93 ± 0.27	7.94 ± 0.63	7.97 ± 0.41 ***	15.01 ± 2.34	11.42 ± 1.40	2.22 ± 0.01 ***	2.97 ± 0.16 ***
2nd group	1	39.67 ± 1.44	4.49 ± 0.15	6.78 ± 0.27	14.24 ± 0.56	19.56 ± 0.93	7.56 ± 0.25	1.24 ± 0.08	1.96 ± 0.01
	3	35.02 ± 1.16 *	4.85 ± 0.43	7.45 ± 0.48	9.86 ± 0.34 ***	27.67 ± 2.97 *	8.10 ± 0.53	1.51 ± 0.12	1.83 ± 0.05 ***
	7	35.83 ± 1.32	4.77 ± 0.08	8.55 ± 0.58	10.33 ± 0.95 **	24.82 ± 0.66 ***	10.79 ± 0.36 ***	1.85 ± 0.09 ***	2.45 ± 0.26 ***
	10	36.83 ± 1.59	4.12 ± 0.38	7.15 ± 0.50	10.13 ± 1.18 **	22.43 ± 1.16	10.41 ± 0.43 ***	2.13 ± 0.07 ***	1.92 ± 0.18
	14	36.00 ± 1.08	4.12 ± 0.21	6.41 ± 0.39	6.64 ± 0.64 ***	18.52 ± 2.26	8.11 ± 0.83	2.24 ± 0.04 ***	3.25 ± 0.34 **
3rd group	1	40.77 ± 2.08	4.45 ± 0.25	7.18 ± 0.35	14.74 ± 0.86	20.62 ± 1.12	8.07 ± 0.36	1.37 ± 0.06	1.98 ± 0.02
	3	39.84 ± 1.08	4.98 ± 0.57	6.87 ± 0.29	10.50 ± 1.35 *	19.28 ± 3.23	6.66 ± 0.60	1.60 ± 0.07 *	4.10 ± 0.99
	7	38.43 ± 2.26	4.37 ± 0.43	8.25 ± 0.50	10.12 ± 0.98 **	23.90 ± 1.16	8.91 ± 1.01	2.00 ± 0.04 ***	3.13 ± 0.20 ***
	10	40.56 ± 0.75	3.72 ± 0.26	6.10 ± 0.39	8.06 ± 1.24 ***	19.92 ± 1.93	7.00 ± 0.53	2.17 ± 0.03 ***	1.75 ± 0.20
	14	29.79 ± 3.79 *	4.84 ± 0.42	7.46 ± 0.48	9.64 ± 0.27 ***	25.99 ± 3.34	7.70 ± 0.66	2.42 ± 0.04 ***	3.88 ± 0.05 ***
4th group	1	44.28 ± 0.64	5.00 ± 0.18	7.84 ± 0.20	16.43 ± 0.42	22.36 ± 1.07	9.01 ± 0.30	1.43 ± 0.07	1.96 ± 0.07
	3	45.00 ± 1.74	4.46 ± 0.18	6.94 ± 0.38	15.87 ± 0.73	20.14 ± 1.02	8.07 ± 0.66	1.75 ± 0.08 *	3.92 ± 0.05 *
	7	35.00 ± 1.52 ***	4.06 ± 0.35 *	6.19 ± 0.45 **	9.27 ± 1.12 ***	19.92 ± 1.93	9.28 ± 0.47	2.03 ± 0.07 ***	3.18 ± 0.19
	10	35.32 ± 1.01 **	3.05 ± 0.56 **	6.01 ± 0.43 **	6.70 ± 1.63 ***	12.61 ± 1.73 ***	4.77 ± 0.61 ***	1.92 ± 0.18 *	2.66 ± 0.33
	14	36.90 ± 2.69 ***	4.67 ± 0.39	7.30 ± 0.22	9.74 ± 0.99 ***	23.16 ± 3.10	6.13 ± 0.58 ***	2.41 ± 0.05 ***	3.06 ± 0.26 **

*Note: the difference in values in comparison with the 1st day of the study: *** - p <0.001, ** - p <0.01, * - p <0.05*

The α1-globulin fraction includes acute-phase proteins: α1-antitrypsin (the main component of this fraction) - an inhibitor of many proteolytic enzymes - trypsin, chymotrypsin, plasmin, etc., as well as α1-acid glycoprotein (orosomucoid). It has a wide range of functions, in the area of inflammation it promotes fibrilogenesis.

Globulins include transport proteins: thyroxine-binding globulin, trancortin (functions - binding and transport of cortisol and thyroxine, respectively), α1-lipoprotein (function - participation in lipid transport).

When researching α2-globulins in the first group on the third day showed an increase of 40%, in the second experimental group increased by 9%, in the third group decreased by 4%, in the fourth group by 11%.

On the 14th day of the experiment compared with the 1st day of the experiment, the levelα2 globulins in the first group increased by 10.7%, in the second group decreased by 5.4%, in the third group increased by 3.89%, in the fourth group decreased by 6.8%.

The α2-globulin fraction predominantly includes acute-phase proteins - alpha2-macroglobulin, haptoglobin, ceruloplasmin. Alpha2-macroglobulin (the main component of the fraction) is involved in the development of infectious and inflammatory reactions.

In the study of β-globulins on the third day, a decrease was noted in all groups, in the first group by 50.9% ($p<0.001$,), in the second group 30.7% ($p<0.001$,), in the third group 28.7% ($p<0.05$), in the fourth group by 3.5%.

In the study of modified serum on day 14 compared to the first day, the following data were obtained: in the first group, a decrease in the level of β-globulins by 50.8%, in the second group - 53.3%, in the third group - 34.5% ($p<0.001$), in the fourth by 40.7% ($p<0.001$.)

In the study of γ-globulins on the third day, an increase of 8.7% was noted in the first group, an increase of 41.4% in the second group ($p<0.05$), in the third group there was a decrease of 6.4%, in the fourth group there was a decrease of 9.9%.

In the study of modified serum on day 14 compared with the first day, the following data were obtained: in the first group there was a decrease by 25.8%, in the second group a decrease by 5.3%, in the third group an increase by 26%, in the fourth group an increase of 3.5%.

In the study of immunoglobulin G on the third day, an increase of 61.2% ($p<0.01$), in the second group there was a decrease of 6.6% ($p<0.001$), in the third group there was an increase of 107%, in the fourth group by 100% ($p<0.05$).

In the study of modified serum on day 14 compared to the first day, the following data were obtained: an increase was noted in all groups, in the first group by 62.2% ($p<0.001$), in the second group 65.8% ($p<0.001$), in the third 95.9% ($p<0.001$), in the fourth 56.1% ($p<0.001$).

In the study of immunoglobulin A on the third day, an increase of 2.4% was noted in the first group, an increase of 7.14% in the second group, a decrease of 17.4% in the third group, and a decrease of 10.4% in the fourth group. In the study of modified serum on day 14 compared to the first day, the following data were obtained: in the first group, an increase of 37.4% was noted, in the second group, an increase of 7.2%, in the third, a decrease of 4.5%, in the fourth decrease by 31.9% ($p<0.001$).

In the study of immunoglobulin M on the third day, an increase was noted in all groups, in the first group by 26% ($p<0.05$), in the second group 21%, in the third group by 16% ($p<0.05$), in the fourth by 22% ($p<0.05$).

In the study of modified serum on day 14 compared with the first day, the following data were obtained: an increase was noted in all groups, in the first group by 86.5% ($p<0.001$), in the second group by 80.6% ($p<0.001$), in the third group by 76.6% ($p<0.001$), in the fourth group by 68.5% ($p<0.001$).

Immunoglobulin A protects the mucous membranes of the eye from pathogenic microorganisms, potential allergens and autoantigens.

By binding to antigens, it inhibits their adhesion to the surface of epithelial cells and prevents their penetration into the internal environment of the body.

IgA deficiency leads to repeated infections, autoimmune disorders, and allergies.

CONCLUSIONS

In ophthalmic diseases, there is a violation of the ratio of plasma protein fractions (dysproteinemia).

Dysproteinemias are observed more often than changes in the total amount of protein and, when observed in dynamics, can characterize the stage of the disease, its duration, and the effectiveness of the therapeutic measures.

The results of the study of immunoglobulins A, M, G in the blood serum of sick calves showed a slight increase in their concentrations, which indicates the activation of the humoral mechanisms of immunopathogenesis.

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BIOAVAILABILITY OF HEAVY METALS (Pb AND Cd) IN WILD ROE DEER MEAT

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Abstract

Intense concern in recent years for quantitative and toxicological identification and evaluation, especially for heavy metals from animal origin products represent a side that needs to be studied continuously, especially for game meat, nutritional toxicity of those mineral elements (mainly Pb and Cd) being directly influenced by their variations in habitat water, air and soil. The goal of the study is motivated by the inexistence of information regarding appreciation of contamination degree with heavy metals of game meat, having in view the limited checkout of feeding of game animals exploited in their natural environment, as well as the polluting environmental factors. The analytical results regarding the quantitative evaluation of the Cd concentration of the muscle samples indicate that the average values found in male carcasses are higher than those in female carcasses, except the Trapezius cervicalis muscle. The average concentration of Cd residues ranged from 0.020 ± 0.0028 to 0.040 ± 0.017 mg/kg DM, both limits being attributed to the Triceps brachii muscles. The range of averages corresponding to the other muscle groups are described by an amplitude of the variation of 0.018 mg/kg DM.

Key words*: Roe deer, toxicology, xenobiotic.*

INTRODUCTION

Heavy metals are components naturally present in the environment, having a dual role in this regard. Although low levels of some of them have been considered essential for animal health and growth, having multiple functions in the body (Theron et al., 2012; Govind & Madhuri, 2014; Neila et al., 2017), intensification of human activities and complexity modern industries have led to a continuous increase in their environmental concentrations, with important ecotoxicological effects on wildlife and implicitly on human health (Perez-Lopez et al., 2016).

Even as essential elements for maintaining physiological processes, some heavy metals can be dangerous to the matrices of the ecosystem by their presence in excessive concentrations, so that numerous studies have highlighted their particular toxicity for the entire food chain.

According to Baker et al. (2003), given their structural complexity and their properties, the transfer of metallic elements can be genuinely done from the environment to the biota, being favoured by different parameters. Therefore, considering their persistence and the peculiar capacity for bioaccumulation in trophic elements, concerns about them have been arisen due to their expression within the food chain structure.

Ali et al. (2019) carried out reviews in this subject on various peculiarities of heavy metals, namely their persistence in the environment, toxicity to living organisms and bio-cumulative potential for all elements of the food chain, in this way, highlighting the characteristics of each compound and the subsequent physiological mechanisms developed by the body for their removal.

The animal organism is not exposed to the action of a single chemical substance, but a whole series of chemical compounds present in food, water and air. Each chemical element in the group of heavy metals has its specificity as a way of presentation, manifestation, level of contamination and toxicity on the health of consumers.

According to Aprile & De Bellis (2020), metallic contaminants bind to cellular structures in the human body, mainly affecting

the performance of essential biological functions. The toxic effects caused by heavy metals are enhanced by the level of exposure to the source (Wang & Fowler, 2008), being materialized mainly by changes in physiological processes, combined with several other important disorders in cardiovascular or bone systems (Bouchard et al., 2009). In the same context, Ismail et al. (2017) described the toxic impact on human health and the prevalence level of different metals (Cd, Pb, As, Hg, Ni, Co and Cu) assessed by researchers in food matrices. Since the animal organism functions as an absorbent of all these compounds (Aftab et al., 2011; Ismail et al., 2014), through this study, high proportions of them were found in animal products, especially meat and milk.

To assess the degree of contamination with heavy metals, as well as to quantify their impact, a key element could be their transfer to animals and subsequently, through their production, to humans. Mammals such as wild boar (*Sus scrofa*), deer (*Capreolus capreolus*), foxes (*Vulpes vulpes*) or red deer (*Cervus elaphus*) can be excellent bioindicators of heavy metal contamination, as well as for the concentrations in which they are found in the environment (Srebocan et al., 2011; Bakowska et al., 2016; Friends et al., 2012).

From the environment, the transfer of heavy metals to animal tissues takes place mainly through the digestive tract, mainly due to the intake of contaminated feedstuffs (Latif et al., 2013); in this point of view, for wild animals, food has a particular role (Bakowska et al., 2016). Exposure of plant matter, part of wildlife feed to the many substances with potential environmental pollutants, makes it difficult to manage the generated risks, especially to quantify the diversity of ecosystem contaminants (Voda et al., 2019).

Concerning game animals, assessments on their tissues can provide a relevant perspective on the degree of environmental pollution. Given the specificity of game animals and their living in a free environment, the presence of high levels of toxic elements in their tissues may be a genuine indication for quantifying pollution threats to human health (Amici et al., 2012). The assessment of heavy metals in different tissues can be performed through various techniques, adapted to a specific analyte or desired level of detection. Thus, the concentrations identified in the evaluated matrices could serve to evaluate more accurately the humans' potential exposure to heavy metals and several prevention and reduction actions could be implemented (Demirel et al., 2008; Kazi et al., 2009).

The study of game populations in certain environments that could potentially be altered or damaged by anthropogenic activities provides relevant information on the viability and balance of these ecosystems. Knowledge on the degree of diffusion of heavy metals in soil, water and air, as well as into animal tissues and organs is an important aspect to be taken into account when the safety of game meat products is assessed (Danieli et al., 2012).

Studies related to the proportions of heavy metals identified in tissues of game mammals sampled from heavily polluted areas, specified levels of 1.6-2.1 mg/100 g Pb and 1.6 to 3.0 and 10 mg/100 g Cd, particularly in certain internal organs, such as liver and kidney. In slightly polluted areas, the concentrations ranged between 0.1-0.5 mg/100 g Cd or within the 0.4-1 mg/100 g limits for Pb (Rajaganapathy et al., 2011). For other game species, Florijancic et al. (2015) evaluated the heavy metals content in wild boars muscles and found concentrations of 0.0107-0.0209 mg Pb/100 g, of 0.1425-0.1799 mg Cd/100 g, as well as lower concentrations of Hg (0.0041-0.0097 mg/100 g) or As (0.0130-0.0158 mg/100 g live weight). Similarly, Durkalec et al. (2015) studied the level of bioaccumulation of Pb, Cd and Hg in the muscle tissues of deer and wild boars in areas with different levels of toxic metal pollution and found average concentrations of 0.07-0.43 mg Cd /100 g and of 0.12-0.57 mg Pb /100 g. Mitrănescu et al. (2011) investigated the occurrence of Cd and Pb residues in game meat and found average values of 0.254 mg Cd/100 g and 2.079 mg Pb/100 g, within the maximal limits specified by the Regulation (EC) 1881/2006 establishing maximum levels for certain contaminants in foodstuffs.

The intense concern in recent years for the identification, quantification and toxicological evaluation of heavy metals in animal products is an aspect that needs to be studied

continuously, especially for game meat, because their nutritional toxicity is directly influenced by variations of their proportions in water, air and soil habitat. In animals, the accumulation of heavy metals depends on their concentration in feed, on the duration of exposure or on the age of the animal (Pokorny et al., 2000; Taggart et al., 2011; Lehel et al., 2015; Giżejewska et al., 2017; Pilarczyk et al., 2020).

Given that game species are known as bioindicators for heavy metal toxicity (Millan et al., 2008; Perez-Lopez et al., 2016), both in terms of their distribution areas and in terms of feeding specificity (Schley & Roper, 2003; Baubet et al., 2004), they can be used in various biomonitoring studies (Froslie et al., 2001).

MATERIALS AND METHODS

The purpose of the research is motivated by the lack of information on the assessment of the degree of heavy metal contamination of game meat, taking into account the limited control of the diet of game exploited in its natural environment, as well as other environmental pollutants.

To achieve the proposed goal, investigations were performed on a total of 11 adult individuals of deer (*Capreolus capreolus* L.), out of which 6 males and 5 females. Four muscle areas were taken in the analysis (*Longissimus dorsi*, *Semitendinosus*, *Triceps brachii*, *Trapezius cervicalis*). The individuals were harvested in hunting parties organized in the N ÷ E area of Romania (Suceava Forestry Department, 24 Frasin Hunting Fund) in the hunting seasons 2018-2019 and 2019-2020, according to the provisions of Law no. 407/2006 amended and supplemented by G.E.O no. 102/2010. Analysis of the metal content of the lyophilized muscle tissue samples was performed by the atomic absorption spectrometric method (AAS) on a GBC-AVANTA type atomic absorption spectrometer, provided with burners for flame analysis and a gas source. The principle of the method consists in calcining the samples dissolving the ash in HCl and evaporating to dryness the obtained solution. The final residue is redissolved in 0.1 mol/L nitric acid and the metal content is determined by the atomic absorption method, according to RS EN 14082/2003. The working method included the separation of metal cations from muscle samples (the stage of transition of metals into the ionic state) and their actual determination (instrumental analysis by spectrometer), working simultaneously in duplicate each muscle sample analyzed. The separation of the metals consists of the calcination operation of the biological samples of lyophilized muscle tissue, a process that determined the passage of the metals in ionic or ionizable form. This process allowed the global gravimetric evaluation of the mineral content, because the calcination residue contains all the mineral substances in the form of salts.

After complete calcination of the muscle tissue sample, 5 mL HCl (6 mol/L) was added. The resulting mixture was evaporated in a sea bath and subsequently dissolved in a set volume of HNO3 (0.1 mol/L). The melting pot with the resulting solution was left to stand up for 2 hours, after which, the solution was transferred to a plastic container. The solutions thus prepared were introduced into the apparatus to be read from the constructed calibration curve.

The construction of the calibration curve initially involved the achievement of the optimal operating parameters, provided in the device manual. This step was followed by the successive measurement of the absorbance of each standard solution. The data were statistically processed to achieve the main descriptors (mean, standard mean error, variation coefficient) and were subsequently introduced to analysis of variance, for males vs. females comparisons, using the ANOVA single factor algorithm, under the GraphPad Prism 8.0 statistical analysis software for Windows.

RESULTS AND DISCUSSIONS

Cd is a microelement that is part of the heavy metal group, specifically the seventh most toxic heavy metal (Jaishankar et al., 2014). Rarely present in nature, it is considered one of the most noxious elements, along with Pb, Hg and As. On the other hand, Pb is also considered a metal compound with high toxicity, the sources of contamination, in this case, being more frequent.

The main criteria for which heavy metals are considered dangerous are bioaccumulation, toxicity and persistence, which are considered "risk elements", with toxic effects on both animals and humans. Contamination of animal tissues with Cd occurs as its transmission from the soil, air or water to heavily industrialized areas. The statistical estimators of the values attributed to the Cd and Pb concentration defined an extended dispersion of the data compared to the average, with limits of the coefficient of variation of 45.97 ÷ 114.79% for Cd (Table 1), respectively 82.97 ÷ 162.45% for Pb (Table 2).

The analytical results of the Cd concentration in muscles indicate higher concentration of this element in males samples, in comparison with the females ones, the single exception occurring in *Trapezius cervicalis* samples. For the analyzed muscles, the average concentration of Cd residues showed values in the range 0.020 ± 0.0028 to 0.040 ± 0.017 mg/kg DM, both limits reached in the *Triceps brachii* muscles, the range of averages corresponding to the other muscle groups being described by an amplitude of the variation of 0.018 mg/kg DM.

Compared to the maximum allowed limit of 0.05 mg/kg, regulated for the farm animals meat (EC Reg. No. 1881/2006), the average Cd concentration in game meat was lower.

Table 1. Cd content (mg/kg DM) in deer meat (males and females)

Muscles		Gender	$\bar{x} \pm s_{\bar{x}}$	V%	Min.-Max.	The significance of the differences (Males vs. Females)
Cd	LD	M	**0.031**±0.017	71.685	0.008 – 0.062	$\hat{F} = 0.475$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.022**±0.012	91.957	0.001 – 0.048	
	ST	M	**0.034**±0.008	65.825	0.009 – 0.056	$\hat{F} = 0.578$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.023**±0.015	114.795	0.001 – 0.067	
	TB	M	**0.040**±0.017	45.976	0.02 – 0.060	$\hat{F} = 2.697$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.020**±0.028	75.458	0.009 – 0.041	
	TC	M	**0.027**±0.024	79.309	0.005 – 0.062	$\hat{F} = 0.215$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.036**±0.013	62.421	0.018 – 0.070	
MAL* = 0.05 mg/kg (ppm)						

[1]**LD** = m. *Longissimus dorsi,* [2]**ST** = m. *Semitendinosus,* [3]**TB** = m. *Triceps brachii,* [4]**TC** = m. *Trapezius cervicalis;*
[5]**M** – males, [6]**F** – females, [7]**MAL*** = maximum allowed limit Reg. EC no. 1881/2006.

Table 2. Pb content (mg/kg DM) in deer meat (males and females)

Muscles		Gender	$\bar{x} \pm s_{\bar{x}}$	V%	Min.-Max.	The significance of the differences (Males vs. Females)
Pb	LD	M	**0.045**±0.017	82.971	0.003 – 0.084	$\hat{F} = 0.275$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.070**±0.0275	85.781	0.003 – 0.13	
	ST	M	**0.051**±0.028	126.784	0.005 – 0.16	$\hat{F} = 0.549$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.025**±0.017	162.453	0.004 – 0.101	
	TB	M	**0.037**±0.023	128.596	0.001 – 0.112	$\hat{F} = 0.054$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.044**±0.024	114.585	0.009 – 0.137	
	TC	M	**0.041**±0.025	144.57	0.001 – 0.133	$\hat{F} = 0.075$; $F_{0.05}(1;19) = 4.38$; $\hat{F} < F_{0.05} \Rightarrow$ **ns**
		F	**0.053**±0.031	132.142	0.006 – 0.165	
MAL* = 0.1 mg/kg (ppm)						

[1]**LD** = m. *Longissimus dorsi,* [2]**ST** = m. *Semitendinosus,* [3]**TB** = m. *Triceps brachii,* [4]**TC** = m. *Trapezius cervicalis;*
[5]**M** – males, [6]**F** – females, [7]**MAL*** = maximum allowed limit Reg. EC no. 1881/2006.

Lead is another chemical element with a metallic character. In small quantities, it does not have an essential function for plants or animals even though it is naturally and permanently found in the body tissues. As it is not a constituent of living matter, Pb is considered a non-essential element in food. Lead is a toxicant with a certain aggressiveness towards living organisms, being found on the list of carcinogenic elements. Quantitatively,

the *Longissimus dorsi*, *Triceps brachii* and *Trapezius cervicalis* muscles in carcasses expressed higher average Pb concentration than in males. In the shoulder muscles, the values were quite reversed, and the maximum difference between genders was attributed to the shoulder muscles (Table 2).

The statistically processed data indicated the average value obtained within the interval 0.025 ± 0.017 (m. *Semitendinosus*) to 0.07 ± 0.027 mg/kg DM (m. *Longissimus dorsi*), the amplitude of variation reaching 0.034 mg/kg DM. The statistical significance of the differences between males and females for the values of Cd and residual Pb concentrations was tested through the ANOVA dispersed unifactorial analysis. Thus, for both pollutant heavy metals, the samples did not differ significantly between genders. For both elements, cadmium and lead, the differences between males and females for each type of muscle were analyzed, respectively for m. *Triceps brachii*, m. *Trapezius cervicalis*, m. *Longissimus dorsi* and m. *Semitendinosus*, were 100% statistically insignificant.

CONCLUSIONS

From a toxicological point of view, the analyzes confirmed the presence of heavy xenobiotic metals in deer meat (Cd and Pb), while the average concentrations were LMA compliant, ranging between 0.020 ÷ 0.040 mg/kg DM for Cd and 0.025 ÷ 0.07 mg/kg DM for Pb. Despite the identified proportions, the sources of exposure to heavy metal contamination of wild animals could not be easily quantified, thus highlighting the outstanding persistence and storage capacity of these xenobiotics in the environment. Between the sexes, the differences for the cadmium and lead concentration values were insignificant, for the four analyzed muscle areas.

Although the data obtained are a direct consequence of their bioaccumulation, their low level does not endanger the health of animals, and consequently of the consumer.

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RESEARCHES REGARDING THORACIC PERIMETER AVERAGE PERFORMANCES IN ROMANIAN HUCUL HORSE BREED - HROBY BLOODLINE

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Abstract

Study of average performances in a population is very important. Regarding to a population, the average of phenotypic value is equal with average of genotypic value. That minds that the studies of the average value of characters offer us an idea about the population genetic level. The biological material is represented by 177 Hucul horse from HROBY bloodline divided in 6 stallion families analyzed at 18, 30 and 42 months old, owned by Lucina Hucul stood farm. The average performances for thoracic perimeter was 149.58 cm. at 18 months, 160.21 cm. at 30 months old and 166.97 cm. at 42 months old. We can observe a good growth rate from one age to another and significant differences between sexes. The average performances of the character are between characteristic limits of the breed.

Key words*: bloodline, horse, Hroby, Hucul, Lucina.*

INTRODUCTION

The individual can no longer be a reliable source of information on genetic determinism or in mechanisms of phenotypic manifestation for the quantitative character considered, which makes the unit of study for these characters extend to the population level. Also, in order to study the nature of the quantitative differences regarding the manifestation of the same character in different individuals in different populations, measurements are required which generally do not express the character itself but its value.

The character's average performances, in a population, have a great value because it can offer an overview of the genotypic value. All this is possible because, regarding to a population, the average phenotypic values are equal with the average of genotypic values (Maftei, 2015). More than that, the study of average values of characters, in a population, can offer an idea about populational genetic level (Maftei, 2019).

Tracking of body growth can be done by periodic determination of body weight and body dimensions. As a rule, there is a direct relationship between the weight of an animal and its volume, which means that the dynamics of the weight will, indicate also the dynamics of the dimensions. Determining only the body weight can not always indicate the clearest picture of the evolution of the growth process, as it may happen when the weight remains the same between two determinations (Popescu-Vifor, 1978, 1985).

The growth process can be followed by: growth energy, growth rate, growth intensity, and growth coefficient.

Perhaps more than in other species of economic interest, in horses, phenotypic characters occupy an important place in the breeding programs, as they play an essential role in the expression of production characters.

In this group of characters, the characters expressing the growth process (height, cannon bone perimeter, thoracic perimeter) and those expressing the body conformation specific to the production specificities (running, sports,

jumping, recreation, traction) are predominantly included. These characters belong to the group of morphological characters and are determined by somatometry. Somatometry is the most objective method of assessing the exterior of the horses. In principle, it consists in direct measurement, on the live animal, of the dimensions of the different body regions, or even the characteristic size of the species. In this study we use the cannon bone perimeter values.

MATERIALS AND METHODS

The purpose of using somatometry in assessing the exterior of the horses is to determine, first of all, body development, but also to establish the overall harmony of the specimen (Marginean et al., 2005).

In this study we analyze the thoracic perimeter, measured with the ribbon and representing the thoracic circumference.

Body size judgments (valid for both young and adult animals) are usually based on the scales set for the standard of each breed, or according to the scales set by the breeding program.

The characteristic limits for each character are different from a breed to another and also between the two sexes. To reach the maximum limit, note 10 is given. For the minimum limit and below this limit, note 4 is given. The exceeding of the maximum values is penalized by subtraction of the note.

For realising purposed objectives, the biologic material became from Lucina stood farm. It is a sample of 177 horses from Hucul breed - Hroby bloodline (figure 1), divided in 6 stallions families: Hroby XVI, Hroby XVII, Hroby XVIII, Hroby XIX, Hroby XX, Hroby XXI, presented in Table 1. It was 84 males and 93 females analyzed at three different ages: first grading at 18 months old, second grading at 30 months old and third grading at 42 months old. After the third grading the individual will be tested for energetic capacity. The sample of 177 horses was extracted from population in according with registered performances, for all three ages, in order to have one balanced experimental plan (Popa, 2009).

The individuals were studied at three different ages: 18, 30 and 42 months old.

We had calculate statistics like average, variant, average error, standard deviation, and coefficient of variability. We applied significance tests like Student. The Fisher test was applied to the case of several samples, preceded by a variance analysis. The calculated F value was obtained by reporting the average squares value between the samples at the average squares from sample. The Tukey test involves calculating a statistic, noted

$$w = q_{(p;GL_e;\alpha)} \times s_{\bar{X}}$$

where q represents the standardized amplitude read from the table at the desired significance level (α), p being the number of groups, and GLe - degrees of freedom from the intragroup component of the variance analysis table. The value is obtained by the fact that MPe is the intragroup squares average value, and n is the average size of the groups. Applying Fisher or Tukey tests had the advantage to highlight, to allows us to see between which families we recorded significant differences.

Figure 1. Ranking of sire stallions in Lucina studfarm

Table 1. Analyzed biological material

HROBY Families	Individuals	Males	Females
Hroby XVI	10	3	7
Hroby XVII	13	6	7
Hroby XVIII	3	1	2
Hroby XIX	31	15	16
Hroby XX	54	29	25
Hroby XXI	66	30	36
TOTAL	**177**	**84**	**93**

RESULTS AND DISCUSSIONS

The average performances for thoracic perimeter, in Hroby bloodline, is presented in Table 2, and the dynamics of the same character can be observed in Figure 1.

Analyzing Table 2 and Figure 1, we can observe an important growth from one grading to another, in both sexes. Also we distinguish insignificant difference between sexes for mentioned character, with a small plus for females. Anyway, the differences between sexes of 0.3 cm, at this age, is insignificant to put in discussion some differences in energetic capacity between sexes (Popa R. et al., 2004).

Analyzing the data presented, there is a more pronounced variability of the thoracic perimeter, in the Hroby bloodline at the first ranking (18 months old), in males. This is most likely due to the environment, or possibly intangible factors. From the analysis of the datas can notice the existence of differences with a high degree of significance between individuals belonging to the two sexes.

For statistical testing of significance of differences between tested families of halfsibs from Hroby bloodline it was applied Fisher test.

The calculated Fisher test scores reveal distinctly significant differences between halfsibs (males and females) families, in the Hroby blooline for the thoracic perimeter cannon bone perimeter, but only at the second ranking, at age of age of 30 months old (F = 2.94).

Tukey's test calculated values show that at the age of 30 months old there are significant differences between the performance of the Hroby XIX and Hroby XX.

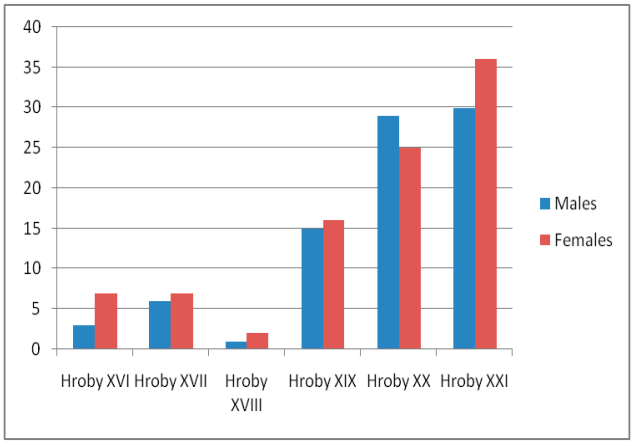

Figure 2. Distribution of individuals by families in Hroby bloodline

Table 2. Average performances for cannon bone perimeter in Hroby bloodline

Family	Sex	Age (years)											
		1.5				2.5				3.5			
		n	$\bar{X} \pm S_{\bar{X}}$	s	v%	n	$\bar{X} \pm S_{\bar{X}}$	s	v%	n	$\bar{X} \pm S_{\bar{X}}$	s	v%
H XVI	M	3	154.67 ± 2.9	5.03	3.25	3	166 ± 2	3.46	2.08	3	174.67 ± 2.19	3.79	2.17
H XVII		6	149.83 ± 2.3	5.64	3.76	6	162.5 ± 2.47	6.06	3.73	6	166.67 ± 1.94	4.76	2.86
H XVIII		1	157	-	-	1	171	-	-	1	166	-	-
H XIX		15	148.73 ± 1.55	6.02	4.05	15	158.67 ± 1.59	6.16	3.88	15	163.53 ± 0.88	3.4	2.08
H XX		29	151 ± 1.22	6.59	4.36	29	162.69 ± 0.75	4.04	2.48	29	167.48 ± 1.01	5.43	3.24
H XXI		30	145.5 ± 1.9	10.39	7.14	30	157.1 ± 1.25	6.83	4.35	30	166.9 ± 0.84	4.58	2.74
Total M		**84**	**148.75 ± 0.9**	**8.29**	**5.57**	**84**	**160.18 ± 0.69**	**6.31**	**3.94**	**84**	**166.75 ± 0.56**	**5.10**	**3.06**
H XVI	F	7	148 ± 1.72	4.55	3.07	7	159.57 ± 0.97	2.57	1.61	7	164.57 ± 1.53	4.04	2.45
H XVII		7	149 ± 1.65	4.36	2.93	7	161 ± 2.12	5.60	3.48	7	166.29 ± 1.7	4.50	2.71
H XVIII		2	153.5 ± 2.5	3.53	2.3	2	164 ± 3	4.24	2.59	2	165.5 ± 2.5	3.54	2,14
H XIX		16	148.69 ± 1.5	5.99	4.03	16	156.81 ± 1.65	6.59	4.2	16	166 ± 1.17	4.69	2,83
H XX		25	152.6 ± 1.26	6.30	4.13	25	160.28 ± 1.09	5.47	3.41	25	169.24 ± 0.87	4.37	2,58
H XXI		36	150.03 ± 0.92	5.51	3.67	36	161.53 ± 0.85	5.11	3.16	36	167 ± 1.02	6.13	3,67
Total F		**93**	**150.33 ± 0.6**	**5.76**	**3.83**	**93**	**160.25 ± 0.57**	**5.53**	**3.45**	**93**	**167.16 ± 0.54**	**5.24**	**3.13**
Total bloodline		**177**	**149.58 ± 0.53**	**7.10**	**4.75**	**177**	**160.21 ± 0.44**	**5.89**	**3.68**	**177**	**166.97 ± 0.39**	**5.16**	**3.09**
Significance of the observed differences between sexes (Student)		1.58 NS				0.08 NS				0.56 NS			

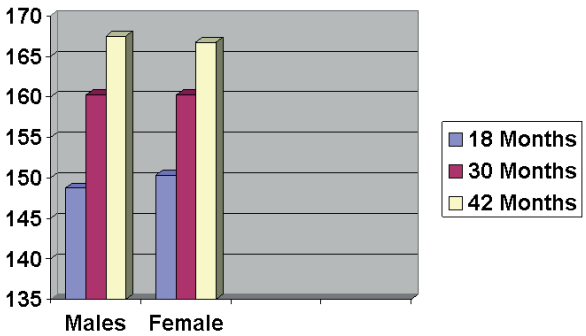

Figure 3. Dynamics of cannon bone perimeter

CONCLUSIONS

The data presented above show as values of this character, thoracic perimeter, that are between the characteristic limits of Hucul horse breed. It is easy to observe a small degree of variability, with an increasing tendencies from one successive age to another, for both sexes that was analyzed.

The evolution of growth process, for this character (thoracc perimeter) is normal, without significant differences between males and females.

We reveal an important growth of character, from one age to another, especially in stallion case (11.43 cm between first and second grade of stallions). Significant differences were recorded between the individuals from both sexes, but only at the second grading.

The calculated values for Fisher test reveal the existence of some distinctly significant differences between half sibs families from

Hroby bloodline, for thoracic perimeter, but only at 30 months old (F = 2.94). Values of Tukey test shows that at 30 month old are significant differences between performances of families Hroby XIX and Hroby XX.

From the data presented in for the thoracic perimeter, it is observed that, at these three ages, the average values of the character are approximately equal, at all the genealogical lines, for both sexes.

Regarding the absolute speed growth of thoracyc perimeter, in Hroby bloodline, this study reveal that this indicator had different levels, levels that was influenced by age, and showing decreasing values in relation to it.

After analyzing the presented data, in connection with absolute growth rate of thoracic perimeter, the following ideas can be deduced:

- ✓ The growth rate of the thoracic perimeter decreases in relation to age;
- ✓ The absolute growth rate has a small variability from one stallion family to another.

In the first growth period analyzed (from birth to the first ranking - 18 months), the individuals from Hroby bloodline recorded the highest absolute rate of growth of the thoracic perimeter, comparative with other Hucul horse bloodline.

The relative growth rate of character (%) - from the analysis of the presented data it is observed that the relative speed of thoracic perimeter is, like the absolute speed, influenced by the age of recording values of character (thoracic perimeter) a function of the age of character determination.

Regarding the relative growth rate of the thoracic perimeter, we can conclude:

- ✓ In the first period of growth, the relative growth rate had the highest value, in entire

Hucul breed also for individuals belonging to the Hroby line;

- ✓ The analyzed individuals, belonging to Hroby bloodline, had the largest thoracic perimeter at the end of the period, the initial value of the character being considered the same for all analyzed individuals.

Regarding the growth intensity, from the analysis of the presented data it is observed that, after birth, the young horses of the Hroby bloodline register a normal growth, the highest growth intensity manifesting itself until the age of 18 months (1.5 years).

It is very clear that in the post uterine period, the parameters of the growth process for the thoracic perimeter vary in close dependence with age. Their highest values are found in the first part of life, they show a decreasing trend in relation to the age factor. As a result, any deficiencies in the technology of breeding young horses during the period of maximum intensity of this process, has extremely serious repercussions on the productive life of the animal, especially on the energy capacity, which is the main production of horses.

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ANALYSIS OF CALF MANAGEMENT PRACTICES IN DIFFERENT DAIRY CATTLE FARMS

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Abstract

Given the lack of knowledge on the effects of farm size on the rearing practices of calves, the aim of the current study was to evaluate husbandry practices in small (<25 heads), medium (26-100 heads) and large (>100 heads) sized dairy farms from Romania. The current survey was conducted online and/or via telephone interviews in 2020, on a number of 58 dairy farms, representing an overall number of 12.721 dairy cattle. Regarding calving pens, large dairy farms used them in a significantly higher proportion than small farms (75% vs. 30.43%, p≤0.01) or medium farms (75% vs. 40%, p≤0.05), respectively. Only 4.34% of the small farms were using colostrum banks, while these were used by 6.66% medium sized farms and 55% large farms. The use of colostrum banks was significantly lower in small farms compared to medium (p≤0.05) and large (p≤0.001) farms, with differences (p≤0.001) being observed between medium and large farms. Current results highlight the differences in rearing practices of dairy calves, based on farm size.

Key words*: colostrum, dairy calves, management practices, survey.*

INTRODUCTION

In dairy cattle farms, un-weaned calves represent the main vulnerable category due to the immunity assimilation, which occurs exclusively throughout colostrum feeding and the exposure to stressors after calving (Sadar et al., 2019). Previous studies outlined the high morbidity in pre-weaned calves, with reports up to 35% being affected (Windeyer et al., 2014). Morbidity and mortality in calves bring important negative aspects in the farm economy (Vasseur et al., 2010; Mohd Nor et al., 2012), good rearing and management strategies reducing the risks of developing diseases in calves (Mee, 2008; Irimia et al., 2020).

On the other hand, the rearing technologies adopted during the calves suckling period, have been shown to influence the productive and reproductive performances further in the animals' adult life. In this regard, researchers such as Vasseur et al. (2010a) demonstrated the existence of critical points in rearing technologies, starting from calving, colostrum feeding, cow-calf separation and calf housing. Over the time, various research groups from Europe and North America have studied the management implications on calf's health and behaviour, in order to improve the calf's overall welfare and the farm incomes (Vasseur et al., 2010; Stanek et al., 2014). Due to the development of new intensive agriculture systems, each country has adopted different practices in calves rearing, depending on their breed (EFSA, 2006) or their destination (veal calves, replacement, fattening). However, significant differences in the rearing practices can be observed within the same region or country (Svensson et al., 2006; Stanek et al., 2014). For instance, in the United States there are marked differences in calves rearing, most of the variables in the rearing systems being dependant on the size of the farms (USDA-APHIS, 2012). Thus, identifying the main risks in animal welfare is the first step in adopting different and effective practices for each farm (Whay, 2007; Stanek et al., 2014). Livestock

welfare and animal health can be monitored by using sensor tehnologies which is widely adopted in dairy farms (Kelemen et al., 2016; Mihai et al., 2020).
Good practices should be implemented in all farms, although, significant differences in management exist, particularly when conventional and organic farms and compared (Klein-Jobstl et al., 2015; Pempek et al., 2017).
Klein-Jobstl et al. (2014) found the size of farms to be among the risk factors for calves to contract disease, with a strong correlation between farm size and the incidence of the main infectious diseases.
In Romania the majority of dairy farms (roughly 90%) is represented by small family farms (≤25 heads) (EC, annual report, 2019). Between 2005 and 2016, the largest reductions in farm numbers within the EU-27 was recorded in Romania, with a reduction of 0.8 million farms, representing roughly 20% of the total number of farms (EC, annual report, 2019).
The Romanian cattle sector has 1,241,059 breeding cows, with a total number of 1,914,602 cattle (Eurostat, 2020). The percentage of birth rates in dairy cows in Romania ranges based on our estimates from 80 to 85%, regarding the reproduction efficiency of each individual farm, with an estimated number of 990,000 calves being born annually.
Given the lack of knowledge on the effects of farm size on the rearing practices of calves, the aim of the current study was to evaluate husbandry practices in small (<25 heads), medium (26-100 heads) and large (>100 heads) sized dairy farms from Romania.

MATERIALS AND METHODS

Study population
The survey was conducted across Romania between May and November 2020, on a number of 58 dairy farms, representing an overall number of 12,721 dairy cows. The survey was focused on the following indicators: the general herd descriptors; cow-calf separation; existence and use of birth pens; colostrum quantity and quality; colostrum bank; navel hygiene; weaning methods and strategies; calves' milk, hay, water and concentrates administration.

Questionnaire design
The online questionnaire was disseminated throughout the use of iSondaje.ro platform (iSondaje, 2020).
The questionnaire had 39 questions and was divided into five sections containing 8, 10, 4, 10 and 13 questions, respectively.
Section 1 was focused on herd description with questions designed to capture general farm details, such as herd size, breed(s) composition of the herd, time for pasture allowance/year and the geographic position (lowland, hill or mountain), etc.
Section 2 was focused on the organization of the farm around calving, including existence of infrastructures such as calving pens, colostrum banks, quality colostrum checks, cow-calf separation and naval disinfection practices.
Section 3 was focused on housing, section 4 was describing the feeding regime of un-weaned calves, section 5 followed health indicators and veterinary care in dairy calves.
Results from sections 1, 2, 3 and 4 are being presented in the current paper, a comparative study based on the farms size being employed.
The questions were multiple choice, open questions, semi-closed and closed questions, dependant on the specificity of each of the indicators studied. The initial testing and validation of the questionnaire was performed on a number of 3 farms, for a good clarity and conciseness of the questions addressed.

Data analyses
A total of 71 farmers answered the questionnaire, 4 filled-out questionnaires were removed due to inconsistences and 9 questionnaires were described rearing practices of beef calves, as a result, data from a total of 58 farms were used in the final analysis.
Chi-square test of independence was performed to determine the relationships between the farm size and calving management, housing and feeding practices.
Decisions about the acceptance or rejection of the statistical hypothesis have been made at the 0.05 level of significance.

RESULTS AND DISCUSSIONS

Use of colostrum banks, colostrum quality and colostrum administration

Results concerning colostrum management in the studied farms are summarised in Table 1. In the majority of the surveyed farms (56.89%), the time from calving to the first colostrum administration was one hour, while 34.48% of farms administered the first colostrum between 1 and 4 hours after calving. A smaller percentage of farms, 3.44% and 5.17% administered colostrum after 4 hours and 6 hours *postpartum*, respectively. The farm size had no significant influence ($p>0.05$) on the moment of colostrum administration.

Current results suggest that Romanian farmers are aware of the importance of the correct time for colostrum administration, which is in accordance with the recommendations from the technical and scientific literature. Colostrum management practices in Romania are in line and comparable with recent research studies from the Czech Republic (Stanek et al., 2014) and Austria (Klein-Jobstl et al., 2015). Furthermore, according to a study conducted by Fisher et al. (2018), the IgG absorption levels were the most effective during the first 45 minutes after calving.

Regarding the use of colostrum banks, overall, 22.41% of the farms included in the study implemented colostrum freezing in their farms, conversely to data from the Czech Republic, where 73.5% of the farmers used frozen stocks of colostrum (Stanek et al., 2014).

In small and medium size farms, the existence of frozen colostrum banks is a minority practice, while on large farms, over half of the farmers are implementing this practice. This indicator is of high importance for feeding orphaned calves with good quality colostrum, ensuring higher survival rates throughout the immunological contribution of colostrum (Campbell et al., 2007; Godden et al., 2019). However, in the current study, a disparity was observed for the existence of colostrum banks among small and medium sized farms ($p\leq0.05$) and medium and large sized farms ($p\leq0.001$).

More than half of the studied farms (53.44%) practice colostrum quality evaluation, with the most used method being represented by colostrometer, followed by visual assessment and, to a lesser extent, refractometry. Current results are in accordance with those reported for the Czech Republic (Stanek et al., 2014), however, divergent from the Austrian colostrum quality assessment practices, where less than 5% of the cattle farms are evaluating the colostrum quality (Klein-Jobstl et al., 2015). Farm size influenced the practice of colostrum quality check in our study, with large farms practicing the procedure to a significantly higher extent ($p\leq0.05$), when compared to small sized dairy farms. No statistical significances ($p>0.05$) for the use of colostrum quality assessment among small and medium sized farms and medium and large farms were found.

Karamaev et al. (2021) found that immunoglobulins can be traced in the sanguine circulation of calves one hour after consuming the first colostrum.

Table 1. Influence of farm size on colostrum administration, colostrum bank and colostrum quality check practices

Farm size	Time of first colostrum administration (%)				Colostrum bank (%)		Checking colostrum quality (%)	
	0-1h	1-4h	4-6h	>6h	Yes	No	Yes	No
Small farms (5-25 heads)	60.86	34.78	0	4.34	4.34	95.65	39.13	60.86
Medium farms (26-100 heads)	60	26.66	6.66	6.66	13.33	86.66	53.33	46.66
Large farms (>100 heads)	50	40	5	5	55	45	70	30
Total	56.89	34.48	3.44	5.17	24.13	75.86	53.44	46.55
Small vs. medium	NS (0.638)				* *(0.015)*		NS (0.168)	
Small vs. large	NS (0.712)				*** *(0.000)*		* *(0.042)*	
Medium vs. large	NS (0.731)				*** *(0.000)*		NS (0.137)	

*NS: $p>0.05$; *$p\leq0.05$; **$p\leq0.01$; ***$p\leq0.001$.*

Colostrum and milk administration methods

Data regarding colostrum and milk administration methods are showed in Table 2.

Colostrum artificial feeding, especially with the oesophageal tube, is regarded as the optional practice, also being recommended in the case of ill and weakened calves, which are unable to suckle themselves (Zwierzchowski et al., 2020). Therefore, in small and medium dairy farms from Romania, the administration of the first colostrum with the help of the oesophageal feeder is not practiced, while in large size farms this practice was found in a proportion of 25% respondents. Current results are in accordance with those of Stanek et al. (2014), where half of the investigated farmers described they are using an oesophageal tube for calves with a low viability.

Regarding colostrum administration methods, a significant difference ($p \leq 0.01$) was detected in small farms compared to large sized farms, in small farms the natural method is still used in large proportion, while in large farms the bucket and the natural method are used in equal proportions. This aspect, regarding the practice for colostrum administrations is attributed to the difference of rearing system adopted in small farms which are commonly traditional, while in large sized farms is predominantly intensive or semi-intensive. Moreover, the intensification of dairy farming, especially in large farms, where higher levels of mechanization and automation of technological practices are predominant, as outlined by Batanov et al. (2020) and Karamaev et al. (2021).

The significant differences between medium and large sized farms ($p \leq 0.05$) could be attributed to different technologies implemented to the farm level, including the lower number of calves in medium farms. Our data shows that in small, medium and large farms, teat buckets are most commonly used (65.2%, 73.3%, 55%, respectively) for colostrum administration, which contributes to the welfare of calves, satisfying their suckling innate behaviour. Current results are in accordance with previous studies by Stanek et al. (2014), who found that calves were fed using a teat bucket in a proportion of 77.1%.

The most commonly used practice to administer milk was teat bucket, with a percentage of 44.82%, followed by open bucket with 25.86%. Direct dam suckling is the third practice used in Romania, ranking with a percentage of 24.13%, and the feeding machine being the least encountered, used in 5.17% of farms.

Farm size significantly influenced ($p \leq 0.05$) the methods for milk administration in calves between small and large farms, in small farms the natural method is still used in large proportion, while in large farms the bucket and the natural method are used in equal proportions. This could be attributed to the availability of resources and the use of modern agricultural production practices in large farms, as previously published by Butanov et al. (2020). According to previous studies, the practice of calves feeding directly from their dams was adopted in proportion of 58.8%, while feeding from an open bucket had an average use of 41.2% in commercial dairy farms from the Czech Republic (Stanek et al., 2014).

Table 2. Influence of farm size on colostrum and milk administration methods in un/weaned calves

Farm size	Colostrum administration method (%)				Milk administration method (%)			
	Esophageal tube feeder	Teat bucket	Open bucket	Natural sucking	Open Bucket	Teat bucket	Automated milk feeder	Natural sucking
Small farms (5-25 heads)	0	65.21	4.34	30.43	17.39	39.13	0	43.47
Medium farms (26-100 heads)	0	73.33	13.33	13.33	33.33	40	6.66	20
Large farms (>100 heads)	25	55	10	10	30	55	10	5
Total	8.62	63.79	8.62	18.97	25.86	44.82	5.17	24.13
Small vs. medium	NS (0.101)				NS (0.105)			
Small vs. large	** *(0.009)*				* *(0.020)*			
Medium vs. large	* *(0.023)*				NS (0.190)			

*NS: p>0.05; *p≤0.05; **p≤0.01; ***p≤0.00*

Individual housing period, calving pens and cow-calf separation

Data on calves individual housing period, existence of calving pens and cow-calf separation are given in Table 3.

Across all surveyed farms in the current study (53.44%) the most widespread practice regarding calves housing was in individual pens up to 3 months of life, for 1-2 weeks in 22.41% of farms and 12.06% more than three months of age, while 12.06% of farms do not use individual calf pens. Farm size seemed to significantly influence ($p \leq 0.05$) the individual housing period of calves. This could be attributed to the need of farmers to minimize viral and bacteriological infections in the un-weaned calves (Bertoni et al., 2021). Moreover, European legislation, through Council Directive 119/2008, does not recommend the individual maintenance of calves over 8 weeks of age, which supports the results obtained by us. On the other hand, pair housing can be considered as a good option for calves rearing, because can develop a higher behavioural flexibility for environmental changing and future mixing and grouping of calves after weaning (Mahendran et al., 2021). Current results are similar to those of previous studies, where individually housing in dairy calves is a common practice, being used in proportion of more than 95% (Stanek et al., 2014).

Over the last decade, many researches have been focused on issuing recommendations about individual or group housing of dairy calves. Reducing the risk of spreading pathogens, weight gaining and avoiding cross-suckling were the main reasons for recommending the rearing of calves in individual pens (Mahendral et al., 2021).

In our survey, the existence of calving pens was adopted in 48.27% of the farms. This is in accordance with previous studies where 47.0 % of the farms has available calving pens (Vasseur et al., 2010; Klein-Jobstl et al., 2015). Furthermore, the use of individual calving pens is a common practice for German dairy farming, where it is being encountered in all commercial enterprises (Heuwieser et al., 2010). However, a significant difference ($p \leq 0.01$) was noticed between small and large farms, in large farms the proportion of calving pen is higher than in small farms. These findings are in accordance with those of Klein-Jobstl et al. (2015). Significant differences ($p \leq 0.05$) could be observed between medium farms and large farms which was using calving pens in a higher proportion than the medium farms. This being attributed to the modern agricultural practices implemented in large farms, compared to smaller ones, as previously reported by Butanov et al. (2020). Moreover, to reduce the stress after birth and for a good farm sanitation, is it recommended to use calving pens in the farm. However, proper hygiene and regular surveillance of the calving pens are recommended (Vasseur et al., 2010).

Table 3. Influence of farm size on individual housing period, calving pens and cow-calf separation

Farm size	Individual housing period (%)				Existence of calving pens (%)		Cow-calf separation (%)					
	1-2 weeks	≤ 3 months	> 3 months	No	Yes	No	≤2h	After 2h	After 12h	>12h	After 7days	Other
Small farms (5-25 heads)	4.34	56.52	26.08	13.04	30.43	69.56	30.43	17.39	0	0	13.04	39.13
Medium farms (26-100 heads)	20	60	0	20	40	60	40	20	0	13.33	6.66	20
Large farms (>100 heads)	45	45	5	5	75	25	60	20	5	15	0	0
Total	22.41	53.44	12.06	12.06	48.27	51.72	43.10	18.96	1.72	8.62	6.89	20.68
Small vs. medium	* *(0.019)*				NS (0.067)		NS (0.105)					
Small vs. large	** *(0.003)*				** *(0.003)*		** *(0.003)*					
Medium vs. large	* *(0.025)*				* *(0.013)*		NS (0.080)					

*NS: p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001.*

Over the last years, the separation of calf from the dam has constituted the subject of a great deal of studies, with a focus on animal welfare and implications of cow-calf contact systems (Knierim et al., 2020). Our results shows that the cow-calf separation is most frequently implemented immediately after calving, with 43.10% after 2 h *post-partum*, while 6.89% of

farmers separate the calves from their dams after the age of 7 days.

A significant difference ($p \leq 0.01$) for cow-calf separation moment was found between small and large sized farm, in large farms there is a higher proportion in the case of cow-calf separation earlier, while in small farms a larger proportion for later separation. Moreover, this could be attributed to disease transmission risk management and colostrum administration practices that are implemented in larger farms (Trotz-Williams et al., 2008). However, if this practice of cow-calf separation is adopted soon after calving, it is recommended to maintain calves in a clean and disinfected pen to prevent infections. On the other hand, this practice deprives the animals of emotional bonds and reduces the stress of separation (Pempek et al., 2017).

Milk feeding regime and quantity

Milk feeding regime and administrated quantity is presented in Table 4.

Across all surveyed farms, almost three quarters of the farms (71.41%) feed the calves with whole milk, 25.86% with milk replacer and just 1.72% with mixed (replacer + whole) milk. According to previous studies, in the Czech Republic, 35.3% of calves were fed with milk replacer (Stanek et al., 2014), a higher percentage than in our study. Significant differences were observed between large and medium farms ($p \leq 0.001$) and between large and small farms ($p \leq 0.01$), respectively. In large farms being preferred to use whole milk in dairy calves feeding. According to the literature, the results from our survey are similar with previous studies where milk replacer was significantly more often fed on large farms (Klein-Jobstl et al., 2015), the difference could be attributed to the fact that it is easier to handle and has optimal balanced nutrients (Vasseur et al., 2010).

In one third of farms (32.75%) milk was offered 8 litres/day, followed by *ad libitum* practices with 27.58% and 18.96% with 4 l/day, respectively. Significant differences between small and medium farm ($p \leq 0.05$), between small and large farms ($p \leq 0.05$), and between medium and large farms ($p \leq 0.05$) were found. These results are not supported by findings of previous studies, were milk or milk replacer feeding at herd level had a median of 6 l/day in two meals (Stanek et al., 2014).

Calves feeding practices during the first weeks of life, with the optimal amounts of milk or milk replacer, has an important role both for their growth and development (OIE, 2017), and for expressing the natural suckling behaviour (Miller-Cushon & DeVries, 2015). Milk feeding level has great potential to influence the development of feeding behaviour during the preweaning period (Miller-Cushon et al., 2013).

Table 4. Influence of farm size on milk quality and milk type based on farm size

Farm size	Milk feeding regime (%)			Milk quantity (litres) (%)				
	Whole	Replacer	Mixt	*Ad libitum*	≤2	4	6	8
Small farms (5-25 heads)	86.95	13.04	0	47.82	0	14.39	13.04	21.73
Medium farms (26-100 heads)	93.33	6.66	0	20	6.66	13.33	6.66	53.33
Large farms (>100 heads)	53.33	55	5	10	0	25	35	30
Total	72.41	25.86	1.72	27.58	1.72	18.96	18.96	32.75
Small vs. medium	NS (0.054)			* *(0.027)*				
Small vs. large	** *(0.001)*			* *(0.028)*				
Medium vs. large	*** *(0.000)*			* *(0.021)*				

*NS: $p>0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.*

Water, hay and concentrates feeding

Details regarding access to water, hay and the concentrates protein percentage are presented in Table 5.

In order for the calves to develop the rumen mucosa and functions, is important to have free access to water, hay and concentrates earlier in life. According to previous studies, a large number of farms adopt free access to hay and concentrates (84.9 and 60.5 %, respectively) for calves, starting with first three weeks after calving (Klein-Jobstl et al., 2015).

Table 5. Influence of farm size on access to hay, water and concentrates in un-weaned calves

Farm size	Access to hay (%)			Access to water (%)			Concentrates protein (%)					
	1-3w	4-8w	>8w	Ad libitum	2l/day	5l/day	<14%	14-16%	16-18%	18-20%	20-22%	>22%
Small farms (5-25 heads)	34.78	34.78	30.43	95.65	0	4.34	17.39	13.04	39.13	21.73	0	8.69
Medium farms (26-100 heads)	40	53.33	6.66	100	0	0	13.33	33.33	6.66	26.66	13.33	6.66
Large farms (>100 heads)	50	35	15	95	5	0	0	5	40	25	15	15
Total	41.37	39.65	18.96	96.55	1.72	1.72	10.34	15.51	31.03	24.13	8.62	10.34
Small vs. medium	NS (0.143)			*NS (0.396)*			NS (0.062)					
Small vs. large	NS (0.271)			*NS (0.233)*			NS (0.102)					
Medium vs. large	NS (0.280)			*NS (0.344)*			**(0.033)*					

*NS: p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001.*

Our data shows a non-significant (p>0.05) difference for access to hay and water between the three different farms size categories. Water was in 96.55% of the farms offered *ad libitum*, while less than 4% of the farms offered restricted 2 l/day and 5 l/day, respectively. Data concerning calves' access to water, were in accordance with previous studies.

Water access of calves is an overall disputed topic, given that many farmers do not give access to water for calves for a long period after birth (Relic et al., 2020). On small farms, for example, in the USA, calves have first access to water between day 15 and day 20 of life (USDA, 2016). Consuming water immediately after birth could improve calf's growth and rumen development, and thus increasing nutrient digestibility (Wickramasinghe et al., 2019). Moreover, there is the believe among farmers that milk contains enough water, and the calves during suckling do not need to consume additional water to the milk diet.

In contrast, access to concentrates is regarded as a crucial need for successful calf rearing (Khan et al., 2011). Our data shows a preference of farmers (31.03%) to use concentrates with a 16-18% protein content (PB) for calves feeding, followed by 24.13% which use 18-20% protein content for the concentrates and 15.51% administer 14-16% PB, respectively. However, across all surveyed farms, we obtained a significative difference between medium and large sized farms (p≤0.05), lower PB concentration in medium farms, while higher PB concentration in large farms preferred. The practice changes among farms could be attributed to the different economic weights and implications in calves feeding (Batanov et al., 2020). In a study conducted by Stamey et al. (2021) testing 3 different concentrates with protein ranging between 21.5% and 26%, no influence of the feeding regime on body weight and starter intake up to the age of weaning was found.

Practices, such as naval disinfection was common in all farms (42%), however, we found no significant difference (p>0.05) based on the farm size. In contrast, in countries such as the Czech Republic navel disinfection has higher importance, being practiced in 88.2% of the farms, using methods such dipping or spraying (Stanek et al., 2014). Compared with other studies from different countries, calves were usually weaned between week 7 and 10 (Vasseur et al., 2010; Stanek et al., 2014), in Romania farmers adopted the same practice and they were weaning the calves around three months of age. Age of calves at weaning was not influenced (p>0.05) by the farm size in our study.

CONCLUSIONS

Regarding the use of certain infrastructures on farms, calving pen use is low in small and medium sized farms, compared to large sized farms. The verification of colostrum quality prior to calf administration is being performed on almost a half of the small and medium sized farms, being widely used in large farms.

Colostrum banking does not represent a common practice in Romanian dairy farms, with significant disparities being found between small and larger farms.

Cow-calf separation is most frequently done immediately after calving or after 2 hours *post-partum*, a minority of farms practicing the cow-calf contact during the sub-colostral period (first 5-7 days after calving), respectively.

The most widespread practice regarding calves housing was in individual pens up to 3 months of age, which poses animal welfare concerns and could lead to significant post weaning stress in dairy calves, with negative consequences on their immune functions and growth rates.

The milk feeding regime and milk quantity presents the furthermost differences between small, medium and large farm. With large farms adopting the most economical practices for calves rearing, which is represented by the use of milk replacers. The use of milk replacers is not recommended under organic production systems, although, it is allowed according to the European Directive for organic production.

Access to water, hay, concentrates and weaning age of calves was similar to other European countries and generally respect the conventional rearing practices.

Our initial hypothesis that differences in rearing practices of dairy calves can exist, based on farm size is partly supported by the results.

Results obtained in this study provide data on calf practice management in dairy farms from Romania and this data could help to further point out levels and practices to be improved at farm level. Furthermore, significant differences could be determined between small, medium and large sized Romanian farms, suggesting a higher degree of specialisation on large farms.

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INFLUENCE OF ESSENTIAL OILS ON BIOPRODUCTIVE INDICES AND HEALTH OF BEE COLONIES

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Abstract

The productivity of bee colonies is strongly influenced by genetics and age of the queen, by health status of bee colonies, the honey potential of the area where they are maintained, but also by the meteorological factors that condition the capitalization of the honey source. Scientific research in beekeeping, carried out in recent years, has been aimed at improving the genetics of existing biological material in order to improve productive performance and disease resistance, but also to use technological measures of maintenance to achieve these objectives. The use of essential oils as a technological measure in the stimulation of bee colonies has shown favorable effects on the prolificacy of the queen (essential oils of thyme, basil, oregano, mint, rosemary, juniper), improvement of the health of bees (essential oils of juniper, thyme, basil, oregano, cinnamon) correlated with the increase in honey production.

Key words: *bees, bioproductive indices, disease prophylaxis, essential oils.*

INTRODUCTION

The intensification of agriculture, pollution and climate change have led to the increase of factors with a negative influence on bee families, which favor the emergence of their diseases. The use of drugs in the treatment of diseases in bees should be limited due to the negative effects on the longevity of bees, the vitality of the brood, the emergence of resistance to them (Boudegga et al., 2010), but also to the negative effect on the quality of bee products (Isman, 2000). It is necessary to find alternative solutions to the use of antibiotics (El Shafai, 2012; Pătruică & Moț, 2012).

Essential oils are found in almost all plants (Imdorf et al., 1999), the components being very different, varying from one species to another, but the composition can be very different even in the case of the same essential oil, due to genetic and environmental factors (Flamini, 2003).

The researches conducted by Bakkali et al. (2008) shown that essential oils and the main compounds in their composition represent a broad spectrum of bioactivity. Studies on the use of essential oils on health of bees have shown the important role in reducing mites (Damiani et al., 2009). Also, Bailac et al. (2006) showed that essential oils have a high content in benzene compounds, and these determine their antimicrobial activity. Porinni et al. (2017) highlighted the effects of the use of essential oils on the productivity and survival of bee families. In the studies performed by Arbia & Babbay (2011), we may note that essential oils can be an important method in preventing the development and spread of pathogens.

The aim of this paper is to investigate the influence of some essential oils administered in the supplementary feeding of bee families, in the spring, on the stimulation of the egg laying of queen, the improvement of health and productivity, materialized in the production of honey.

MATERIALS AND METHODS

The researches were undertaken in Murani locality, Timiș County, România, on 90 bee colonies of medium power and queen of the same age, and each experimental group was represented by 10 bee colonies. Eight experimental batches were fed with sugar syrup and essential oil, and the control group was fed only

with sugar syrup. The 1:1 sugar syrup was administered over 3 weeks in an amount of 1 l/week/family, the dose of essential oil being 2 drops/l syrup. The essential oils of thyme, basil, rosemary, juniper, oregano, mint, cloves, cinnamon were used.

Before testing on bees, the essential oils were analyzed in terms of chemical composition, in the chemistry laboratory of the Banat's University of Agricultural Sciences and Veterinary Medicine "King Mihai I of Romania" from Timisoara, the Interdisciplinary Research Platform "Ecological agriculture and food safety". For their chemical examination we used the gas chromatograph-mass spectrometer, model GCMS-QP2010PLUS, equipped with 4000 GC/MS/MS system and flame ionization detector (FID). The chromatogram interpretation was performed using the NIST database identifying the compound for each drop of essential oil.

From each family of bees we collected, at the beginning of the experiment and at 21 days, 10 working bees in order to perform the microbiological examination of the small intestine. The microbiological examination was performed according to the method described by Pătruică & Moț (2012).

In order to evaluate the influence of essential oils on the degree of development of bee families, at the beginning of the experiment, at 10 days and 21 days, the area occupied with brood was assessed using the Netz frame.

The evaluation of the rapeseed honey production was made by appreciating the surface occupied with honey on honeycombs with the help of the Netz frame and its transformation into kilograms.

The data obtained from the experiments were processed using the program IBM SPSS Statistics Version 21. For statistical analysis we applied Paired-Samples T-Test with 95% Confidence Interval of the Difference.

RESULTS AND DISCUSSIONS

Following the chemical analyzes performed on the 8 essential oils, it was found that: thyme essential oil has in its composition Borneol 17.15%, Alfa terpineol 6.05%, Camphene 14.41%, and the rosemary essential oil 52.82% Eucalyptol, Alfa pinene 17.56%, Camphor 10.01%. Basil essential oil contains Estragol 55.73%, Linalool 38.64%. According to the results, juniper essential oil has as main components Alfa-pinene 49.75%, Beta-pinene 16.70%, Beta-myrcene 9.27%, and the oregano essential oil contains Karvacrol 33.42%, O-cymene 22.98%, Gamma terpinene 17.44%. The peppermint oil has within its content Menthone 30.73%, Neomenthol 17.37%, Limonene 9.52%. Clove essential oil contains active compounds such as Eugenol 85.17%, Carryophillene 8.15%, Eugenol acetate 5.44%, and cinnamon essential oil has in its composition Cinnamaldehyde (E) 69.28%, Cinnamaldehyde-o-methoxy 14.30% and Cinnamil acetate 6.55% (Figure 1).

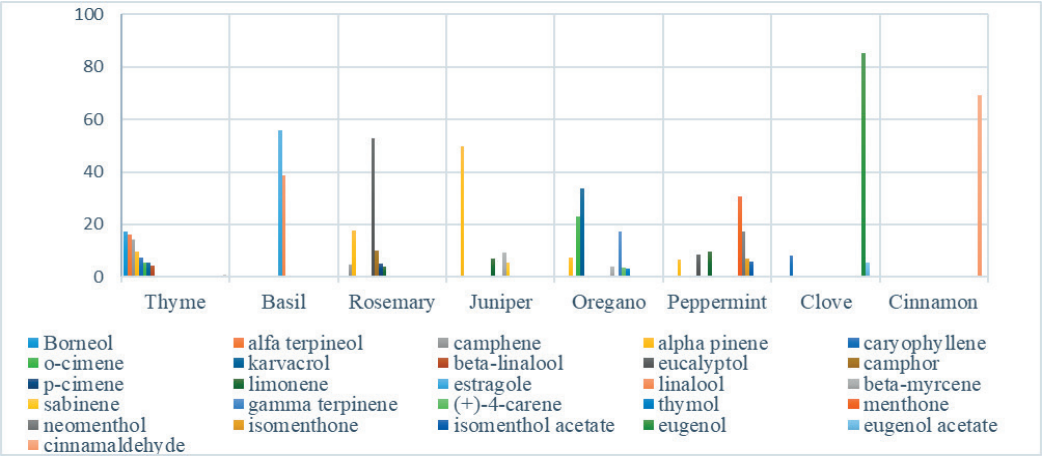

Figure 1. Chemical composition of the analyzed essential oils

Similar studies conducted by Porrini et al. (2017) highlighted in the composition of oregano essential oil 27.7% Karvacrol, in rosemary essential oil 15.2% Camphor, and in the cinnamon essential oil, identified as the main active compound Cinnamaldehyde (79.3%). Feeding bee colonies in the spring with sugar syrup in which we incorporated an essential oil (thyme, basil, rosemary, oregano, juniper, mint, cloves, cinnamon) had the effect of reducing the total number of germs in the intestine of worker bees, stimulating the queen prolificity, correlated with higher honey production (Table 1).

Table 1. The results of the use of essential oils in the supplementary feeding of bee colonies

Experimental variants	Essential oil used	Tracked indicators						
		Total no. of germs		Bee family development			Honey production	
		At the beginning of experiment	At 21 days	At the beginning of experiment (cells)	At 10 days (cells)	At 21 days (cells)	At 21 days (kg)	After rapeseed picking (kg)
Control group	---	664.40	719.00	2110	4290	7200	1.590	25.096
EG_1	Thyme	644.00	472.80*	1900	4800	10800***	2.145	31.753***
EG_2	Basil	598.80	479.20*	2050	6000**	10800***	2.590	31.685***
EG_3	Rosemary	872.40	574.20	1770	5030	8530*	1.683	30.013
EG_4	Oregano	734.60	474.00*	1780	5220	10150**	2.720	32.161***
EG_5	Juniper	881.80	618.60**	1970	5350	9150*	1.596	27.196
EG_6	Mint	861.60	658.40	1920	5160	9350**	2.003	32.905***
EG_7	Clove	831.00	624.20	1890	4730	8410	1.746	27.156
EG_8	Cinnamon	654.80	491.40*	2080	4830	8080	1.893	26.987

$p<0.001$***
$p<0.01$**
$p<0.05$*

From a microbiological point of view, after 3 weeks of administration of sugar syrup with essential oils, a significant reduction in the total number of germs was observed, namely: in the groups fed with thyme essential oil, the number of germs in the intestine decreased by 34.25% compared to the control group, oregano essential oil had an effectiveness of 34.08%, basil reduced the number of germs by 33.36%, cinnamon oil caused a decrease in germs by 31.66% compared to the group witness. Similar results were obtained with the use of oregano essential oil, which reduced the total number of germs by 24.26% and thyme, which reduced the total number of germs in the small intestine by 20.68% (Pătruică et al., 2018). The administration of essential oils of thyme, basil, rosemary, oregano, juniper, mint, cloves, cinnamon in the supplementary feeding of bee colonies in autumn, reduced the total number of germs in the intestine of bees by 32.54% in the case of essential oil of oregano, 29.95% in the case of peppermint essential oil, and thyme essential oil reduced the total number of germs in the intestine by 25.43% (Lazăr et al., 2021). The administration of rosemary essential oil had the effect of reducing by 20.14% the total number of germs compared to the control group, juniper oil reduced the total number of germs by 13.97%, followed by clove essential oil by 13.19 %. Peppermint essential oil reduced intestinal germs by 8.43% compared to the control group (Figure 2).

The reduction in the total number of germs in the intestine of worker bees is correlated with a better health of bee colonies.

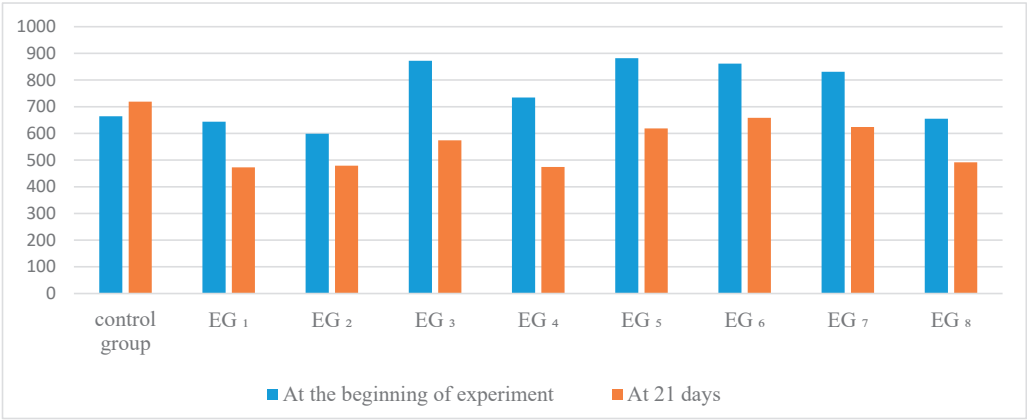

Figure 2. Evolution of the total number of germs during the experiment

Regarding the influence of essential oils on the queen's egg, there was a significant increase in the number of brood cells after feeding them with sugar syrup and essential oils (Figure 3), so we can say that the use of essential oils of thyme, basil, oregano, mint, rosemary and juniper showed a stimulating effect on queen prolificacy.

After 10 days of administration of sugar syrup with addition of essential oils, there was an increase in the number of cells with brood by 11.88-39.86%, the best results being obtained when we administrated basil essential oil ($p<0.001$).

At the end of the experiment, the administration of thyme and basil essential oil resulted in an increase in the area occupied by brood by 50% compared to the control group.

Oregano essential oil increased the number of brood cells by 40.97%, followed by peppermint essential oil by 29.86%. Juniper essential oil increased the amount of brood by 26.08%, and in the case of rosemary essential oil there was an increase in the number of brood cells of 18.47% compared to the control group. In the case of the other essential oils used, there was an increase in the number of brood cells by 12.22% (cinnamon) and 16.80% (cloves) compared to the control group, at statistically insignificant differences.

Similar results were recorded following the research undertaken by Pătruică et al. (2018), succesive to the use of oregano and basil essential oils.

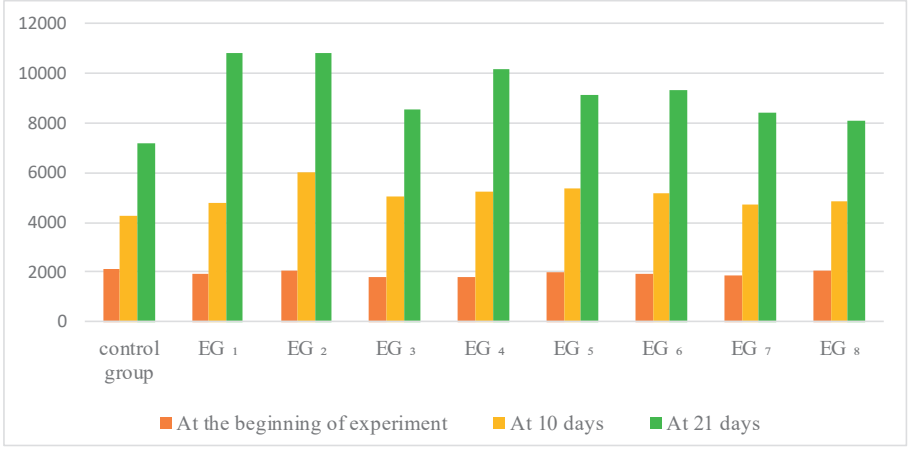

Figure 3. The evolution of the number of brood cells following the administration of essential oils

After the control carried out 10 days after the administration of the supplementary feeding, it was found that the essential oil of oregano caused an increase in the number of frames occupied by bees by 50%, compared to the control group, followed by the essential oils of thyme, basil and mint, which increased the amount of bees by 33.33%. In the case of the other oils used (rosemary, juniper, cloves, cinnamon) no statistically significant results were shown.

At the control performed at the end of the experiment, the amount of bee increased by 66.66% in the case of batches where thyme, basil and oregano essential oil was administe red. Peppermint essential oil increased the amount of bees by 50%, and the oils of rosemary, juniper, cloves and cinnamon by 33.33%, compared to the control group (Figure 4).

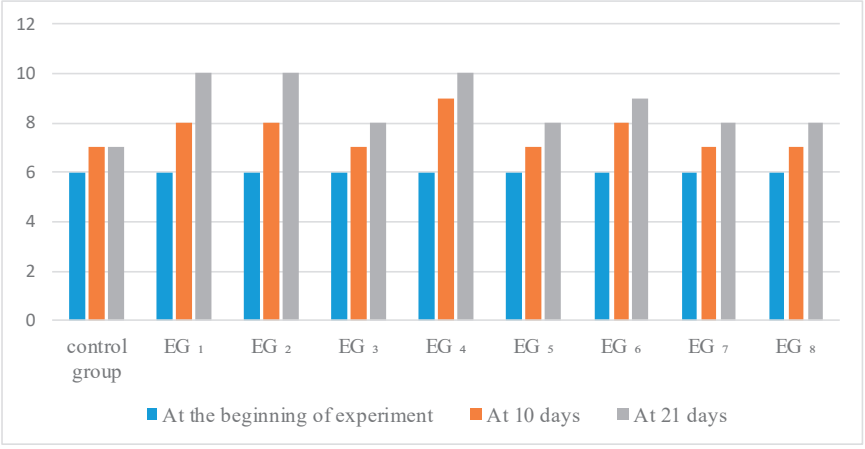

Figure 4. The evolution of the development of bee families following the administration of essential oils

Regarding honey production, after the end of the rapeseed harvest, a significant increase was observed in the groups which were given essential oil of thyme, basil, oregano and mint (Figure 5).

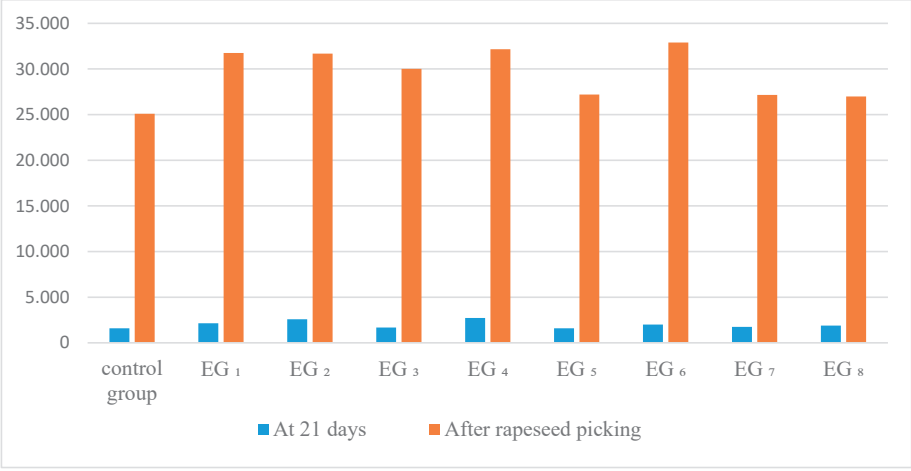

Figure 5. Honey production obtained from rapeseed harvest

According to the results obtained, all essential oils had a positive influence on honey production by increasing it, compared to the control group. Peppermint essential oil increased honey production by 35.41%, oregano essential oil increased honey production by 27.76%, and basil essential oil obtained an increase in honey production by 26.06%, compared with the control group. In a percentage of 25.82%, there was an increase in the production of honey in the case of the thyme essential oil. Honey production, in the case of experimental groups in which rosemary essential oil was administered, increased by 17.66%, followed by juniper essential oil which caused an increase in honey production by 7.77%. In the batches in which cinnamon essential oil was administered, an increase in honey production was obtained by 6.03%, and clove oil determined a percentage increase of 5.54%. The increase in honey production is correlated with the development of bee colonies that participated in rapeseed harvesting with a significantly higher number of working bees.

CONCLUSIONS

1. Following the chemical analyzes performed, it was observed that the essential oil of thyme is predominated by Borneol 17.15%, that of basil by Estragole 55.73%, the essential oil of rosemary has a content of 52.82% Eucalyptol, the essential oil of juniper contains 49.75% Alpha pinene, oregano essential oil contains 33.42% Karvacrol, mint essential oil contains 30.73% Menthone, clove essential oil has 85.17% Eugenol, and cinnamon oil contains 69.28 Cinnamaldehyde.

2. The administration of sugar syrup with addition of essential oil had the effect of significantly reducing the number of germs in the intestine, correlated with a better health of bee colonies. The experimental variants with the best results were the batches fed with sugar syrup with addition of juniper essential oil ($p<0.001$), thyme ($p<0.05$), basil ($p<0.05$), oregano ($p<0.05$) and cinnamon ($p<0.05$).

3. Basil essential oil administered in the supplementary feeding of bee colonies determined after 10 days a statistically significant increase ($p <0.05$) in the number of cells occupied with brood. At the end of the experiment, statistically significant differences compared to the control group, regarding the number of cells with capped and non-capped brood were recorded in the groups fed with sugar syrup and thyme essential oil ($p<0.001$), basil ($p<0.001$), oregano ($p<0.01$), mint ($p<0.01$), rosemary ($p<0.05$) and juniper ($p<0.05$).

4. All the essential oils used had positive results, statistically significant in terms of rapeseed honey production. The best results were recorded when using thyme essential oil ($p<0.001$), basil ($p<0.001$), oregano ($p<0.001$), mint ($p<0.001$).

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HEMATOLOGICAL PROFILE OF CARPATHIAN GOAT BREED MOTHERS ACCORDING TO AGE AND PHYSIOLOGICAL STATUS - CASE STUDY

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Abstract

Goats are extremely prone to many metabolic diseases that lead to disturbance milk production and their general health. The purpose of this paper was to highlight the particularities of hematological profile Carpathian goat breed according to age and physiological status. The study was conducted in a farm in Poiana Aiudului area, Livezile county of Alba, during July 2020 - March 2021. Blood samples were collected from the jugular vein into EDTA bottles in the period preceding the mount, at the beginning of the gestation and postpartum period. Blood samples were transported in refrigerated conditions (4 degrees C) to the USAMV Cluj-Napoca, Veterinary Medicine Laboratory. The hematological profile was structured in the following elements: White blood cells (WBC-10^9/l), Lymphocyte (LYM-10^9/l), Monocyte (MON-10^9/l), Neutrophils (NEU-10^9/l), Lymphocyte percentage (LY-%), Monocyte percentage (MO-%), Neutrophils percentage (NE-%), Red blood cells (RBC-10^12/l), Hemoglobin (HGB-g/l), Hematocrit (HCT-%), Mean corpuscular volume (MCV- fl), Mean corpuscular hemoglobin (MCH-pg), Mean corpuscular hemoglobin concentration (MCHC-g/dl), Red cell distribution width (RDWc-%). The results obtained were statistically processed, being beneficial in making faster and more efficient decisions to ensure the welfare and health of goats.

Key words*: antepartum, Carphatian goat breed, complet blood count (CBC), postpartum.*

INTRODUCTION

In animal science the goat sector has recently experienced a very good development for increase goat population and the interest given in research studies.

The last thirty years gives a considerable attention to hematological profile of goats because it is desired a standardization of the normal hematological values and variability of hematological parameters. Parameters variability of CBC can be influenced by internal factors (age, sex, race, health) and external factors (environmental differences, climate, etc.) (Kramer J.W., 2000).

The goat sector has always been closely linked to the sheep sector, so information on the hematological profile and immune system of the goat is very limited (Smith et al., 2009). American Dairy Goat Association developed a program blood typing to improve the techniques for breeding and identifying the origin of goats (Smith et al., 2009)

The periparturient period in the life of any animal is very stressful because there are many endocrine and metabolic changes. This period requires the greatest approach to be able to understand the biochemical phenomena which occur in the animal body (Guo et al., 2007). The metabolic changes in goat`s body have been briefly studied over time. Studies show that the metabolism of goats during the transition period are based on only a few measurements (Khan et al., 2002a; Skotnicka et al., 2011). The complete blood count (CBC) is very important in the transition period of animals, because it captures the dynamic changes that occur during the periparturient period. The hematological parameters vary from one breed of goat to another (Azab et al., 1999; Daramola et al., 2005; Opara et al., 2010). The most numerous studies on goat species have been started in African and Asian countries, so this study propose to highlight the changes in the hematologic profile of a Carpathian goat population during the

transition period before mounting to the postpartum period.

MATERIALS AND METHODS

The study was conducted in a farm, individual enterprise Pacurar Emilia, in Poiana Aiudului area, Livezile county of Alba, during July 2020 - March 2021, on Carpathian goat female entered in the genealogical register of the goat species. Thirty percent of goat female population (n = 30) % consisted of young female goats born in 2020 (who have reached the age of 6 months and weighed over 35 kg), primiparous female goats and multiparous female goats. During the monitoring period the maintenance and feeding conditions were the same for the entire study population. The individualization of the goats was done with plastic straps applied to the base of the neck of different colors according to the age of goat.

Blood samples were collected individually from the experimental group at three different times of the year, namely: the period before breeding (June-July), gestation (November-December) and postpartum (February-March). Blood sampling was performed at the same time in the morning to minimize the influence of circadian rhythms (Piccione et al., 2002).

Blood samples were taken from each animal that have the general health good. (livelybehavior, lack of internal and external parasites), in 6 ml vacutainers containing ethylenediaminetetraacetic acid (EDTA) through the jugular vein puncture.

Blood samples were transported in refrigerated conditions (4 degrees Celsius) approximately two hours after collection to USAMV Cluj-Napoca, Veterinary Medicine Laboratory.

The hematological profile was structured in the following elements: White blood cells (WBC-10^9/l), Lymphocyte (LYM-10^9/l), Monocyte (MON-10^9/l), Neutrophils (NEU-10^9/l), Lymphocyte percentage (LY-%), Monocyte percentage (MO-%), Neutrophils percentage (NE-%), Red blood cells (RBC-10^12/l), Hemoglobin (HGB-g/l), Hematocrit (HCT-%), Mean corpuscular volume (MCV- ƒl), Mean corpuscular hemoglobin (MCH-pg), Mean corpuscular hemoglobin concentration (MCHC-g/dl), Red cell distribution width (RDWc-%).

The obtained data were centralized and statistically processed with Excel program and GraphPad, T test and ANOVA test was used to calculate the significance of the age and physiological status between the CBC in goats.

RESULTS AND DISCUSSIONS

The hematological profile of goats depending on the breed, age, sex, physiological status and environmental conditions (altitude, climate, farm management) (Arfuso et al., 2016).

The most important hematological changes produced in the goat species during the transition period from breeding to parturition are related to neutrophilia, monocytopenia and degrease erythtrocyte count. These hematological changes are associated with stress caused by parturition and lactation (El-Ghoul et al., 2000).

White blood cells, lymphocyte, monocyte, neutrophils show significant variations in physiological status, both among animals of the same age depending on physiological status, but also between animals of different ages (Tabel 1).

In organisms, the number of red blood cells is the dynamic activity of the bone marrow, which is released into the peripheral circulation and stored in various organs or basins (Habibu et al., 2018).

Tabel 1. Variation of CBV according to age and physiological status for Carphatian goat

category	ANOVA test	WBC 10^9/l	LYM 10^9/l	MON 10^9/l	NEU 10^9/l	LY %	MO %	NE %
TF	F	29.77	56.45	33.99	45.81	0.008	11.99	24.26
	P value	****	****	****	****	ns	***	****
	R square	0.68	0.8	0.71	0.77	0.006	0.47	0.64
P	F	79.47	36.74	13.62	96.33	2318	5.09	132.9
	P value	****	****	****	****	****	*	****
	R square	6.85	0.73	0.5	0.87	0.99	0.27	0.9
M	F	112.3	24.14	35.96	22.12	129	2.31	93.54
	P value	****	****	****	****	****	ns	****
	R square	0.89	0.64	0.72	0.62	0.9	0.14	0.87
		RBC 10^12/l	HGB g/l	HCT %	MCV ƒl	MCH pg	MCHC g/dl	RDWc %
TF	F	166.4	18.42	6.58	9.73	1.19	6.48	19.26
	P value	****	****	**	***	ns	**	****
	R square	0.92	0.57	0.32	0.41	0.08	0.32	0.58
P	F	713.6	13.31	5.51	9.84	1.09	5.51	1.61
	P value	****	****	**	***	ns	**	ns
	R square	0.98	0.49	0.29	0.42	0.07	0.28	0.1
M	F	132.8	23.76	3.46	14.51	9.18	15.46	1.4
	P value	****	****	*	****	***	****	ns
	R square	0.9	0.63	0.2	0.51	0.4	0.53	0.09

ns: p<0.5; *p>0.5;**p>0.1; ***p>0.01; TF - younger goat femele; P - primiparous goat femele; M-multiparous goat femele; WBC-white blood cells; LYM-lymphocyte; MON- monocyte; NEU-neutrophils; LY - lymphocyte; MO-monocyte; NE -neutrophils; RBC- red blood cells; RBC- red blood cells; HGB- hemoglobin HCT -Hematocrit; MCV - mean corpuscular volume; MCH-mean corpuscular haemoglobin; MCHC- mean corpuscular hemoglobin concentration; RDWc- red cell distribution width.

According to Tables 1 and 2, the WBC values are significant (***p>0.01) between goat of the same age in different physiological statuses, but also between goats of different ages in the same physiological status.

The increase in hemoglobin levels could be due to the increase in free radicals on the erythrocyte membrane, which is rich in lipids, as well as the final erythrocytes lysis, in which case the animal consumes more feed or decreases voluntary intake under heat stress (Srikandakumar et al., 2003).

Neutrophils are the body's main defender against infections and antigens. Elevated neutrophil levels may indicate an active infection; a low neutrophil count may indicate a compromised immune system. Lymphocytes are involved in protecting the body against viral infections. Elevated lymphocyte levels may indicate a depleted immune system. Monocytes are useful in fighting severe infections and are considered the body's second line of defence against infection and the largest cells in the bloodstream (Alam et al., 2011). All these hematological parameters were within normal limits during the period studied, which indicates a proper state of health of the animals. RDW - red cell distribution is useful for identifying animals with anemia and is interpreted together with the other parameters, namely with MCV. Regarding the RDW variation during the transition before breeding to the postpartum period, it is significant for the young female and insignificant for primiparous and multiparous.

Tabel 2. Variation of hematological parameters according to physiological status for Carpathian goat

physiological status	Groups	WBC 10^9/l mean±SD V%	WBC 10^9/l Min/ Max	LYM 10^9/l mean±SD V%	LYM 10^9/l Min/ Max	MON 10^9/l mean±SD V%	MON 10^9/l Min/ Max	NEU 10^9/l mean±SD V%	NEU 10^9/l Min/ Max	LY % mean±SD V%	LY % Min/ Max	MO % mean±SD V%	MO % Min/ Max	NE % mean±SD V%	NE % Min/ Max
normal	TF	10.86±0.31 9.9	10.43 11.41	8.18±0.22 4.9	7.85 8.54	0.29±0.04 0.19	0.22 0.37	6.27±0.53 28.49	5.67 7.12	6.15±3.32 111.05	57.6 66.6	1.51±0.15 2.3	1.3 1.8	36.96±0.89 79.6	35.2 38.2
	P	9.54±0.31 9.76	8.99 9.94	7.98±0.29 8.4	7.48 8.43	0.19±0.04 0.16	0.11 0.24	7.42±0.2 4.2	7.11 7.77	60.32±0.72 52.62	59.2 61.4	1.29±0.37 14.3	0.8 0.9	55.86±0.63 40.26	55.2 56.8
	M	8.74±0.25 6.46	8.39 9.1	6.87±0.29 8.68	6.26 7.23	0.32±0.75 0.56	0.2 0.41	5.34±0.43 18.77	4.55 6.07	60.78±0.72 52.4	59.4 61.8	1.65±0.26 69.44	1 1.9	37.79±0.40 16.76	37.2 38.5
Level of signficance		****		****		****		****		ns		*		****	
gestation	TF	11.09±0.11 14.21	10.84 11.29	7.19±0.30 94.70	6.79 7.74	0.21±0.03 1.3	0.16 0.28	7.65±0.12 16.45	7.43 7.86	61.15±1.66 278	58.9 63.2	1.71±0.08 7.66	1.6 1.8	38.32±0.54 29.51	37.3 38.9
	P	10.29±0.19 3.84	9.96 10.63	7.07±0.4 16.13	6.65 7.95	0.14±0.02 0.06	0.1 0.19	7.08±0.11 1.22	6.92 7.23	44.95±0.52 27.16	44.1 45.7	1.65±0.19 3.61	1.3 1.9	57.86±0.36 13.04	57.3 58.6
	M	9.60±0.18 3.6	9.3 9.87	6.31±0.11 1.29	6.12 6.46	0.21±3.46 0.12	0.17 0.26	6.15±0.16 2.72	5.87 6.42	60.56±0.45 20.48	60.1 61.4	1.65±0.19 3.61	1.3 1.9	39.02±0.76 58.62	37.5 40.3
Level of signficance		****		****		****		****		****		ns		****	
postartum	TF	11.63±0.20 4.3	11.27 11.87	8.26±0.20 4.3	8.02 8.63	0.16±0.02 0.05	0.12 0.2	0.82±0.11 1.35	6.56 6.96	61.26±26 0.46	60.1 61.8	1.44±0.13 1.82	1.3 1.7	36.17±0.6 37.12	35.2 37.5
	P	10.86±0017 2.91	10.54 11.1	8.12±0.14 2.14	7.98 8.45	0.12±0.01 0.02	0.1 0.15	6.41±0.16 2.76	6.16 6.75	45.08±0.46 21.28	44.2 45.7	1.5±0.11 1.33	1.3 1.7	53.51±0.87 75.7	51.6 54.5
	M	10.02±0.11 11.36	9.78 10.13	7.08±0.31 9.65	6.36 7.46	0.13±0.01 0.02	0.11 0.16	5.96±0.16 2.86	5.53 6.12	57.09±0.51 26.76	56.1 57.6	1.18±0.13 1.95	1.3 1.7	35.82±0.28 8.17	35.4 36.2
Level of signficance		****		****		***		****		****		ns		****	

ns: p<0.5; *p>0.5;**p>0.1; ***p>0.01; TF - younger goat female; P - primiparous goat female; M-multiparous goat femele;WBC-white blood cells;LYM-lymphocyte; MON-monocyte; NEU-neutrophils; LY - lymphocyte; MO-monocyte; NE -neutrophils; RBC- red blood cells;

physiological status	Groups	RBC 10^12/l		HGB g/l		HCT %		MCV ƒl		MCH pg		MCHC g/dl		RDWc %	
		mean±SD V%	Min/ Max	mean±SD V%	Min/ Max	mean±SD V%	Min/ Max	mean±SD V%	Min/ Max	mean±SD V%	Min/ Max	mean±SD V%	Min/ Max	mean±SD V%	Min/ Max
normal	TF	15.53±0.29 8.9	15.03 15.91	10.47±0.13 1.8	10.26 10.71	28.01±0.37 14.2	27.5 28.84	19.9±0.37 14.06	16.23 17.36	6.03±0.16 2.6	5.8 6.25	34.38±0.93 87.51	33 35.8	33.55±0.82 67.83	32.4 34.7
	P	15.67±0.17 3.19	15.3 15.9	8.75±0.41 0.17	8.7 8.82	25.86±0.47 22.72	25.13 26.42	17.81±0.44 19.89	16.99 18.46	6.00±0.15 2.3	5.8 6.25	36.26±0.6 36.93	35.4 37.5	37.37±0.87 76.9	36.5 39.5
	M	16.45±0.32 10.52	15.85 17.06	8.36±0.08 0.65	8.25 8.51	26.61±0.61 38.09	25.66 27.73	16.08±0.27 7.6	15.65 16.45	5.77±0.23 5.5	5.3 6.1	37.45±0.49 24.54	36.7 38.2	36.33±0.51 26.45	35.4 36.9
Level of segnificance		****		****		****		****		**		****		****	
gestation	TF	17.39±0.16 26.98	16.99 17.57	11.01±0.25 63.22	10.7 11.4	27.65±0.09 0.83	27.52 27.82	17.29±0.11 13.38	17.08 17.42	6.13±0.14 19.98	5.97 6.4	35.16±0.61 37.37	34.3 35.9	35.28±0.67 45.28	34.1 36.1
	P	17.6±0.09 0.83	17.57 17.84	9.15±0.32 10.72	8.8 9.9	25.42±0.24 6.07	25.12 25.97	18.44±0.18 3.29	18.16 18.67	6.09±0.16 2.67	5.86 6.34	36.71±0.43 19.21	36 37.5	37.88±0.48 23.95	36.9 38.7
	M	17.87±0.06 0.37	17.76 17.96	9.19±0.42 18.98	8.6 9.7	26.21±0.11 1.22	26.12 26.41	16.61±0.28 8.17	16.2 16.9	6.12±0.20 4.18	5.83 6.6	38.09±0.28 8.1	37.6 38.5	36.34±0.43 18.84	35.6 36.9
Level of segnificance		****		****		****		****		ns		****		****	
postartum	TF	16.7±02 4.24	16.43 17.01	10.85±02 4.05	10.47 11.12	28.05±0.26 7.08	27.56 28.45	17.3±0.08 0.75	17.1 17.42	6.11±0.14 2.04	5.87 6.28	34.03±0.54 30.01	33.4 35.2	35.32±0.67 45.95	34.3 36.1
	P	17.8±0.05 0.34	16.98 17.2	9.38±0.34 12.09	8.83 9.86	25.93±0.36 13.21	25.26 26.28	7.81±0.41 17.08	17.06 18.34	6.06±0.09 0.82	5.91 6.25	35.86±0.65 42.26	35.1 37.4	37.71±0.49 24.76	36.7 38.3
	M	17.3±0.08 0.68	17.21 17.46	9.15±0.28 7.5	8.65 9.45	26.73±0.48 23.56	26.07 27.64	16.1±0.17 3.1	15.76 16.34	6.05±0.11 1.29	5.82 6.17	37.19±0.28 8.1	36.8 37.6	36.67±0.51 26.67	35.7 37.3
Level of segnificance		****		****		****		****		ns		****		****	

ns: p<0.5; *p>0.5;**p>0.1; ***p>0.01; TF - younger goat female; P - primiparous goat female; M-multiparous goat female;RBC- red blood cells; HGB- haemoglobin; HCT -Hematocrit; MCV - mean corpuscular volume; MCH-mean corpuscular hemoglobin, MCHC- mean corpuscular hemoglobin concentration; RDWc-red cell distribution width.

CONCLUSIONS

It can be concluded that age and physiological status produce significant changes on some blood parameters of the Carpathian goat raised in the Poiana Aiudului area.

Measurements made on this goat population can improve future studies for a better understanding of the biochemical phenomena that occur in the animal body and ensure a farm management that contributes to animal welfare and ensuring conditions for them, adapted to physiological status.

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MORPFO-PRODUCTIVE CHARACTERISTICS OF AUBRAC CATTLE BREED: A SISTEMATIC REVIEW

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Abstract

This work reviews the most important aspects of the characterization of the Aubrac beef cattle breed. Into the Romania, this cattle breed was first imported in 2013, from France, after an extensive initial documentation. According to data published by M.A.D.R. in 2018, 808 Aubrac heads were registered, currently more than 2000 heads were registered. Aubrac is an old breed from France, originating from the Aubrac Mountains (southern Central Massif), which in the last 4 years has become an important object of interest for beef cattle breeders in our country. The meat obtained from Aubrac cattle has a beautiful red, intensely marbled, with a high protein content and its subtle aromas makes it one of the tastiest and most appreciated beef. This paper wants to emphasize that the exploitation of this breed in our country could bring great benefits to farmers, being a breed adaptable to environmental conditions in Romania and with extraordinary meat qualities. The paper also reviews the most relevant information in the literature on the Aubrac beef cattle breeds.

Key words*: Aubrac breed, beef, farms.*

INTRODUCTION

Originally from the Aubrac Mountains (southern Central Massif), the Aubrac Breed cow is an old breed in France. Grown mainly in the department of Aveyron, Cantal, Lozere and Haute-Loire, over time it has spread especially in difficult areas, unsuitable for other breeds: the mountainous area (Aude, Puy-de-Dôme, Pyrenees Orientales, etc.) and the plateaus high limestones (Gard, Hérault, Landes, etc.).

Initially bred as a mixed breed later after World War II it was bred mainly for meat production. Although the genealogical register dates from 1893, the selection began many years before (17th century, Benedictine monks who lived in the monastery of Aubrac).

The breed is exploited mainly for meat production, although initially in the nineteenth century, this breed was highly valued for the quality of milk, with a production of about 2,200 kg of milk/lactation, containing about 4.13% fat. Due to the extraordinary organoleptic properties of milk, a type of high-quality was created, famous in France under the Laguiole brand, which is still very popular today. The meat obtained from Aubrac cattle has a beautiful red, intensely marbled, with a high protein content and its subtle aromas makes it one of the tastiest and most appreciated beef.

Due to its hardiness, resistance to a certain hilly and mountainous climate, with a remarkable longevity and survival, excellent recovery of plant nutrients, very low maintenance costs, obtaining a consistent carcass of meat with special organoleptic properties, this breed has become the target of local farmers, being described as a simple, rustic and unpretentious breed.

The main objective of this study was to provide useful information about the morpho-productive characteristics of the Aubrac cattle breed and the future growth prospects.

MATERIALS AND METHODS

To achieve the objectives of this study, several bibliographic sources from the literature were consulted. The main issues addressed concern the morphological and productive evaluation of the Aubrac breed, especially in our country, as well as the adequacy of the adaptation of this breed to the conditions in our country.

The research methods used in this study were the observation, analysis and graphical interpretation of data from the literature on the numerical evolution of our country but also the morpho-productive characteristics (especially meat production) of the Aubrac cattle breed.

RESULTS AND DISCUSSIONS

1. The morphological and productive characteristics of the Aubrac cattle breed

Aubrac breed, the old breed from France, originating from the Aubrac mountains (south of the Massif Central), which in the last 4 years has become an important object of interest for beef cattle breeders in our country.

The breed is of medium size, the cows reaching 550-800 kg and the bulls 900-1200 kg. They have a reddish-brown color, darker around the eyes and snout that continues with white, black skin, lyre-shaped horns. Short neck, wide chest, muscular rump, short and strong legs. The Aubrac breed is distinguished by several characteristics that it possesses and that make it unique.

Rusticity: Aubrac cattle are easy to raise and do not require much intervention from the farmer. Being native to the mountain area, it adapts easily to any climatic conditions, it is resistant to cold and humidity and due to its characteristic color (wheat color) it easily withstands exposure to the sun during summer and extreme heat. It is an unpretentious breed in terms of food. Consume large amounts of coarse fodder even if they are not of the best quality, make excellent use of natural pastures. Aubrac animals are disease resistant, have a very resistant black hoof, can travel long distances to graze (Wegner et al., 2000).

Reproductive performance: fertility is one of the most important indicators in any cow farm. Obtaining one calf a year from each cow is the wish of every farmer. In the Aubrac breed this objective is achieved very easily without treatments and interventions from the farmer. In France the interval between calvings is on average 375 days. Calving is mild 98% of cows face alone without the assistance of the farmer. After calving, cows possess an impressive maternal instinct, defends and take care of her calf, then breastfeed it until the age of 7-8 months.

Longevity: longevity is another characteristic of the Aubrac breed on average, an Aubrac cow ends her productive life at the age of 11 after giving birth to 8-9 calves (5% of cows under official production control in France have a productive life of 12 years).

Productive performance: the Aubrac breed is unique because although it is a rustic breed without great pretensions in terms of feeding and maintenance conditions, it produces a calf that manages to make productive performances similar to the super specialized meat breeds. Thus, the male calves, purebred, easily reach the weight of 275-280 kg at the age of 210 days. Purebred females weigh 240-250 kg at the age of 201 days, average daily increase 1300 g, slaughter efficiency 60% and excellent adaptability to environmental conditions, very resistant to cold. To increase the production performance, crosses can be made with other breeds of meat.

The first Aubrac cows in Romania were purchased in 2013, directly from France. The acquisition of this breed was not a coincidence, but made after extensive documentation. The most important characteristics of the Aubrac breed were the extraordinary qualities of adaptation, the superior taste of the meat, as well as due to the excellent growth increase.

Initially, 15 Aubrac heads were bought, then another 14 pregnant women and a bull were brought, and then another 30 one-year-old heifers. In total reaching many 60 heads.

Then, at the end of 2018, according to statistics published by the C.O.P.C. (M.A.D.R. - A.N.Z.) at the level of our country there was a staff of 808 Aubrac heads.

Nr.crt.	RASA	ASOCIAȚIE C.O.P.C.							Total
		A.C.A. ARAD	A.ABERDEEN ANGUS SIBIU	A.ANUGUS RO SUCEAVA	A.C.B.C.R. SUCEAVA	A.C.B. NARCISA	C.O.O.P. CLUJ	A.C.V.B R	
1	Aberdeen Angus	11	31721	46	203	267	360		32610
2	Aubrac	10	0	708		0			808
3	Blonde D'Aquitaine				91				91
4	BB								
5	Charolais	740	61		2850	338	101		4090
6	Galloway		95	253		29			377
7	Hereford			0					0
8	Highland		59	312					371
9	Limousin	13	360		2557	30	97	10	3067
10	BR-Simmental	365	43		101	4	948	6	1467
11	Romagnola								0
12	Grey Steppe			811	5				816
13	Salers		45						45
TOTAL R.C.		1139	32384	2222	5807	668	1506	16	43742
TOTAL METISI R.C.		168	5240	124	1601	296			7429
TOTAL		1307	37624	2346	7408	964	1506	16	51171

Figure 1. Cattle herds specialized for meat production, by breeds and associations C.O.P.C. (source: http://www.anarz.eu/)

There are currently an estimated 2,000 head of Aubrac cattle in Romania. At present, very little data about this breed are recorded in our country, given the fact that it is a new breed, but I consider that it is in a process of continuous development.

2. Meat production of the Aubrac breed

Meat is an important part of the human diet, with strong implications for health, economy and culture (Web, 2008). The beef production in the EU fell in 2019 due to lower prices and reduced herds. It fell further in 2020 due to the same factors and the COVID-19 pandemic, which led to a reduced in slaughter in the second quarter and narrowed production and demand for high-quality tranches. A small recovery is expected in the second half of 2020, with the overall reduction being 1.7% for the full year (Bendikas et al., 2009).

The United States, Brazil and the European Union produce about 47% of the world's beef, with about 19%, 15%, and 13%, respectively. On average, beef consumption in Europe is around 16 kg per capita (20% of total meat consumed). This proportion of beef consumption is lower than that observed in Argentina, Brazil, the United States and Australia, where beef accounts for 55%, 41%, 34%, and 37% of total meat consumed, respectively (Vandendriessche, 2008). Total beef consumption is likely to increase from 2011 to 2025 with the lowest increases in Australia and the European Union and the highest increases in Brazil and China. Similarly, the largest increases in production are likely to occur in Brazil and China from 2011 to 2025 and the lowest in the European Union and especially in the United States, which will probably produce less beef in 2025 compared to 2011. Beef exports are likely to increase from 2011 to 2021 in major countries, except the European Union and China and especially the United States (Stimbirys et al., 2016).

Romania, through the potential for grazing and raising beef cattle in an extended system, is ideally placed to respond to these fashionable market signals. Romanian farmers have the ability to sustainably increase production to meet global demand.

In our country, in November 2020, 51,000 head of cattle were slaughtered, increasing by 8.5% compared to October 2020, but decreasing by 7.3% compared to November 2019. Meat production obtained in November 2020 from slaughtered animals reached 8,330 tons, compared to 7,583 tons in October 2020 and 9,274 tons in November of the previous year (www.fao.org).

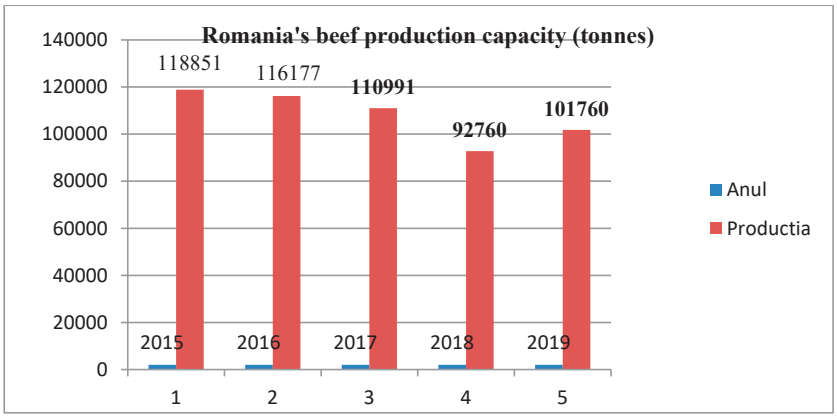

Figure 2. Romania's production capacity for beef (source: made by the author based on FAO data, 2015-2019)

Also, based on the graph, a slight decrease in beef production can be observed in the period 2015-2019.

The chemical composition of meat depends on the species, age and sex of the animals, feeding conditions and health (Boișteanu et al., 2002). Assessments of the physical properties of meat are made according to color, pH, water retention capacity, shear loss of strength and cooking (Bendikas et al., 2008; Jukna et al., 2009 a;). The water retention capacity is an important technological feature in the meat industry (Vavrišinova et al., 2013). The color and appearance of the meat is its commercial image. The pH level determines the suitability of the meat for sale and is associated with the color of the meat and the water retention capacity (Oury et al., 2009).

In a research conducted by Lithuanian researchers in 2017 on the quality of meat of different breeds of cattle, they showed that intramuscular fat influenced meat flavor and juiciness. Thus, the beef from the Charolais and Aubrac breeds was of the highest quality, while the meat from the Limosine breed was of a lower quality. In terms of meat consistency, the samples from the Aubrac breed were harsher and the meat from the Charolais breed was softer in consistency. The difference reached up to 0.9 kg cm^2 ($P<0.01$). The differences in meat water content and water loss through cooking were not significant.

In French cattle breeds, Mordenti et al., in 2018, demonstrated that selection on muscle mass was associated with a decrease in intermuscular and intramuscular fat content, but also collagen. For example, the main meat breeds Charolaise, Limousine and Blonde d'Aquitaine have less intermuscular and intramuscular fat than environmentally resistant breeds such as Aubrac and Sales.

Gagaoua et al. in 2018 showed that in the case of Aubrac bulls, slaughtered at the age of one year, there was a slaughter yield of 58%, with an average carcass weight of 262 kg, higher values than other breeds such as Charolais or Normandy.

Jukna et al. studied in 2017 the quality of meat from 6 breeds of cattle specialized for meat production, including meat from the Aubrac breed.

Table 1. The chemical composition of beef from different breeds of meat (after Junka et al., 2017)

CHEMICAL COMPOSITION				
BREED	**Dry matter**	**Protein**	**Fat**	**Mineral substances**
Charolais	24.87 ± 0.25	21.60 ± 0.14	2.19 ± 0.27	1.09 ± 0.10
Simmental	23.35 ± 0.54	21.77 ± 0.27	1.83 ± 0.17	1.10 ± 0.03
Limousine	24.50 ± 0.26	22.10 ± 0.28	1.24 ± 0.15	1.15 ± 0.09
Hereford	25.17 ± 0.54	21.44 ± 0.27	2.51 ± 0.09	1.20 ± 0.07
Aubrac	**25.43 ± 0.23**	**23.23 ± 0.34**	**1.03 ± 0.05**	**1.17 ± 0.11**
Angus	24.26 ± 0.11	20.47 ± 0.81	1.58 ± 0.14	1.15 ± 0.12

Aubrac meat was determined to have the highest rate of meat protein sufficiency, whereas the lowest rate was found in Hereford meat. The difference reached up to 1.79% ($P<0.05$). Differences between breeds were also observed for meat sensitivity. The hardest meat was from Aubrac cattle, while the tenderest was from Charolais. The difference reached up to 0.9 kg cm^{-2} ($P<0.01$). There were no significant differences between breeds for meat water content, water retention capacity. Intramuscular fat levels varied by race. Hereford meat has had the highest level of intramuscular fat, while the lowest level has been found in Aubrac meat. The difference was 1.48% ($P<0.05$). Differences between breeds were also observed for the physical quality of the meat. The meat of the French breeds (Charolais and Limousine) was lighter and had a higher water retention capacity - 0.78-0.82% ($P>0.05$) compared to the meat of the English breeds (Hereford and Angus).

In terms of protein content, beef is superior to pork or battalion. The protein/lipid ratio varies from 1/0.5 for veal to 1/1.8 for fatty beef (Chambaz et al., 2003).

Meat, in general, has a high content of essential amino acids, and beef in particular, is richer in the following amino acids: lysine, methionine, glutamic acid. The content of essential amino acids is significantly equal to that of chicken and higher than that of pork and sheep. Pork is richer in valine and arginine, and sheep in leucine and thiamine. For example, the consumption of 100 g beef provides an intake of 26 g protein. Aubrac beef is known for its very high protein content.

The biological value of the meat varies according to age, fattening status and region of slaughter. Meat from adult animals has a lower biological value than youth, semi-fat and fatty meat has a higher content of amino acids. The amount of essential amino acids is higher in regions rich in musculature.

The energy value varies greatly in relation to the state of fattening, age and anatomical or butchery region. Fat and very fatty meat have an energy value of 50-60% respectively, 80-110% higher than the lean one. Butcher regions rich in muscle have a lower energy value than regions of poorer quality rich in adipose tissue.

While in pork, the share of protein in ensuring energy levels is low, in beef it is higher. Protein calories represent 45-46% in veal, 33-35% in lean beef and 20-22% in fatty beef.

Meat is an important source of B vitamins (B1, B2, PP, B6, folic acid, pantothenic acid, B12). Beef covers 100% of the daily requirement of human B12 vitamins, 63% of the need for vitamin PP, 22.24% of that of vitamin B6 and 10% of that of vitamin B2. Internal organs and intermuscular fat have a higher content of fat-soluble vitamins. Beef is poor in vitamin C and vitamins K and D.

Meat is an important source of minerals: iron, sodium, potassium (Jurie et al., 2006). Phosphorus, sulphur and chlorine are found in large quantities, which determine the acidifying action of meat in the human body. The nutritional value of beef is given by the high iron content, respectively 2.1-4.0 mg/100 g muscle and 7-8 mg/100 g liver and fresh kidney. Of the total amount of iron, 80-85% is found in the form of heme and 15-20% ferritin. Along with iron, potassium, phosphorus, sulphur and chlorine are found in large quantities in beef. Copper, zinc, magnesium, selenium, manganese, cobalt, aluminium, etc. are found in small but sufficient quantities in beef (Soulat et al., 2016).

CONCLUSIONS

The meat from Aubrac cattle has special features, having a beautiful red, intensely marbled, with a high protein content and its subtle aromas make it one of the tastiest and most appreciated beef.

Objectively, the quality of meat is the sum of five major complexes of properties, which are the result of the physico-chemical and morphological composition of the meat, as well as its microbiological properties.

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EFFECTS OF EXTRACT *ORIGANUM VULGARE* L. ON *BOMBYX MORI* L. ADDED TO WITH ARTIFICIAL FOOD

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Abstract

Originated in ancient times, mulberry silkworm farming is a cost-effective sub-sector of agriculture. The strong dependence on food needs and especially the seasonality and distribution area of mulberries are limiting factors in the rearing of larvae.In our country Morus alba grows well and gives high yields of foliage in certain seasons of the year due to its characteristics. The resulting leaf mass has a high nutritional value. An alternative to nutrition is artificial food. It allows for growing in any season of the year, regardless of external climatic conditions. Some plant extracts are food stimulants and improve food intake, growth and even disease resistance. The aim of the present study was to test a hybrid I1xVB1xH2xHB2 *created in Scientific Center on Sericulture, Vratsa, Bulgaria, for susceptibility to artificial food with added extract of Origanum vulgare L. as a growth stimulant. Tracking the most important biological, reproductive and technological features of silk larvae and butterflies; Artificial food was readily accepted by Bombyx mori L. Higher values were observed in the experimental groups fed with artificial food and added extract, we observed the growth intensity of the larvae and their viability.*

Key words*: artificial diet, Bombyx mori L., Morus alba, mulberry silkworm, Origanum vulgare* L.

INTRODUCTION

The silkworm (*Bombyx mori* L.) is a fully domesticated insect and mulberry leaves are its only food that is very important for proper growth and development (Legay, 1958; Kumar, 2013). Nutrition is the only factor that almost individually increases the quality and quantity of production and productivity of the silkworm cocoon (Laskar & Datta, 2000)

In their studies, Gobena & Bhaskar (2015) found that larvae fed on mulberry leaves and added plant extracts had better growth and development compared to control groups.

Mulberry leaves treated with plant extracts have different effects on growth, development and reproduction. Aqueous extracts of *Lantana camara*, *Parthenium hysterophorus* and *Tridax procumbens* (Hipparagi et al., 2001), *Tribulus terrestris* (Murugesh & Mahalingam, 2005), *P. hysterophorus* (Rajashekaragouda et al., 1997), *P. hysterophorus* and *Tridax procumbens* (Mahesha et al., 1999b), *Psoralea coryleifolia* and *Phyllanthus niruri* (Shubha, 2005), *Withania somnifera* (Bhaskar et al., 2004) have a beneficial effect on the species *Bombyx mori* L. Sangamithirai (2014) found that larvae fed on mulberry leaves treated with spirolin extract gave better results. All the signs related to the extraction of cocoons and their quality are significantly affected.

Bombyx mori L. can also be fed with artificial food. The introduction of technologies for the use of artificial mixtures in the practice of sericulture, testing and creation of high-yielding hybrids suitable for cultivation with artificial food, expands the area and opportunities for cultivation and experimentation with laboratory conditions of *Bombyx mori* L.

The use of artificial food has some advantages, such as reducing the care and costs of large mulberry plantations, expanding the range and opportunities for growing the species *Bombyx mori* L. regardless of the season, increasing economic efficiency, reducing the cost of the final product - silk.

The creation and use of semi-synthetic compound feed makes it possible to obtain high results in the development, viability and productivity. In many ways artificial food may be more favorable than natural food (Oatmeal, 2000).

In recent years, the efforts of a number of authors have focused on the search for natural, natural and ecological products that are an alternative to the previously used growth stimulants (Grela, 2000; Meriden, 2000; Ratcliff, 2000).

It has long been known that some herbs stimulate the appetite of animals, increase metabolic and immune status and improve their general condition and productivity (Hammer et al., 1999; Close, 2000; Toncheva et al., 2004; Dimitrova et al., 2004; Dimitrova, 2009).

Plant extracts and essential oils from savory, chestnut, oregano, thyme, nettle and others have been studied (Adams, 1999; Delacon, 1999; Lyons, 2001; Spring, 2002).

There is a lot of information in the literature about the action of essential oils, especially oregano oil. It has antimicrobial (Dorman & Deans, 2000), fungicidal, antioxidant, cytostatic and antiparasitic activity (Force et al., 2000).

Oregano oil is mainly used in the pharmaceutical industry and as a spice. Carvacrol has antimicrobial, antitumor, antimutagenic, analgesic, antispasmodic, anti-inflammatory, antiparasitic, insecticidal and antihepatotoxic effects, which largely explains the in vivo mechanism of action of carvacrol (Can Baser, 2008).

One of the most widely tested and used herbs in pig farming is oregano (*Origanum vulgare*), due to its antimicrobial (Burt & Reinders, 2003; Dorman et al., 2000; Lambert et al., 2001; Sivropolou et al., 1996), anti-inflammatory (Ariza-Nieto et al., 2003), antioxidant (Lagouri et al., 1993; Milos et al., 2000; Vekiari et al., 1993), fungicidal (Adam et al., 1998; Daouk et al., 1995; Stiles et al., 1995), cytotoxic (Sivropolou et al., 1996), antiparasitic (Forse et al., 2000), insecticidal, anticoccidial and immunostimulatory effects (Park & Bilkei, 2004). In studies performed by Donev (2001) with essential oil of oregano (*Origanum vulgare*) on broiler chickens, a good nutritional effect was reported due to the biologically active substances contained in it: carvacrol, thymol, pinene, lemon and borneol.

In Bulgaria, Nicheva (1985), Gurgulova (1996), Malinova (2003) found that the essential oils of savory, mint, anise, eucalyptus and thyme have a relatively high activity against some pathogenic bacteria. In the treatment of suckling pigs with Bioxan-emulsum at a dose of 11.25 mg of oregano essential oil per 1 kg. t.m., Dimitrova et al. (2004) reported a reduction of about 50% in pigs with gastroenteritis and weaning until weaning.

There are studies that show the successful replacement of antibiotics, such as growth stimulants with oils, extracts or oregano-based products in suckling, growing and fattening pigs (Capms, 2005; Thomke & Elwinger, 1998; Günter & Bossow, 1998; Ingram, 1997), as well as in our country (Kanev et al., 2002; Toncheva et al., 2004).

Oregano essential oil contains monoterpenoid phenols (carvacrol and thymol), phenolic acids (rosemary acid), monoterpenes and other active ingredients. The main pharmacologically active components in its composition are first carvacrol, followed by thymol and rosemary acid.

Xu et al. (2008) tested the antibacterial activity of carvacrol and thymol against *Escherichia coli*. The author concluded that carvacrol and thymol were effective in inhibiting growth.

In a study, Lopez et al. (2007) monitored the antibacterial efficacy of essential oils of oregano, cinnamon and thyme against the bacteria *Escherichia coli*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Salmonella enterica Serotype Choleraesui*s, etc., concluding that the minimum inhibitory concentrations of oil (MIC) Ritan are lower than other oils. Avtoran concluded that the carvacrol and thymol oil of oregano and cinnamaldehyde have the strongest antimicrobial activity.

In similar studies of 5 essential oils against avian and porcine strains of enterotoxigenic *Escherichia coli*, Penalver et al. (2005) found that *Origanum vulgare* showed the highest antimicrobial activity against all strains of Salmonella spp. A study conducted in Greece on the effect of Greek plant products, including oregano oil, on stomach diseases and peptic ulcer, found that some herbs, including *Origanum vulgare*, were active against one reference strain and 15 clinical isolates of *Helicobacter pylori* (Stamatis et al., 2003).

Yan et al. (2009) in an experiment conducted in Korea showed that with good nutrition of pigs at the beginning of the fattening period (24 kg) and the addition of essential oil in a

concentration of 0.01% of the ration, a significant increase in average daily growth and -good utilization of feed (P<0.01), increase in daily feed consumption and nitrogen uptake (P<0.01) and energy (P<0.05) during the first 6 weeks of weaning, compared to the group with low nutrition density and without the addition of essential oil. During the next period of experience (up to the 16th week) no significant differences were found.

MATERIALS AND METHODS

The research was conducted in the educational-experimental base of the Faculty of Agronomy at the University of Forestry, Sofia, in February 2021. The eggs were laid for incubation on 02.02.2021.

The tetrahybrid I1xVB1xH2xHB2 created in the Scientific Center for sericulture - Vratsa was used. Three variants were tested, respectively without added oregano extract and with extract. The laid eggs were in a volume of 3 g. After the third sleep, the beetles from the experimental groups were counted in 50 larvae reared until the cocoons were twisted.

During the experiment for feeding the silk larvae, artificial food containing flour from dried mulberry leaf, provided by Scientific Center on Sericulture, Vratsa, Bulgaria, was used. The artificial food is prepared according to the methodology recommended by the manufacturer.

Extract of *Origanum vulgare* L. B 1 liter of water is added/10 g of the herb in experimental group 1, in experimental group 2, 15 g/Place for 1 hour, distilled water is added to the solution in a liter, after which it is added. 24 hours. Strain through a filter cap and store at low temperature.

Method of preparation of artificial food

Distilled water or herbal extract is added to the dry substance. 1 kg dry substance + one l extract of *Origanum vulgare* L. Homogenize with a mixer and put in a box with a layer thickness of 2 cm. The resulting mixture was subjected to a heat treatment of 850 KW for 10 minutes and cooled. The prepared food is stored in a closed container at a temperature of 2-5°C until the moment of feeding. The food can be stored for up to 40 days without losing its nutritional qualities. Before giving the larvae, the prepared food is removed from the refrigerator and tempered. Cut into strips 2 cm thick

An extract of *Origanum vulgare* L. with different concentrations was added in the preparation of the artificial food for the larvae from the experimental group.

Control larvae are fed artificial food without additives, in which the nutrient mixture is prepared only with distilled water.

Incubation and rearing of larvae was performed according to the generally accepted methods in our country (Petkov, 1982; Petkov, 1995), which aims to accelerate the development of the embryo in the egg. After reaching stage IV, the eggs are placed again at a storage of 2-5°C.

Hatching began 11-12 days after their incubation. Growing parameters: first and second age t-30°C and relative humidity 85%, in the third age t-27°C and relative humidity 80%, fourth age t-26°C and relative humidity 75%, in the fifth age and when turning the cocoons t-25°C and relative humidity 70%.

Studied were the most important productive features, the data were processed by the conventional methods.

Table 1. Larvae fed on artificial diet

Instar	Temperature, °C	Relative humidity
I	29–30	90
II	29–30	90
III	27–28	80
IV	26	70-75
V	24	70
Cocooning	25–27	55–60

RESULTS AND DISCUSSIONS

Pupation rate is one of the most important biological indicators, with a special contribution to the formation of productivity. It affects the yield of cocoons and raw silk.

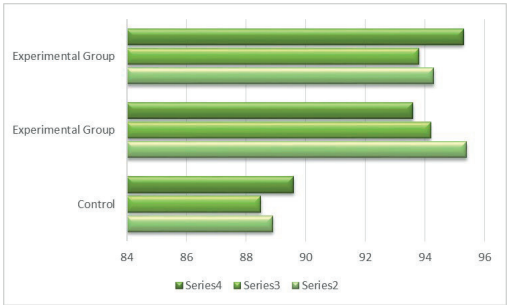

Figure 1. Pupation rate, %

Figure 1 presents the data for Pupation rate, there are small differences between the control and experimental groups. In the control, the values were 88.5 to 89.6% and higher in the experimental groups from 93.6 to 95.54% in experimental group 2 with a more concentrated extract in the food mixture. From the obtained results it can be said that the extract of *Origanum vulgare* L. in the diet slightly increases the viability of the beetles from the experimental groups.

In determining the susceptibility of silkworms to artificial food, the main feature is the number of normally developing individuals, calculated as a survival rate.

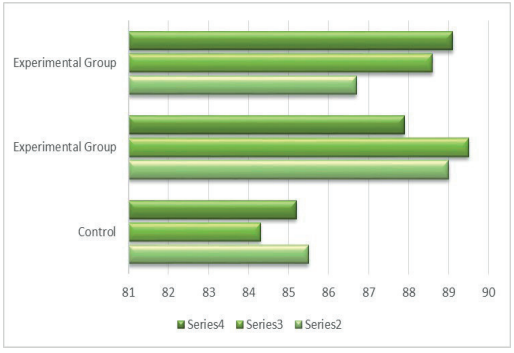

Figure 2. Survival of larvae, %

Figure 2 shows that the survival of larvae in the experimental groups is from 86.7 to 89.5, the susceptibility of artificial food with added extract is high. During the control, lower but normal values of the symptom are observed.

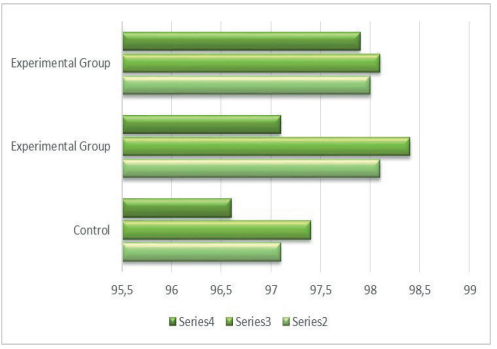

Figure 3. Hatching of the beetle seed (%), average for the period

The hatchability of eggs is largely determined by the technology of their production, storage and incubation. (Petkov, 1989). Figure 3 shows the average values of the sign of hatchability of the beetle seed in percent. No significant differences were observed between the control and the experimental groups. High values of the trait from 96.6 to 98.4% were reported. The extract added to the food of the beetles does not affect the trait.

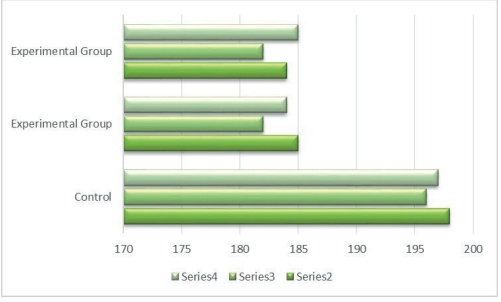

Figure 4. Vth instar duration, h

In Vth instar duration higher values were observed in the control group with values from 196 to 198 h (Figure 4). In the experimental groups, the duration of V-age is shorter, the beetles reach maturity after 182 to 185 h. From the obtained results it can be said that the extract of *Origanum vulgare* L. in the diet has a positive effect on this trait.

CONCLUSIONS

Artificial food was readily accepted by *Bombyx mori* L. Higher values were observed in the experimental groups fed artificial food and added extract, we observed the growth intensity of the larvae and Pupation rate.

The effect of the extract is most clearly manifested at V^{th} instar duration. The effect of the extract is most clearly manifested at duration of V-age.

The results obtained by us are close to those of Gobena & Bhaskar (2015). The larvae fed with artificial food and added plant extracts have better growth and development compared to the control groups. The extract of *Origanum vulgare* L. in food improves the nutritional intake and growth of the beetles from the experimental groups. It has a beneficial effect on the species *Bombyx mori* L.

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ONTOLOGICAL AND LOGICAL RELATIONS IN ANIMAL SCIENCE LANGUAGE

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Abstract

Our present work has as first objective an overview of the conceptual terminology following an onomasiological approach which allows us to work with the ''term'' as main linguistic tool in the animal science language. Due to the fact that animal science terminology is a ''weak'' terminology, most of its terms being taken from the general language, acting as terms only in specialized contexts, our second objective will be to make an analysis of animal science terms using not only onomasiology but also conceptual frames. Thus, we shall analyse the notions of domain shown by different theoretical models, such as the domain of knowledge, the domain of application, the domain of origin, etc. Afterwards, we will present logical and ontological relations in the conceptual system of animal science language.

Key words*: conceptual frames, domain, logic, onomasiology, ontology.*

INTRODUCTION

Onomasiology deals with meanings and meanings relations which exist between individual terms. Conceptual frames which are linguistic representations of the expected relations between cause and effect complete the onomasiology picture which helps us analyse the significance relations among terms within the animal science language. Considering an appropriate approach to new terminological approaches, we shall attempt an outline of conceptual systems in the field of animal science.

We follow the axes according to which the classification process centers upon the knowledge domain and the terms distribution is done by means of the existing relations within the domain, such as: logical relations (generic-specific, coordinating), ontological relations (partitive, associative), etc.

Our work is initiated by a presentation of the *domain* notion and of the *domain types* identified by different theoretical models.

MATERIALS AND METHODS

In terminology, the domain represents the base of an imaginary terminological triangle which also includes the concept and the definition. The domain is equivalent to the conceptual field to which a set of terms belongs.

It is already established that the domain indicates the membership of the concept in a conceptual system, while the definition differentiates between concepts within this system. Thus, the domain represents a cognitive system, a conceptual delimitation being the only way to identify or to denominate a cognitive structure (a conceptual structure, a conceptual system) (Bessé, 2000).

The same author organises the domains into three major categories:

- *A domain of knowledge* represents structured knowledge according to a theme. Thus, Bessé considers the following fields of knowledge: mathematics, law, physics, zoology, botany, economics, linguistics, mechanics and philosophy;
- *A domain of activity* represents the mirror of a human activity, whatever its nature, be it a trade, a practice or an industry;
- *A domain of discourse* is the object of 'meta', scientific discourse, which offers us clues to the nature of the field of knowledge or activity.

Animal science would be in this perspective a domain of activity that falls under a more general domain of knowledge and can become a domain of discourse in a perspective like ours.

Another categorization of domains belongs to Maryvonne Holzem (1999):
- *domain of activity:* for example, the domain of animal science. As such, animal science is a whole that includes several domains of activity: animal nutrition, animal physiology, animal welfare, etc.
- *domain of origin:* The domain of origin (of a term) is the domain where the concept corresponding to the term under analysis originates.
For example: *Animal science is the domain of origin for animal husbandry, the main term used in animal science to refer to its main areas of activity.*
The concept corresponding to a term is used. For example, *cheese* refers to a type of food product obtained by coagulating milk. The word is used in general language, but its extension (term) has the food industry as its field of application.
«Il faut noter que le domaine d'application renvoie à la notion de secteur d'activité. On distingue les concepts en les opposant ou en les associant les uns aux autres. Les relations entre les concepts mènent à la création des systèmes de concepts. Pour le système conceptuel, il correspond à l'ontologie des domaines de spécialité. Les rapports hiérarchiques entres concepts sont très importants, car ils permettent de séparer les différents éléments composant un ensemble organisé de termes en ayant recours aux relations » (Holzem, 1999)
Terminology completes its functional table by describing the types of existing relationships in a specific language. A conceptual relationship establishes a notional link between several concepts, allowing the creation of a conceptual tree in a given domain that appear between concepts, for a better understanding of the studied domain but also for structuring its terminological fields. Our descriptive approach will follow the work of Depecker (2000), Otman (1991, 1996), ISO 704 and Silvia Pavel (2009). Therefore, we understand a conceptual system as a set of concepts structured by their mutual relations:
«*Les concepts n'existent pas en tant qu'unités de connaissance isolées mais sont toujours en relation les uns par rapport aux autres. Que l'on en ait formellement conscience ou non, on crée et on affine constamment les relations entre concepts par le biais de processus mentaux. Un ensemble de concepts structurés en fonction des relations qui les lient est considéré comme formant un système de concepts*» (ISO 704)
According to the above-mentioned sources, we will group conceptual relations into:
- Logical relations and ontological relations (Depecker 2002);
- Associative relations and distinctive relations (Otman 1996) or
- Hierarchy relations and associative relations (Silvia Pavel 2009, ISO 704).

LOGICAL RELATIONS

«Les relations logiques sont les relations qui s'établissent entre concepts d'un point de vue formel. On peut citer comme relations logiques la relation d'identité, la relation d'implication, la relation d'inclusion etc.» (Depecker, 2002).
These relations can be generic, specific and coordinating (ibid.: 51). They represent abstraction relations between concepts that have at least one character in common. For example, cow belongs to the category *bovidae*; belonging to this category implies that it has properties. The concept of //bovidae// covers the common properties of cow species that we can recognise. The domain specialist will certainly understand the content and definition of the concept easily, but the non-specialist must understand the meaning of the concept //bovidae// to understand the definition. This relationship has also been called the TYPE-OF relationship (Figure 1).

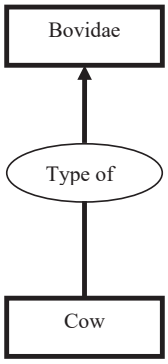


Figure 1. Type- of (gender-species)

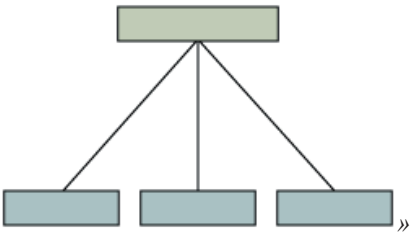
This example illustrates the logical genus-species relation or *"generic relation" (cow is a bovidae species and the intension of //bovidae// is included in the intension of //cow//, a subordinate concept)*

Gender-species relations are very common in terminology. Thus, for example*, mammal - vertebrate - sheep - ovine; mammal - vertebrate - felid - cat, etc.* (the examples follow the sequence of order, *phylum, family* and go up to the last element, which is the term under analysis).

According to ISO 704, a series of concepts that are linked by generic relationships form a vertical sequence, while coordinated concepts with the same level of abstraction form a horizontal sequence.

We consider Silvia Pavel's (2009) and Felber's (1987) view of the types of relationships discussed important.

« *les relations génériques sont représentées par un arbre conceptuel à l'aide de nœuds (rectangles) et de branches (angles aigus)* (Pavel, 2009[1])*:*



»

For Felber, the genus-species relationship is framed in logical subordination, the species being subordinate to the genus:

«Lorsqu'une notion possède tous les caractères d'une autre et au moins un caractère en plus on dit que l'une est une espèce d'une autre, le genre. Du point de vue de la supériorité logique, une notion (le genre) possède un ou plusieurs caractères de moins que l'autre (espèce)» (Felber, 1987: 102)

At the same level of generic relations we find the TYPE-OF relation, a partitive relation where the super-ordinate concept represents a whole and the subordinate concepts represent parts of this whole.

[1] http://www.bt-tb.tpsgc-pwgsc.gc.ca/btb-pavel.php?page=chap2-4-4&lang=fra&contla, page consulted on February 21st 2021

«*Le concept super-ordonné d'une relation partitive est appelé concept intégrant et le concept subordonné est appelé concept partitif»* (ISO 704)

ONTOLOGICAL RELATIONS

Depecker (2002) considers ontological relations as relations that are established by virtue of the structuring natural objects in the world. Thus, there are all-part relations between concepts: the skin is a (detachable) part of the cow, and so are the feet, and these parts are not mutually exclusive, but are in a relationship of co-presence, and consequently there are "relations between concepts whose objects they refer to are in a relationship of presence or contiguity". As a result, the PART-OF relationship takes on two different aspects, depending on whether it is logical or ontological in nature.

For constructed objects, it is always Depecker who envisages another type of relation, namely the TYPE-PRODUCT relation: "thus, an airbus is a type of aircraft, the Airbus A-320 being a particular product in the range of Airbus Industries" (ibid.2002: 87)

We can also provide an example, *hard cheese* as defined by the GDT (the curd is pressed and heated; salting is done for several days with dry salt; they are mainly protected by effect. They are mainly protected by effect. The conservation goes from a few months to a few years. They are cheeses for keeping). It is a foodstuff obtained firstly by the coagulation of the milk and secondly by industrial processes of pressing and salting. Hard cheese is therefore a particular type of cheese - dairy product obtained from the curd.

ASSOCIATIVE RELATIONS

According to Chaumier (1988), associative or "neighbourhood" relations are non-hierarchical relations in which concepts are associated by their spatial or temporal neighbourhood, existing in a natural association.

"The main associative relations are of the type:

Producer-product: baker-baguette;

Product-region of origin: wine-Beaujolais;

Action-result: election-electors;

Action-tool: bludgeon-bludgeon;

Container-content: bottle-milk;
Cause-effect: moisture-mould;
Opposites: heat-coldness" (Pavel, 2009 [Ibid.])

Following the model provided by Silvia Pavel, we will try to list some associative relations that belong to our field of investigation:
Producer - product: cow-milk;
Product - region of origin: Roquefort - Roquefort cheese;
Action - result: milking-milk;
Action - tool: milking- milking machine;
Container - contents: water-trough;
Opposites: lean meat-fatty meat.

Many of the relationships provided by Silvia Pavel are found in Sager (1990) under the heading of "complex binary relationships", described using the following primitive relationships: object, cause, effect, place, form, agent, phenomenon, container, property, product, method, instrument, process, unit of measurement. We have tried to illustrate the relationships proposed by Sager, as far as possible, with examples from our field of study:
Cause - effect: oestrus - reproduction;
Matter - product: milk-butter;
Matter - property: milk-fat content;
Matter - state: milk-milk powder;
Process - instrument: milking-machine;
Process-method: milking-mechanical milking;
Process-object: milking-containers;
Process - object: milking -container for milk;
Phenomenon - unit of measurement: heat-degrees Celsius;
Object - counter-object: poison-antidote.

RESULTS AND DISCUSSIONS

The relation TYPE-OF, a hierarchical relation framed in the series of generic relations, qualifies the majority of relations that characterize the conceptual domain of animal science. A generic relation exists between two concepts when the extension of the subordinate concept includes the extension of the superordinate concept, plus at least one additional distinctive character. As for the extension, that of the superordinate concept includes that of the subordinate concept. The superordinate concept is called a generic concept, while the other is called a specific concept. To account for the functioning of the TYPE-OF relationship, we will apply the validity tests proposed by the ISO 2788(1986) standard, and cited by Otman (1986):
- The first test operates under the name of "all and some". Thus, we will have the following

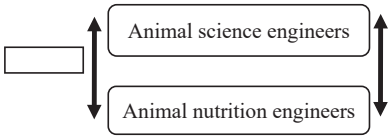


Figure 2. The test "all and some"

This scheme shows us that all animal nutrition engineers are animal science engineers, therefore, animal nutrition engineers are a kind of animal science engineers.
The second test proposed by Ottman is called "concept type", and stipulates that both the hyperonym and the hyponym belong to the same categories. Thus, the concepts cow's milk, buffalo milk, goat's milk represent classes of the concept milk. We will have:
- *Cow's milk is a kind of milk;*
- *Buffalo milk is a kind of milk;*
- *Goat's milk is a kind of milk;*

The relationship TYPE-OF has the characteristic of transitivity: cow's milk is milk while milk can be cow's milk, so the relationships go from generic to specific and from specific to generic. Thus, we can establish that there is contiguity among terms in animal science terminology.

CONCLUSIONS

The results of our analysis are not extensive, however, we can draw the conclusion that the relations existing among the terms in animal science language are quite complex. This way, establishing proper relations can help us in conveying a real conceptual structure of animal science domain. The present analysis, not reaching the dimension of a conceptual map, may help both the specialist and the non-specialist to deal with the concepts proper to animal science field of research.

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BEEKEEPING IN THE CONTEXT OF CLIMATE CHANGE

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Abstract

Climate change is a global phenomenon, driven by industrialization and deforestation, which over time has led to a reduction in the ozone layer and an increase in carbon dioxide. These have led to changes in the integrity of the ecosystem and biodiversity, affecting bee colonies as well. Temperature and humidity play an important role in the secretion of nectar in honey plants, while also influencing the feeding behavior of bee colonies. The study follows the evolution of the meteorological factors mentioned, in the period 2017-2021, during the production harvests of rapeseed, acacia, linden and sunflower, in April, May, June and July. During the analyzed period, statistically significant differences were registered for the analyzed factors compared to their average ($p < 0.05$), with negative influences on the nectar secretion and implicitly on the capitalization of the harvests.

Key words*: climate change, bees, nectar secretion*

INTRODUCTION

Meteorological conditions can influence the capitalization of honey sources by bees, but also of their quality (Bartomeus et al.; Scaven et al., 2013). Temperature variations can change the quantity and quality of nectar provided by honey plants (Le Conte and Navajos, 2008; Genersch et al., 2010; Pătruică et al., 2017). High temperatures cause flower fading and shorten the nectar secretion period (Gordo and Sanz, 2010). At the same time, the temperature influences the feeding behavior, but also the pollination activity of the bees (Reddy et al., 2012). Heavy rains or the lack of them for long periods of time negatively influence the secretion of nectar and the harvesting behavior of bee colonies (Pătruică et al., 2020).

Research has shown that climate change is one of the threats to pollinators (Hegland et al., 2009; Schweiger et al., 2010). Some authors believe that there is a close correlation between climate change and bee colony extinction syndrome (Gordo & Sanz, 2006; Switanek et al., 2017).

In addition to the negative effects on pollinators, climate change negatively affects honey production. This bee product, in addition to its therapeutic qualities, can contribute to Europe's sustainability goals because, compared to sugar, honey production does not require the occupation of agricultural land, the use of mineral fertilizers and irrigation for crops (Kendall et al., 2013).

MATERIALS AND METHODS

The study was conducted 10 km from Timisoara, for a period of 5 years, between the 4th of April and 30th of July, 2017-2021. Temperature and humidity monitoring was performed with the Bee Watch Professional 45726158 system, located under a hive in the apiary of Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania", Faculty of Bioengineering of Animal Resources. The daily evolution, during 24 hours (from hour to hour), of the two meteorological factors was followed, during the production harvests of rapeseed, acacia, linden and sunflower. The statistical processing of the resulting data was performed with the IBM SPSS Statistics Version 21 program, the Anova test, with Tukey.

RESULTS AND DISCUSSIONS

Starting with the second decade of April, depending on the date when the sowing was done, until the end of May, rapeseed blooms in the area of Timişoara. The nectar and pollen

harvest on this plant overlaps in the first decade of May with the acacia harvest. But to capitalize on these harvests, bee families need favorable weather conditions for nectar secretion and flight. The secretion of rape nectar begins at a temperature of 10° C, the optimum value being reached between 16 and 25°C (Farkas & Zajácz, 2007; Pătruică et al., 2017). The temperature lower than 7 °C shows a negative effect on the secretion of nectar, strongly affecting the flight of bees and implicitly the production of honey (Pătruică et al., 2019).

Following the data in Table 1, we observe that in the last 5 years, the average temperatures registered in April presented statistically significant changes, with an average of the differences of 6.28°C between the minimum temperatures and 8.97°C in the case of the maximum ones. The problem in April is that of the minimum temperatures that are below the secretion temperature of the nectar (Pătruică et al., 2017; Pătruică et al., 2019). The average relative humidity registered statistically significant differences between the 5 analyzed years, ranging from 49.96% to 69.23%, the driest month of April being in 2018 and the rainiest in 2017 and 2021 (Table 1).

Beekeepers from Banat region do not show much interest in rapeseed harvesting, especially in case of early flowering, due to unfavorable weather conditions, but also to the reluctance to some pesticide treatments applied to these crops.

In May, in the area of Timiș County, the harvesting of nectar and pollen in rapeseed and the harvesting of acacia continues. Acacia nectar secretion begins at 10°C, reaches an optimum between 16 and 25 and ceases after 35°C (Farkas & Zajácz, 2007).

Table 1. The evolution of temperature and humidity in April and May

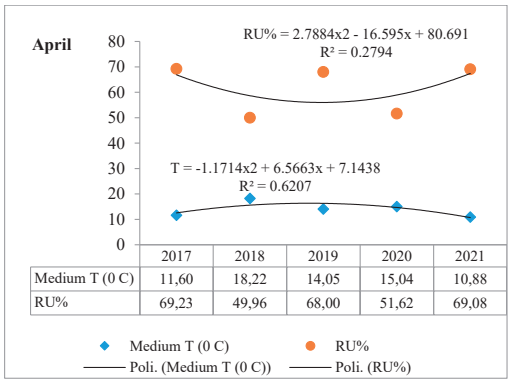
Items	Years					P values
	2017	2018	2019	2020	2021	
April $\bar{x} \pm SD$*						
Min temp (^{0}C)	6.63±4.117^{a}	12.76±2.730^{b}	8.65±3.589$^{a.\ c}$	7.59±4.598$^{a.\ c}$	6.48±4.817$^{a.c}$	0.000
Max temp (^{0}C)	17.29±5.541$^{a.\ c}$	25.10±4.125^{b}	20.36±4.634^{a}	25.29±3.32^{b}	16.32±5.950^{c}	0.000
Min RU (%)	50.58±16.048^{a}	49.96±10.813^{a}	48.96±17.007^{a}	31.77±7.565^{b}	50.54±15.180^{a}	0.000
Max RU (%)	84.65±9.769^{a}	83.58±8.819^{a}	84.42±10.159^{a}	70.81±11.451^{b}	84.23±12.738^{a}	0.000
May $\bar{x} \pm SD$*						
Min temp (^{0}C)	12.33±2.844^{a}	16.10±3.185^{b}	12.75±3.205^{a}	11.90±3.698^{a}	12.25±4.544^{a}	0.000
Max temp (^{0}C)	25.47±4.381$^{a.\ c}$	26.87±3.813^{a}	22.50±4.088$^{b.\ d}$	23.32±3.493$^{b.c}$	24.64±4.405$^{a.c.\ d}$	0.000
Min RU (%)	55.65±13.058$^{a.c}$	50.90±10.768$^{c.d}$	67.13±11.363^{b}	44.48±11.099$^{d.e}$	48.87±13.655$^{a.d.e}$	0.000
Max RU (%)	90.52±5.470$^{a.\ c}$	82.06±8.966^{b}	91.23±4.425^{c}	81.48±9.036^{b}	84.71±12.253$^{a.\ b}$	0.000

$\bar{x}$ Average; **SD**-standard deviation; RU – relative humidity

$^{a, b, c, d}$Values in the same row with a different superscript differ significantly at $p<0.05$

This month, during the 5 years analyzed, we observed that the average minimum temperatures were between 11.9 and 16.1 °C (Table 1), at first sight optimal for the secretion of nectar in the two plants. From the analysis of the daily average values of May, we observed a very large variability of the minimum temperatures from one day to another. The situation of days with minimum temperatures below the nectar secretion value was as follows: 2 days in 2017 (the lowest 4.3°C), 9 days in 2019 (the lowest 5.3°C), 8 days in 2020 (the lowest 7.1°C) and 9 days in 2021 (the lowest 3.3°C), these temperatures being abnormal for this month. The hottest month of May was in 2018 when the lowest daily minimum temperature recorded was 10.1°C. The average relative humidity varied greatly in the analyzed period, ranging between 50.9% and 81.58% at statistically significant differences between the 5 years (Table 1).

The medium temperature and relative humidity in April and May are presents in the Figure 1.



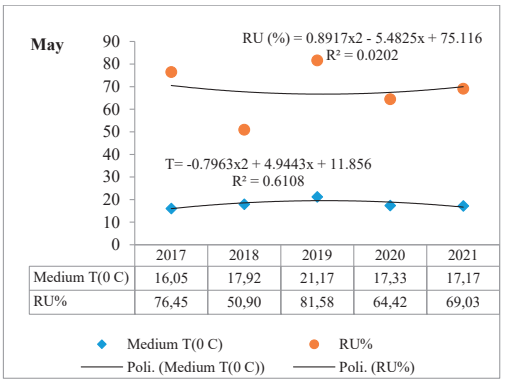


Figure 1. The evolution of the medium temperature and humidity in April and May

Starting with the second decade of June, linden blossoms in the analyzed area, and the sunflower in the third decade, depending on the date of sowing. The optimum secretion temperature of linden nectar is in the range of 18-28°C (Farkas & Zajácz, 2007) and 20-25°C in sunflower (Puškadija et al., 2007).

June 2017-2021 can be characterized by the average minimum temperatures between 15.28°C (in 2017) and 18.44°C (in 2019) and the maximum temperatures between 26.33 (in 2018) and 33.61°C (in 2021) (Table 2). There is a difference between the average maximum temperatures of 7.28°C. Analyzing the daily temperatures, we may observe that, in 2021, the temperature of 33°C was exceeded during 20 days of this month, of which 12 days recorded maximum temperatures above 35°C and 3 days above 40°C. These very high temperatures, unusual for the month of June in Timișoara area, seriously affected the secretion of linden and sunflower nectar.

Table 2. The evolution of temperature and humidity in June and July (2017-2021)

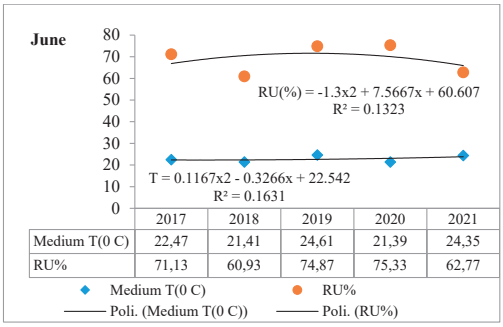
Items	Years					P values
	2017	2018	2019	2020	2021	
June $\bar{x} \pm SD$*						
Min temp (^{0}C)	15.28±2.868^{a}	16.93±3.592 $^{a, b}$	18.44±3.298 ^{b}	17.46±3.199 $^{a, b}$	17.11±5.451 $^{a, b}$	0.031
Max temp (^{0}C)	30.21±3.800 ^{a}	26.33±3.428$^{b, c}$	31.31±2.570$^{a, d}$	26.75±4.402^{c}	33.61±5.635 ^{d}	0.000
Min RU (%)	46.27±11.419^{a}	60.93±10.808^{b}	56.37±8.680^{b}	57.97±14.817^{b}	38.23±5.721^{c}	0.000
Max RU (%)	91.00±5.825^{a}	89.10±5.909$^{a, b}$	87.47±7.951 $^{a, b}$	88.10±7.45$^{a, b}$	84.03±11.247^{b}	0.017
July $\bar{x} \pm SD$*						
Min temp (^{0}C)	15.80±3.325 ^{a}	16.58±2.828 ^{a}	16.48±3.601^{a}	17.353±4.384 ^{a}	20.65±4.045 ^{b}	0.000
Max temp (^{0}C)	32.40±4.650 $^{a, c}$	27.16±2.665 ^{b}	30.67±3.509 ^{c}	31.34±4.144 ^{c}	34.13±3.69 ^{a}	0.000
Min RU (%)	35.57±8.653^{a}	60.93±10.808^{b}	47.03±7.889^{b}	48.67±10.223^{b}	42.80±11.364^{c}	0.000
Max RU (%)	85.50±7.899$^{a, c}$	90.50±3.875^{a}	86.03±7.020$^{a, c}$	89.03±10.036^{a}	82.03±12.104^{b}	0.002

$\bar{x}$ Media; **SD**-standard deviation; RU – relative humidity

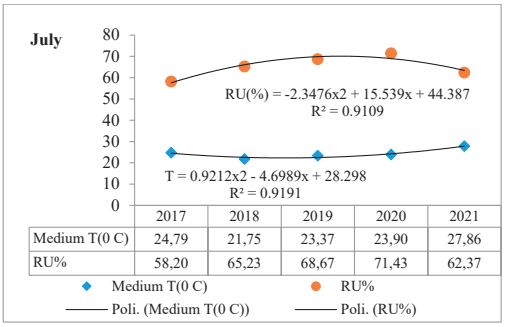
^{a,b,c,d}Values in the same row with a different superscript differ significantly at p<0.05

In July, the main harvest of nectar and pollen is in sunflower. During the analyzed period, the averages of the minimum temperatures were in the range of 15.80-20.65°C, and the maximums in the range of 27.16-34.13°C, with very large daily variations (Table 2). Unusual minimum temperatures were recorded in July 2018 (7.6°C) and 2019 (8.7°C). In terms of maximum temperatures, the warmest month of July was recorded in 2021 (18 days with average maximum temperatures above 33°C) followed by 2017 (13 days with average maximum temperatures above 33°C) and the year 2020 (12 days), consequently so many days unsuitable for nectar secretion. In 2018, the maximum daily temperatures did not exceed the threshold of 32°C, being the month of July with the most precipitation among the analyzed years.

The medium temperature and relative humidity in June and July are presents in the Figure 2.



	2017	2018	2019	2020	2021
Medium T(0 C)	22,47	21,41	24,61	21,39	24,35
RU%	71,13	60,93	74,87	75,33	62,77



	2017	2018	2019	2020	2021
Medium T(0 C)	24,79	21,75	23,37	23,90	27,86
RU%	58,20	65,23	68,67	71,43	62,37

Figure 2. The evolution of the medium temperature and humidity in June and July

CONCLUSIONS

Nectar secretion was severely affected by climate change during the period analyzed. During the rapeseed harvest, during the 5 years, the average of the minimum temperatures registered a difference of 6.28°C and that of the maximum temperatures of 8.48°C, with many days below the minimum nectar secretion temperature. Temperature variations between minimum and maximum averages ranged from 4.2-4.37°C during acacia harvesting, 3.16-7.38°C during linden harvesting and 4.85-6.97°C during sunflower harvesting. Very large differences in daily minimum and maximum temperatures (0.5°C- 30.2°C for rapeseed; 4.3°C-31.9°C for acacia; 7.3°C-42.7°C for linden; 7.9°C-40.9°C for sunflower), to which periods of heavy rain or drought are added, severely affect the behavior of bee colonies, honey production and even the apparition of diseases.

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COMPARATIVE CHARACTERISTICS OF THE GROWTH RATE IN TRADITIONAL AND FEEDING CALVES FOR BEEF TECHNOLIGIES

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Abstract

The use of the technology with feeding calves for beef based on a high-concentration type of feeding (59-61% of the energy nutritional value) on Aberdeen-Angus calves led to an increase in the meat productivity of animals in comparison with the control group kept under the traditional feeding system adopted for beef cattle "cow-calf". At the same time, during the period of growing and fattening (3-12 months), the average daily gain in live weight of bulls in the experimental group was 1359 g, which was 30% higher than these indicators in the control group, and was accompanied by an increase in the absolute gain in live weight of young animals by 81.4 kg as well as slaughter yield by 3.6%. The use of the technology with feeding calves for beef based on the high-concentration type of feeding (65-70% of the energy nutritional value) on Holstein calves led to an increase in the meat productivity of animals in comparison with the control group kept under the traditional feeding system adopted for young dairy cattle with a level of concentrates in the diets more than 50%. At the same time, for the period of growing and fattening (3-12 months), the average daily gain in live weight of bulls in the experimental group was 1359 g, which was 47% higher than these indicators in the control group, and was accompanied by an increase in the absolute gain in live weight of young animals by 120 kg, as well as slaughter yield by 3.1%.

Key words: *Aberdeen-Angus calves, age, average daily live weight gain, concentrates, fattening, Holstein calves, slaughter yield.*

INTRODUCTION

At present, the genetic potential of growing animals, regardless of the direction of productivity, is not fully disclosed, which requires study on the basis of the use of intensive feeding methods.

To realize the growth potential of young animals, it is necessary to create comfortable conditions for keeping and, above all, feeding, which should be not only complete, but also as abundant as possible at all stages of development.

In this situation, the axiom is simple, the earlier the young are accustomed to the abundant consumption of feed, the higher the intensity of its growth, which serves as the basis for high meat productivity. The technology of feeding calves for beef is based on this principle. However, it is practically impossible to increase the increase in live weight of animals, especially ruminants, by simply increasing the consumed feed. Therefore, the feed used must contain physiologically justified norms for the content of nutrients and we are talking primarily about concentrated feed, which are carriers of exchangeable protein and energy necessary for an intensive synthesis of muscle tissue, which ultimately allows you to get valuable young beef. In this case, it is necessary

Feeding calves for fattening calves involves training animals from an early age to a high consumption of concentrates with free access to feed. This is an unconventional approach to feeding ruminants. However, such a reception is justified during the formation of the proventriculus, which leads to a shift in the development of cicatricial digestion towards rennet digestion of nutrients. It is especially important to use feeding calves for beef technology in the absence of pastures, land for the preparation of voluminous feed, with the availability of feed additives, grain purchased or self-produced feed. In the available scientific literature, there is very little data on the effect of feeding a large amount of concentrated feed (more than 50% of the total nutritional value of the diet), especially in a comparative aspect, taking into account the direction of productivity

of fattened livestock. Therefore, high-concentration fattening as a way to increase the meat productivity of fattened animals is gaining even greater interest for science and production throughout the Russian Federation. Thus, to realize the genetic potential inherent in dairy and beef breeds of cattle potential, a scientific substantiation of feeding is required, taking into account the physiological characteristics of the growth and development of young animals (Fisinin, 2003; Strekozov et al., 2006; Amerkhanov et al., 2011)

MATERIALS AND METHODS

The research and production experience was carried out in the conditions of the enterprise LLC "Agrofirma Myaskom" of the Nizhny Novgorod region on bulls of the Holstein and Aberdeen Angus breeds. Before the start of the experiment, four groups of calves at the age of three months, 15 heads in each group, were formed on the principle of analogous pairs. Holstein calves were kept loose in group pens. Aberdeen-Angus calves from three to six months of age were kept in the generally accepted conditions for beef cattle, in feeding areas year-round according to the “cow-calf” system. Holstein bull calves of the control group were fed according to the traditional feeding technology adopted for young dairy breeds, according to which the level of concentrates in the diet was in the range of 43-46%. Young animals of the experimental group were fed intensively on the basis of feeding calves for beef technology on high-concentration rations with a concentrate level of 65-70%. Aberdeen-Angus calves were fed according to the traditional scheme: up to 5 months of age on suckling under cows and then after weaning - loosely in pens. At the same time, the share of concentrated feed, depending on the age of the young, was 36-38% of the total nutritional value of the diet. In the experimental group, bulls had free access to separate feeding of concentrates and bulk feeds, as a result of which the level of concentrated feed in the rations was high and amounted to 59-61%. The experiment lasted 9 months. Watering of animals was organized from group auto-drinkers with heating. The distribution of hay was carried out in group feeders and nurseries. Access to voluminous feed and compound feed was free, with a gradual increase in the level of feed concentrates.

Before the start of the experiment, for the calves of the control groups, feeding rations and compound feed recipes were developed taking into account the age of the animals. To increase the consumption of concentrates, high-protein feeds (fish meal, dry milk, sunflower meal, feed yeast, extruded peas) were added to the composition of the feed of the experimental groups, especially at the beginning of the experiment, with the addition of a premix, flavoring additives, feed chalk. The calculation of rations was carried out using the "Futter-KRS" program. To control the physiological state, blood was taken from the jugular vein in which biochemical parameters were determined. At the end of the experiment, at the age of 12 months, a control slaughter of bulls was carried out.

RESULTS AND DISCUSSION

In the conducted studies, it was found that 6 months old Holstein bulls of the experimental group in terms of live weight significantly exceeded the control animals by 33.6 kg or 22.8% ($P<0.001$). At the same time, the average daily gain in live weight for this period of time was 1523 g in the experimental group, which was 1.6 times higher than in the control (Table 1). The use of intensive technology for fattening bull calves on rations with a high level of concentrates in the experimental group made it possible to bring the animals to slaughter conditions at the age of 12 months. As a result, the live weight of bulls at the end of feeding was 445.3 kg, while in the control this indicator was 37.2% lower and amounted to 324.6 kg with a traditional feeding system. The results of our research on the growth rate of Holstein bull calves on diets with different levels of concentrates are consistent with the data of British scientists (Rutherford et al., 2020).

Table 1. Comparative characteristics of the growth dynamics of calves of different breeds

Indicator	Holstein breed		Aberdeen Angus breed	
	Traditional fattening (the control)	Feeding calves for beef (experience)	Traditional Fattening (the control)	Feeding calves for beef (experience)
Live weight, kg: at the age of 3 months	68.5 ± 1.9	68.8 ± 1.7	86.8 ± 3.2	86.0 ± 2.8 x
at the age of 5 months	118.8 ± 3.1	135.1 ± 3.3	144.7 ± 5.0	154.6 ± 5.3x
in % to control	100.0	113.7	100.0	106.8
average daily gain, g	838	1105	964 ± 56.7	1143 ± 53.7 *
in % to control	100	131.9	100.0	118.4
at the age of 6 months	147.2 ± 4.2	180.8 ± 5.9**	174.4 ± 5.8	195.8 ± 6.2 * x
in % to control	100	122.8	100.0	112.3
average daily gain, g	947	1523 x	991 ± 45.3	1373 ± 55.0 **
in % to control	100	160.8	100.0	138.7
at the age of 9 months	228.7 ± 5.3	314.8 ± 6.6**	270.0 ± 6.3	327.2 ± 8.1 **
in % to control	100	137.6	100.0	121.2
average daily gain, g	906	1489	1062 ± 45.1	1460 ± 51.3 **
in % to control	100	164.3	100	137.5
at the age of 12 months.	324.6 ± 9.6	445.3 ± 11.5**	372.2 ± 8.0	452.8 ± 9.8 **
in % to control	100	137.2	100.0	121.7
average daily gain, g	1065± 32.9	1450	1136 ± 40.1	1396 ± 42.4 **
in % to control	100.0	136.2	100.0	122.8
During the experience: absolute gain, kg	256.1± 7.1	376.5± 11.2 **	282.4 ± 7.9	366.8 ± 8.8 **
average daily gain, g	902	1326**	1045 ± 29.1	1359 ± 32.5 **
in % to control	100	147	100.0	130.0

Note: * P <0.05 ** P <0.001 - significance of differences to traditional feeding;
x P <0.01 - the significance of differences for feeding calves for beef.

Aberdeen-Angus calves of the experimental group at the age of 6 months in live weight exceeded the control animals by 21.4 kg or 12.3%. At the same time, the average daily gain in live weight for this period of time was 1373 g in the experimental group, which was 38.5% higher than in the control (P<0.01). The bulls of the experimental group at the age of 9 months in live weight exceeded their peers from the control (P<0.001) by 57.2 kg or 21.2%. The average daily gain was 1460 g in the experimental group, which is 37.4% higher. The same dynamics of growth persisted in the following months of feeding.

At the end of the experiment, the live weight of bulls in the experimental group reached slaughter conditions at the level of 452.8 kg, which was 21.7% higher than this indicator in the control. At the same time, the average daily gain in live weight for the entire experiment in the control group was 1045 g, which was 30% lower than in the experimental group, the gain in which was 1359 g (P<0.001). The results of our research on the growth rate of Aberdeen-Angus calves in the control group are consistent with the literature data (Bychkov, 2011; Saenko et al., 2016; Shevkhuzhev et al., 2015), in which it was found that with traditional feeding, the live weight of calves raised in different climatic zones of the Russian Federation with different feeding conditions at the age of 12-13 months varied from 363 to 398 kg.

Despite the fact that the bulls of both groups with the feeding calves for beef technology had a high meat productivity, the superiority in live weight in the Aberdeen Angus bulls in comparison with the Holstein bulls was noted both initially and throughout the entire experiment. At the beginning of fattening at the age of 3 to 6 months, the live weight of the Aberdeen-Angus bulls significantly exceeded

their peers of the Holstein breed by 14.4 (P<0.01) and 8.3% (P<0.05), respectively. In other periods of growth, no significant difference was obtained. The dynamics of animal growth showed that the maximum average daily gain in live weight during calves fattening in Holstein bull calves (1523 g) was established at the age of 6 months, in Aberdeen Angus bull calves at 9 months (1460 g). It should be noted that in a comparative aspect, the growth rate of Holstein calves was generally somewhat higher than their peers, they were more efficiently fed by feeding for beef breeders than young beef cattle. This is evidenced by the fact that the average daily gain in live weight during fattening of Holstein calves was 47% higher in comparison with traditional feeding, and in Aberdeen Angus breed it was 30%. According to the literature, specialized meat breeds are characterized by increased early maturity and have higher meat productivity and slaughter yield compared to other combined or dairy breeds (Dunin, 2014; Tikhomirova et al., 2014). At the same time, it has been scientifically proven that with intensive feeding technologies, young dairy breeds are not inferior in productivity to meat breeds (Legoshin & Sharafeeva, 2013).

Meat productivity largely depends on the amount of concentrated feed consumed. The average daily consumption of compound feed by calves of both groups in feeding calves for beef increased with age in comparison with control (Table 2).

Table 2. Feed consumption during the experiment

Indicator	Holstein breed		Aberdeen Angus breed	
	Traditional fattening (the control)	Feeding calves for beef (experience)	Traditional fattening (the control)	Feeding calves for beef (experience)
Consumption of compound feed during the experiment for 1 head:				
total (from 3 to 12 months), kg	635	1561	438	1137
on average per day, kg	2.4	5.8	1.6	4.2
Consumption of voluminous feed during the experiment for 1 head:				
total (from 3 to 12 months), kg	1973	1883	2310	2100
on average per day, kg	7.3	7.0	12.8	11.6

The average daily consumption of compound feed by bulls increased with age from 1.9 to 7.0 kg in Holstein bull calves and from 1.2 to 6.2 kg in Aberdeen Angus bull calves. At the same time, during the experiment, Holstein calves consumed 1.4 times more concentrates than Aberdeen-Angus calves. On average, the consumption of concentrated feed by Holstein and Aberdeen Angus calves was 5.8 and 4.2 kg. High consumption of compound feed led to the saturation of the body with the main nutrients during feeding calves for beef, which in turn led to a decrease in the consumption of bulky feed compared to the control group. In a comparative aspect between breeds, it was found that, regardless of the method of feeding, Holstein bulls tend to consume more concentrates and eat less voluminous feed than animals of the Aberdeen Angus breed. Thus, with traditional and feeding calves for beef, Holstein bulls consumed 45 and 37.3% more concentrates, and, on the contrary, voluminous fodder was 17.1 and 11.5% less than Aberdeen Angus bulls. Consequently, beef cattle consume and utilize voluminous feed more efficiently, but less concentrated than dairy cattle.

According to a number of scientists, it has been established that gradual adaptation to high consumption of concentrated feed does not have a negative effect on the body of young ruminants and can significantly increase the meat productivity of fattening cattle (Galochkina, 2013; Kharitonov, 2015; Ruppe, 1984; Mendel, 1987). The results of the analysis of the blood of bulls, obtained in our studies, confirmed this fact. With feeding calves for beef, a higher level of protein metabolism is observed, which led to an increase in blood total protein by 4.9-5.7% (P<0.05) in comparison with the control (Table 3).

Table 3. Biochemical parameters of blood

Indicator	Holstein breed		Aberdeen Angus breed	
	Traditional fattening (the control)	Feeding calves for beef (experience)	Traditional fattening (the control)	Feeding calves for beef (experience)
Total protein, g / l	63.5 ± 0.9	67.1 ± 1.1 *	73.5 ± 0.9	77.1 ± 0.9 *
Glucose, mmol / l	56.6 ± 0.8	58.3 ± 0.9	54.7 ± 0.8	58.0 ± 0.8 *
Calcium, mmol / l	2.8 ± 0.1	3.2 ± 0.1 ***	12.4 ± 0.17	12.8 ± 0.3
Phosphorus, mmol / l	1.8 ± 0.04	2.0 ± 0.05 **	5.7 ± 0.2	6.0 ± 0.2

Note: *P <0.05; **P <0.01; ***P <0.001- the importance of differences to traditional feeding

Comparative analysis of animal slaughter data indicates that when fattening, bulls of dairy and meat breeds are capable of reaching high slaughter rates by the age of 12 months. However, in terms of slaughter indicators, Holstein calves were inferior to Aberdeen-Angus calves, regardless of the feeding method (Table 4).

Table 4. Comparative indicators of slaughter of calves of different breeds

Indicator	Holstein breed		Aberdeen Angus breed	
	Traditional fattening (the control)	Feeding calves for beef (experience)	Traditional fattening (the control)	Feeding calves for beef (experience)
Pre-slaughter weight, kg	315.5 ± 5.7	436.7 ± 7.5*	370.7 ± 8.7	449.4 ± 9.8 * x
Slaughter weight, kg	177.9 ± 3.2	259.8 ± 4.3*	220.2 ± 5.3	283.0 ± 6.9 * x
Lethal output,%	56.4	59.5	59.4	63.0 * x
Class	"Good"	"Extra"	"Excellent"	"Prima"

Note: *P <0.001 - the significance of the differences to traditional feeding;
x P <0.01 - the significance of differences in feeding calves for beef.

At the same time, the slaughter weight of meat calves with traditional and feeding calves for beef was significantly higher by 17.5 and 9.2% compared to dairy calves (P<0.01). The slaughter yield of the Aberdeen-Angus calves was also 3-3.5% higher (P<0.05) than that of the Holstein calves.

CONCLUSIONS

In the conducted studies, it was found that feeding calves for beef with a high level of concentrate consumption leads to an increase in the growth rate of Holstein and Aberdeen Angus calves compared to traditional feeding by 47 and 30%, respectively. Feeding calves for beef technology, due to the implementation of the high genetic potential for the growth of young animals, can significantly increase the meat productivity of bulls, regardless of breed, and get a high-value product - young beef at the age of 12 months, which is 4-6 months earlier than the accepted deadlines for the delivery of animals for slaughter.

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ANALYSIS OF TRIGLYCERIDES AND CHOLESTEROL OF LAYING CHICKEN CARCASS ON REPLACEMENT OF FISH MEAL WITH DEGRADED MANURE FLOUR (MHD) LARVAE OF *HERMETIA ILLUCENS* L.

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Abstract

This study used an experimental method using a completely randomized design (CRD) consisting of 4 treatments, namely RA = 15% fish meal + 0% MHD meal, RB = 10% fish meal + 5% MHD meal, RC = 5% fish meal + 10% MHD meal and RD = 0% fish meal + 15% MHD flour. The results of the analysis of triglycerides and blood serum cholesterol in a sample of 20 laying hens, the analysis showed that MHD as a result of degradation of black fly larvae (Hermecia illucens L.) had a significant effect on triglycerides, LDL, HDL and total cholesterol but had no significant effect on VLDL (Very Low density). lipoprotein) blood serum of native chicken (p <0.05). RC treatment, namely administration with 10% MHD flour is a treatment with the best response to the content of the blood serum lipid profile, and the use of MHD in laying hens up to 15% is as good / effect as the use of MHD in domestic broilers.

Key words: *cholesterol, H. illucens L., laying free-range chicken, triglycerides.*

INTRODUCTION

Laying free-range chickens are part of local native chickens in Indonesia whose lives are closely related to the community. The appearance of laying free-range chickens is very diverse, as well as their genetic characteristics, their distribution is very wide because the domestic chicken population is found in cities and villages. The potential should be developed to improve community nutrition and increase family income, this can be seen from population growth and increasing demand for meat and eggs from year to year (Nyakeri et al., 2016).

H. illucens L. lives in organic matter, food waste disposal, and manure, produces enzymes such as: amylase, lipase, and protease to hydrate carbohydrates, fats, and proteins into smaller or individual parts such as maltose, fatty acids, glycerol, and amino acids respectively (Kim et al., 2011).

Thus, *H illucens* L. can break down waste disposal into quality feed ingredients and can also reduce pollution (Wang & Shelomi, 2017). *H. illucens* L. has been studied for its ability to convert organic waste into high quality protein, control certain harmful bacteria and insect pests, provide potential chemical precursors for producing biodiesel and for use as feed for various animals (Barbagan et al., 2017).

Considering this potential, it is necessary to seek a way out to increase the population and productivity. Laying free-range chickens have the advantage of high adaptability because they are able to adapt to various situations, environmental conditions and climate change and local weather. One of the contributing factors is the maintenance system which is still traditional in nature, the amount of feed given is insufficient and the feeding does not yet refer to the principles of nutrition, especially the feeding that has not taken into account the need for food substances for various levels of production (Wang & Shelomi, 2017).

In general, the nutritional requirements for chickens are highest during the first week (0-8 weeks) of life, therefore it is necessary to provide adequate rations containing energy, protein, minerals and vitamins in a balanced amount. Another factor is genetic improvement and increased management of native chicken

rearing must be supported by improved feed nutrition (Setioko and Iskandar, 2005).

Feed costs account for 70-75% of the total production cost of poultry farming, particularly native chickens (Teguia & Beynen, 2005; Mupeta et al., 2003).

The price of feed ingredients continues to increase due to an increase in the number of poultry farms (Hassan et al., 2014).

Poultry feed costs reach 70-80% of the total production. Fish meal is one of the ingredients of the ration and has a fairly good source of nutrition, especially as a source of animal protein. Fish meal as a raw material for poultry rations ranks first in the supply of animal protein sources because the very high crude protein based on its use in the composition of poultry ration/feed reaches 10% (Anggorodi, 1985).

One of the alternative feed ingredients that are easily available, cheap and can be used to replace fish meal is the degradation of manure flour (MHD) larva *H. illucens* L. The results of the study by Manangkot (2014) showed that MHD contains 51% protein which competes with fish meal.

Based on the above thinking, research has been carried out to determine the effect of replacing fish meal with manure meal resulting from degradation of *H. illucens* L. larvae on triglycerides and blood serum cholesterol of laying hens. Does the replacement of manure flour degraded by *H. illucens* L. larvae with fish meal affect the quality of carcass on triglycerides and blood serum cholesterol of laying hens? This study aims to determine the effect of replacing fish meal with manure flour from degradation of *H. illucens* L. larvae on blood serum cholesterol and triglycerides of laying native chicken meat.

Previous research has been carried out by Rotinsulu (2020), Exploration of Degraded Manure Flour (MHD) of Black Fly Larvae (*Hermetia illucens* L.) Against Carcass of Layed Buras Chicken with Soaking Sweet Orange (Citrus sinensis). This study aims to determine the effect of replacing fish meal with manure meal resulting from degradation of *H. illucens* L. larvae on triglycerides and blood serum cholesterol of laying native chicken carcasses and testing the carcass quality.

MATERIALS AND METHODS

H. illucens L. was obtained from the chicken farm environment and then thirty pairs of flies were placed in each litter box. The manure box is designed with a size of 100 x 100 x 70 cm, each side made of gauze. Thirty kg of broiler manure as a medium for *H. illucens* L. larvae were placed in this litter box. The flies lay eggs until the fourth day. *H. illucens* L. larvae were reared in this medium for biodegradation of manure for eight days of its life cycle.

MHD was obtained from biodegradation of 8 day old larvae through rearing results of *H. illucens* L. flies with 2 week old broiler chicken manure.

Maintenance was carried out for 6 months. A total of 60 6-month-old laying hens, known as Balinese chickens, are housed in 20 cages, each measuring 50 x 50 x 70 cm. The laying hens used were divided randomly into 20 experimental units, each consisting of 3 chickens and each food was given randomly. Each cage is equipped with separate eating and drinking areas. Drinking water and feed were given ad libitum during the experimental period.

The treatment used a completely randomized design with 4 treatments and 5 replications according to Steel and Torrie (1991).

Table 1. Composition and content of food substances in the experiment

Ingredients	Treatments (%)			
	R1	R2	R3	R4
MHD	0	5	10	15
Fish meal	15	10	5	0
Copra meal	7	7	7	7
Soybean meal	10	10	10	10
Rice bran	11.5	11.5	11.5	11.5
Yellow corn	55	55	55	55
Bone meal	1	1	1	1
Vitamin Premix*)	0.5	0.5	0.5	0.5
The content of food substances				
Crude protein	20.30	20.17	19.83	19.55
Crude fiber	4.56	5.02	5.49	5.95
Ether extract	5.27	5.23	5.20	5.03
Ca	1.00	0.92	0.83	0.67
P	0.74	0.61	0.55	0.42
ME (kcal/kg)	2951.40	2934.60	2917.25	2905.75

Each treatment is formulated in terms of iso-nutrient and iso-calorie compositions. The treatments were formulated as follows: RA as control feed with 15% fish meal + 0% MHD flour; RB, 10% fish meal + 5% MHD flour; RC is a diet with 5% fish meal + 10% MHD flour; and RD is feed consisting of 0% fish meal + 15% MHD flour (Table 1).

Data collection for 4 months and age 12 months after harvest for analysis of triglycerides and blood serum cholesterol in laying hens.

Data analysis. Analysis of variants was performed using the SPSS procedure. The research data was tabulated, then tested according to analysis of diversity to see the effect of treatment. The level of difference for each treatment ration was tested according to Duncan's Multiple Range Test (Steel and Torrie, 1991). TAG and Cholesterol Test using the CHOD-PAP Method. The independent variable is feeding/ration of manure flour as a result of degradation (MHD) of *H. illucens* L. larvae, while the dependent variable observed in this study is the content of triglycerides and cholesterol (VLDL, LDL, and HDL) in mg/dL in chicken blood serum.

CHOD-PAP Method (Enzymatid Calorimetric Method/NS Method), with using Kit/Spectrophotometry Analysis (Biotec England, 2011), as the following:

Triglycerides (TAG)

Blank tubes containing 10 µl of distilled water and 1,000 µl of kit reagent were prepared, standard tubes containing 10 µl of standard triglycerides and 1,000 µl of kit reagents, sample tubes containing 10 µl of serum and 1,000 µl of kit reagents. The mixture was then homogenized, incubated at a temperature of 20-25ºC for 10 minutes. The absorbance is read at the Hg 546 nm wavelength within one hour.

Total cholesterol

Blank tubes containing 10 µl of distilled water and 1,000 µl of kit reagent were prepared, standard tubes containing 10 µl of standard cholesterol and 1,000 µl of kit reagents, and the sample containing 10 µl of serum reagent kit and 1,000 µl of kit reagent. The mixture was then homogenized, incubated at a temperature of 20-25ºC for 10 minutes. The absorbance is read at the Hg 546 nm wavelength within one hour.

HDL (High Density Lipoprotein)

A total of 500 µl of serum was added with 1,000 µl of precipitation, mixed until it was homogeneous, then left to stand for 10 minutes at room temperature. Centrifugate for 10 minutes with 3,500 revolutions per minute. The supernatant was prepared from the precipitate within two hours after centrifugate. A total of 100 µl of supernatant plus 100 µl of CHOD-PAP reagent were mixed, incubated for 10 minutes at 20-25ºC. The absorbance was read within one hour at a wavelength of Hg 546 nm.

LDL (Low Density Lipoprotein) and **VLDL** (Verry Low Density Lipoprotein)

A total of 100 µl of serum was added with 1,000 µl of precipitation, mixed until it was homogeneous, then left to stand for 10 minutes at a temperature of 15-25ºC. Centrifugate for 15 minutes with 3,500 turns per minute. The supernatant was prepared from the precipitate within two hours after centrifugate. A total of 50 µl of supernatant plus 100 µl of reagent kit were mixed, incubated for 10 minutes at 20-25ºC.

RESULTS AND DISCUSSIONS

The response of laying free hens after being given manure flour from degradation results (MHD) of *H. illucens* L. larvae for 6 months, showed different variations in the lipid profile (Triglyceride and Cholesterol content) between treatments. The lipid profile in this study, namely cholesterol (Triglycerides/TAG, LDL, VLDL, HDL and total cholesterol) VLDL, LDL and HDL) was obtained through spectrophotometric analysis of blood serum in laying hens. The results showed that blood triglyceride levels in this study ranged from 49.20-74.86 mg/dL. The highest triglyceride content was in RA (control ration without MHD on the ration) (74.86 mg/dL; while the lowest triglyceride content was found in RC (10% MHD and 5% fish meal on the ration) (49.20 mg/dl) The average triglyceride levels in this study are in line with Tohala (2010) who reported that blood triglyceride levels in broiler chickens ranged from 50.17 ± 1.4 to 52.83 ± 2.44 mg/dL. consumption of feed, especially carbohydrates such as sugar, saturated fat, high levels of free fatty acids, high insulin levels, and low levels of glucagon carbohydrates in the

liver are broken down into fatty acids and converted back into triglycerides. Manure results of degradation (MHD) with *H. illucens* L. larvae in this study were assumed to have high enzyme activity (amylase, lipase, and protease), especially lipase. Early studies (Manangkot, 2014) revealed that on the 7th day of manure after being broken down *by H. Illucens* L. and the 8th day of larval growth in manure, gave the highest activity of amylase, protease, and lipase enzymes.

Blood LDL content in this study ranged from 47.58-82.26 ml/dL. RA (control ration without MHD administration) was the highest blood LDL content around 81.06 ml / dL; whereas RC (10% MHD in the ration) gave the lowest blood LDL level of around 47.58 ml / dL. Statistical analysis showed that the treatment provided a significant difference (P <0.05) to the LDL level of laying native chicken blood in this study. It appears that MHD has the ability to reduce the LDL content of laying hens' blood in this study.

The results of the VLDL experiment showed that the manure treatment resulting from degradation (MHD) of H. illucens larvae had no effect on the VLDL content of chicken blood serum (P> 0.05) RA=9.30, RB=9.24, RC=9.20, RD=9.29 like the TAG content, the RC treatment also showed the smallest average VLDL content compared to all treatments, namely 9.20 mg/dL. RA treatment showed the largest average VLDL, namely 9.30 mg/dL. Very Low density lipoprotein (VLDL) is synthesized in the liver and is rich in endogenous triglycerides. In the blood will be degraded into LDL. The main function is as a carrier of triglycerides that are carried from the liver to other tissues in the body, especially to stored adipose tissue. VLDL contains high concentrations of triglycerides and moderate concentrations of cholesterol and phospholipids (Mokosuli, 2012). The TAG content is related to the VLDL content because the largest charge in this lipoprotein is TAG. The average HDL content in this study was 77.00-87.10 mg/dL. RA (control ration without MHD administration) had a lower blood HDL content (P<0.05) (77.00 mg/dL compared to RB (83.15 mg/dL), RC (87.10 mg/dL), and RD (79.05 mg/dl).

The blood HDL level of the RB (5% MHD) was significantly lower (P<0.05) than the RC (10% MHD in the ration). The blood HDL level of RD (15% MHD in the ration) was significantly lower (P <0.05) than RB and RC while the blood HDL level of RC (10% MHD in the ration) was significantly higher (P<0.05) compared to other treatments. Hariyanto (2017), also reported that blood HDL levels in broilers ranged from 54.20 ± 20.9 to 84.78 ± 23.6 mg/dL. RA gave significantly higher results (P<0.05) compared to the RB treatment. RC, and RD.RB provided significantly higher blood triglyceride content (P <0.05) than RC.

Total blood cholesterol in this study ranged from 128.05 to 163.05 ml/dL. The highest total cholesterol in RA (163.05 mg/dL) of RB, RC, RD because RA is a control diet without MHD and RC gives the lowest total blood cholesterol level (12.05 mg/dL = MHD 10%). The average total blood cholesterol found in this study is in accordance with the findings of Iriyanti et al., (2014) who reported that blood cholesterol levels in native chickens ranged from 123.04 ± 7.07 to 170.27 ± 9.68 ml/dL. Statistical analysis showed that the treatment gave a significant difference (P<0.05) to total blood cholesterol. RA (control ration without giving MHD on the ration) had higher total blood cholesterol (P <0.05) compared to RB, RC, and RD treatments. RB (5% MHD in the ration) also gave higher total blood cholesterol (P <0.05) compared to RC and RD treatments. As mentioned above, the MHD used in this study had high enzyme activity. So that the higher the MHD level in the ration larva growth in manure gave the highest amylase, protease, and lipase enzymes activity. The production of these bile salts requires cholesterol. So, when cholesterol levels in the liver are low, high density lipoprotein (HDL) will mobilize cholesterol from body tissues to meet their needs for cholesterol (Mayes, 1997). The results of the blood lipid indices of the experimental birds are presented in Table 2 and Figure 1.

Table 2. Average content of the blood fat profile of laying hens given manure flour degraded (MHD) larvae of *Hermetia illucens* L.

Treat-ments	Blood Lipid Profiles (mg/dL)			
	Triglycer id (TAG)	LDL	HDL	Tot Cholesterol
RA	74.86	82.26	77.00	163.05
RB	57.87	63.40	83.10	134.00
RC	49.20	47.58	87.10	128.05
RD	50.20	54.00	79.05	130.07

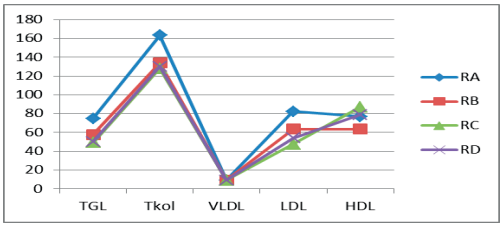

Figure 1. Comparison of Triglyceride Content, Total Cholesterol, VLDL, LDL and HDL between treatment of MHD rations of *H. illucens* L. larvae in laying native chickens

CONCLUSIONS

Analysis of triglycerides and carcass cholesterol of laying hens on replacement of fish meal with Degradation Product Manure (MHD) of *Hermetia illucens* L. larvae can replace fish meal up to 15% in the ration of laying free hens and degraded manure (MHD) of *H. illucens* L. larvae. significant effect on triglycerides, LDL, HDL and total cholesterol but not significant effect on blood serum VLDL of laying hens. MHD rations resulting from degradation of *H. illucens* L. larvae can maintain the triglyceride and total cholesterol content of laying hens in the normal range (MHD RC = 10% treatment), capable of producing low-cholesterol carcasses.

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GROWTH PERFORMANCE OF GOATS IMMUNIZED WITH THORAXIAL ANTIGENS OF *MUSCA DOMESTICA*

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Abstract

The antigen thoraxial extracted from some insects indicated a potential in immunity system amelioration in mammalian. This study aimed to evaluate the growth performance of goats immunized with IATMd (immunogen antigens of Musca domestica). Twelve goats were used in this experiment. Each experiment animal was treated with 10 µl of thoraxial antigens of Musca domestica. The animals were divided into two groups: control group and treated group. The growth performances were evaluated under three parameters: Body weight gain, feed intake and FCR. All animals were fed with the same feed. The data were collected during eight weeks and analyzed by using t-student procedure. The results showed that FCR of the animas in treatment group was significance higher than in the control group (P<0.05) while there has no different statistically on body weight gain (P>0.05). It was concluded that immunization of IATMd extract could improve nutrient metabolism in goats that play a role in FCR value of these animals experiment.

Key words*: insect antigens, Musca domestica, FCR value, goats.*

INTRODUCTION

Goat production is an important source for food in many regions in the world. In Indonesia local goats are cultivated to support meat production. The Ettawa goats breet could be reared for milk production (Salim & Susanti, 2016) and for meat production. The demand of meat production farming has an increasing due to the significance augmentation of human population (Sumardianto et al., 2013).

On the other hand, goat farming with local breed generally is reared with a little amount by household.

Even though the numbers are small, this farming pattern is spread over many locations. Accumulatively, the amount is important by contribution to a provision of food for the community.

The health of goats is one of the determining factors to increase goat production, especially in relation to viral and bacterial infections (Aldridge et al., 2018) and will determine to a level of success in livestock management systems (Silva et al., 2014; Caroprese et al., 2016). To get animal good health, it has to anticipate the bacterial pathogen spread which related to the report of Heidt et al. (2012). The immune system becomes active when exposed to antigens (McGeer et al., 1989).

Antigen derived from insects has begun to be known for its use in improving the immune system of mammalian livestock (Rumokoy et al., 2017) including goats (Toar et al., 2017). Ameri et al. (2008) reported the use of crude extracts gave an immune response in cattle against insect infestations.

Subsequently, a variety of antigens extracted from insect are potential support goats production optimally (Toar et al., 2019).

Good production is also a consequence of the functioning of the immune system properly so as to control bacterial infection or infestation of parasitic organisms such as insects.

This paper presents the results of research activities using thoraxial antigens extracted from *M. domestica* on growth performance of local goats.

This research work is a continuation of previous research that has been done by observing the role of IAMTd on blood serum immunoglobulin levels in goat kids, which showed that this immunogen extract indicated to increase serum IgG of goat kids (Rumokoy et al., 2020).

MATERIALS AND METHODS

Twelve local goats after weaning were used in this experiment. The initial body weight of animals is shown in Figure 1. The animals were divided into two groups: a control group (AK1) and a treatment group (AK2). Animals were reared in experiment cage. All animals were offered various local green forages which were alternately supplied in the same manner. Drink water were available *ad libitum* to all animals observed. The animals of treatment group were immunized with thoraxial antigen extract of *M. domestica* (IATMd). Each experiment animal in AK2 group was treated by subcutaneous injection with 10 µl of IAMTd.

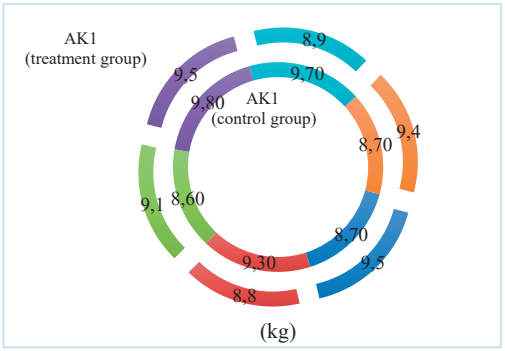

Figure 1. The initial of body weight of animal experimental

Data were collected during eight weeks and analyzed by using t-student procedure to evaluated the effect of IATMd on growth performance of two groups studied.
The effect of the treatment was recorded after two weeks of immunization over an eight weeks periode. The growth performances were evaluated according to the following parametres:

1) Dry Matter (DM) of feed intake calculated as:

$$DMI = \frac{(fo - rf)}{t}$$

which:

fo = total DM weight of feed offered (g);
rf = total DM of residual feed (g);
t = number of days during observation.

2) Daily body weight gain (DWG), calculated as:

$$DWG = \frac{(fbw - ibw)}{t}$$

which:

fbw = final body weight (g);
ibw = initial body weight (g);
t = number of days during observation.

3) Feed conversion ratio (FCR) was calculated as a ratio of dry matter of feed intake and daily body weight gain and expressed in the following formula:

$$FCR = \frac{DMI}{DWG}$$

which:

DMI is dry matter of feed intake (g);
DWG is daily body weight gain (g).

RESULTS AND DISCUSSIONS

The significant role of IAMTd immunization in experimental goats on the measured parameters was obtained in daily body weight gain and FCR values as presented in the graphs (Figures 3 and 4).
The immunization with IAMTd to local experimental animals did not have a significant effect on the average of dry matter intake in treatment group (AK2) compared to the control group (AK1) where P value was higher than 0.05 although the data showed tendency that most of the animals used in this experiment had a higher DMI rates in the treatment group compared to control group as presented in Figure 2. These results remained that at the applied dose of antigen did not provide a negative impact on young goat palatability.
The use of IATMd has an effect positive on immune system of the goat kids that leads to control pathogen agent infection, thereby ensuring metabolic activity in utilizing nutrient intake to be a muscle formation development (Rumokoy et al., 2020)
Conditions can contribute to weight gain of animal treatment as shown in Figure 3.

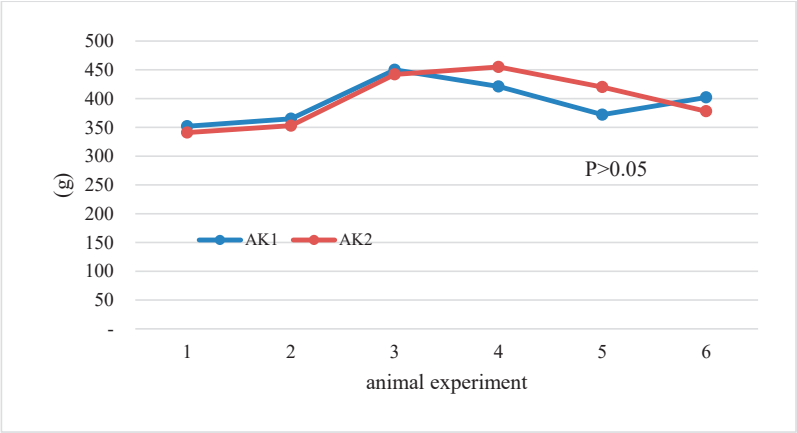

Figure 2. Dry matter intake (gram d^{-1})

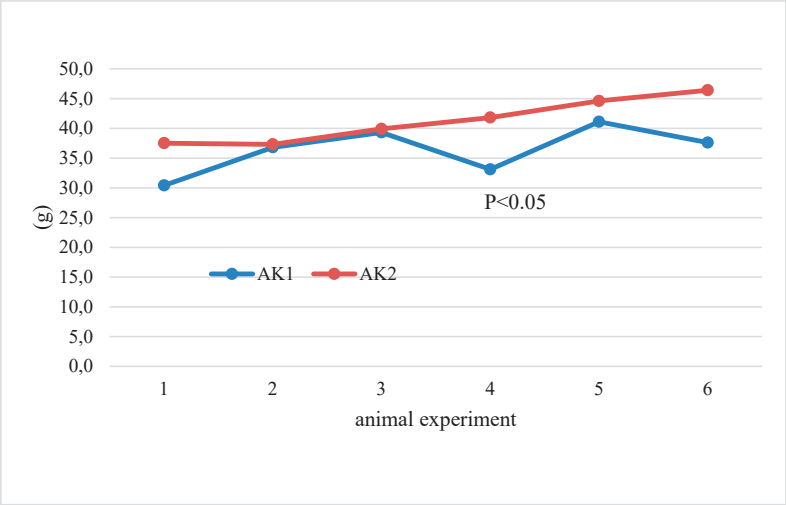

Figure 3. Daiy Weight gain (gram d^{-1})

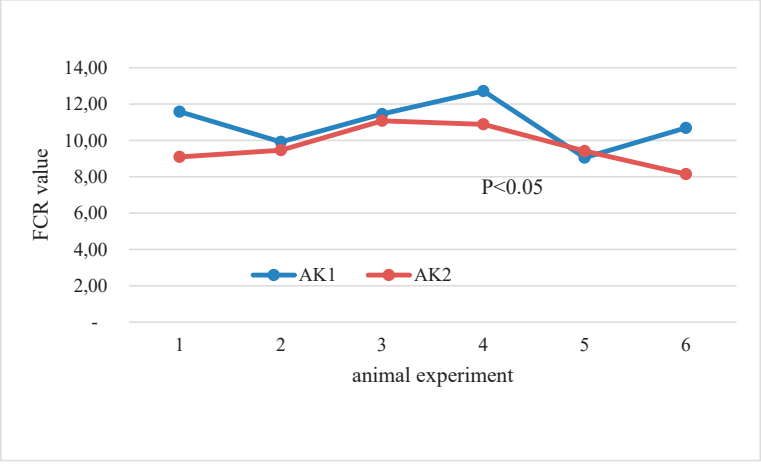

Figure 4. Feed Conversion Value

The average of daily weight gain obtained in AK1 (control group) was significantly lower than in AK2 as treatment group (P<0.05) as shown in Figure 3.

The difference of the groups related to mean FCR value was significant (P<0.5). The treatment animal group had a significantly smaller value than the control animal group. The FCR values of the treatment group ranged from 8.15 to 11.8. These FCR values were better than in the control group with FCR values ranging between 9.05 and 12.72

CONCLUSIONS

The IATmd substance in this study had a positive effect in keeping the palatability of goats, and also to improve body weight gain and the value of the feed conversion ratio of the trial animals

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THE SUSTAINABLE CONTROL OF VARROOSIS (*VARROA DESTRUCTOR*) BY TREATMENT OF CAPPED HONEYBEE BROOD USING ORGANIC VOLATILE ACIDS AND INNOVATIVE PROCEDURES

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Abstract

The varroa mite infestation is a serious cause of honeybee colony loss at a global level. The varroa mite population development in the honeybee colony is the result of its reproduction success and of some favouring factors. Its parasitism model, which rely on capped brood for reproduction, as well as the role as vector of viruses increase the negative impact on honeybee health. Thus, there is clearly a necessity to develop new treatment approaches to interrupt the mite's life cycle, especially before winter honeybee rearing in order to protect it. Except for the formic acid, the substances used today, which generally treat the whole colony, target only phoretic mites. Using the formic and acetic acids' rapid vaporization properties, two procedures were developed and tested for the treatment of capped brood. The results show a high effectiveness in the mortality of mites (90%-100%) in different experimental variants. The capped brood brushing with volatile organic acids represents a highly effective, cost efficient, organic and minimally invasive procedure. It could be applied any time during the active season to decrease the level of infestation before critical moments.

Key words*: brushing, capped brood, honeybee, organic, varroa.*

INTRODUCTION

The worldwide depopulation and mortality of honeybees' colonies in the past decades, caused by different factors, has been widely documented (Potts et al., 2010; Neumann and Carreck, 2010; vanEngelsdorp et al., 2009).

One of the main causes of these mortalities, varroosis, was also largely studied (Traynor et al., 2020; Noël et al., 2020; Nazzi and Le Conte, 2016; Piou et al., 2016; Le Conte et al., 2010), its control being the subject of different, complex strategies (Roth et al., 2020; Dieteman et al., 2012).

Being an important vector for viruses, especially for the deformed wing virus - DWV, (Roberts et al., 2020; Dubois et al., 2020, 2019; Barrosso-Arévalo et al., 2019; Dainat et al., 2012a) and in light of the new findings showing that this parasite feeds primarily on the fat body of honeybees (Ramsey et al., 2018), the negative impact increases substantially, especially on winter honeybees' longevity and immunity (Di Prisco et al., 2016; Annoscia et al., 2015; Francis et al., 2013; Nazzi et al., 2012).

The mite *Varroa destructor* (Acari: Varroidae) (Anderson and Trueman et al., 2000) was described for the first time as the ectoparasite of *Apis cerana*, a species which copes very well with this parasitosis by complex adaptive, naturally selected traits, one of them being the almost exclusive reproduction of the varroa mite in drone brood, (Lin et al., 2018; Beaurepaire et al., 2015; Rath, 1999; Koeniger et al., 1983).

In *Apis mellifera*, varroa mite reproduction takes place in both, drone and worker brood, but there is a preference for drone brood in its rearing period, when the mite population could be 8-10-times greater (Rosenkranz et al., 2010; Boot, 1995; Boot et al., 1995; Boot et al., 1993; Fuchs, 1990). Following the differences in the post-capping period, an average of 1.3 -1.45 new mated females are produced in worker brood and 2.2-2.6 in drone brood (Martin, 1994). The success of its reproduction depends highly on the number of the reproductive cycles per each mated female, with an average of 2-3 reproductive cycles (Donze et al. 1998; Martin & Kemp, 1997; Ruijter et al., 1987), as well as on the type of brood. In the drone brood

it is 95%, while in the worker brood it is 73% (DeGrandi-Hoffman & Curry, 2004).

As it is well known, the life cycle of the varroa mite includes a phoretic phase, visible on adult bees, and a reproductive phase, which takes place in the capped brood, where new generations of mites are reared. Studies show that, in the active season, up to 90% of the varroa mite population can be found within the brood (Rosenkranz et al., 2010). Thus, the reproductive phase of mites has a very important negative impact on honeybees' health as both mature and immature mites feed intensively on brood, affecting the nutritional status and the immunity, as well as transmitting the viruses. As result of this complex varroa-honeybee relationship, combined with seasonal particularities and re-infestation risks, the varroa mite population in a colony is a dynamic process, with different levels of infestation between colonies, regions and time periods (DeGrandi-Hoffman & Curry, 2004; Martin, 1998; Fries, 1994;) which trigger the treatment strategies.

Regarding the reproduction phase, the varroa mite foundress enters a cell just before it is capped, for example in a 0-24 hours interval in the case of honeybee worker brood, and an even longer interval in the drone brood (Donze et al. 1998; Ruijter et al., 1987).

In the post capping period, the honeybee metamorphosis with different undergoing processes such as spinning the cocoon, pupation, moulting or pigmentation takes place under this cap and usually pass unobserved (Snodgrass, 1956; Rembold et al., 1980). In the same situation is the reproductive phase of the varroa mite, which is totally protected by the capping barrier, with negative consequences on the honeybee's natural defending mechanisms, such as grooming or hygiene mechanisms, as well as on the treatments' effectiveness.

Studying the brood capping closely, one can observe the presence of the two layers: (1) the external wax layer, applied by worker honeybees in order to protect the larvae from falling down during the pupation process (Siceanu, 1996), and (2) the internal layer, which is represented by the cocoon tissue formed in the pupation process right after capping (Snodgrass, 1956; Rembold et al., 1980). The external surface of the capping made by wax, which has the color of the neighbouring comb cells as an economic strategy of the honeybee colony, is rough and has small openings visible through a stereomicroscope.

However, the internal surface is smooth and glossy-white, with a relative transparency, allowing the wax colour to be slightly visible (Figures 1 and 2).

Figure 1. The external view of the brood cap in worker brood. In the green background one can notice small openings in the irregular composition

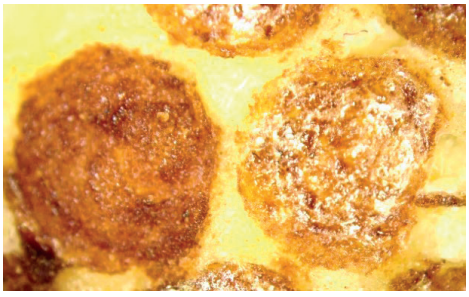


Figure 2. The internal view of the brood caps in worker brood. One can notice the white-shiny cocoon layer (right) and the wax layer after the cocoon was removed (left)

This porous, spongy-like structure of the honeybee brood cap, and the property of some organic substances (especially formic acid) to rapidly volatilise and pass through it, have recently led us to develop new procedures (Siceanu et al., 2019), for varroa mite control in capped brood. By their chemical properties (for example the pungent and irritating smell) (Formic acid-technical evaluation report, 2011), the highly volatile organic acids, like formic and acetic acids, affect the varroa mites through various mechanisms such as breathing inhibition (asphyxiation), disruption of the basic metabolic pathways (Rosenkranz et al.,

2010) and very likely by affecting the soft membranes (e.g., apoteles, intersegmental membranes) as well as by impairment of the sensory organs (e.g., pit organ), considering its chemosensing abilities (Nganso et al. 2020; Plettner et al., 2017).

Today, it is also well known that formic acid is the only substance that acts on brood when applied in the whole colony treatment, its effectiveness being very variable as many studies indicate: 41-95% (Calderón et al., 2010), 94.74% (Amrine & Noel, 2007), >60% (vanEngelsdorp et al., 2008). Some research even focused on brood treatment, separately by honeybee colony, for 1-2 hours, with very good results (up to 100% mite mortality) (Calis, 2001; Fries, 1991) and some practical information and applications were tried and recommended (Guido, 2018). The efficacity of formic acid on phoretic mites is also very variable (at least 40% and even over 95%), showing the importance of many factors involved, products or methods used (Pietrapaoli & Formato, 2019; Underwood & Currie, 2005, 2003; Elzen et al., 2004; Feldlaufer et al., 1997; Mutinelli et al., 1994). Most of these authors recommend the treatments of honeybee colonies with formic acid in long application (7-30 days) at the same time with monitoring the external temperature conditions in certain intervals which helps in vaporization control and reduction of the side-effects on bees. Unfortunately, the long duration of formic acid application can harm honeybees, queens, communications between individuals and the general development of the honeybee colony. These phenomena are highlighted in almost all the above-mentioned researches, as well as in practice. To overcome these problems, some new application methods were developed (Amrine & Noel, 2007; van Engelsdorp et al., 2008) to decrease the concentration and treatment duration, as the external temperature can be better predicted. The use of acetic acid in varroa mite control was also considered by researchers, but its effectiveness by whole colony treatment was lower than that of the formic acid (van Engelsdorp et al., 2008). To have a good effectiveness for varroa mite control, the use of highly volatile acids should be a very reasonable solution as they are also cost effective and organic substances.

Their use is allowed in varroa mite control in organic beekeeping in the European Union, as it is ruled in Council Regulation 834/2007, Regulation (EU) 2018/848 of the European Parliament.

Taking into account the negative effects of these substances on honeybees it is important to develop new methods of treatment, focusing only on capped brood (drone and worker), where the most part of varroa mite population exists in the active season. At the same time, this approach could be included in the sustainable strategies for varroa mite control which may be applied at any moment during the active season or at key moments, especially before rearing winter honeybees, in order to limit the natural development of the mite population, whose peak overlaps with this period.

Another advantage of limiting the treatment with volatile acids to capped brood combs is represented by a lower risk of honey contamination, having in view their hydrophilic properties and the presence of a higher content in honey, over the normal limits, following the conventional treatments.

In order to help the transfer of the volatile acids into brood cells by decreasing the treatment duration (from days or even hours to minutes), new procedures were developed and tested in our laboratory in recent years (artificial brood decapping, closed boxes using pression, brushing brood) (Siceanu et al., 2019). Following these preliminary researches, we focused on those treatment procedures that could be optimised and practically applied in beekeeping with very good results. Thus, the aim of the present study was to evaluate the effectiveness of two procedures for the capped brood treatment in very short time applications, on the mite (*Varroa destructor)* mortality inside the cells (the reproductive phase).

These procedures use highly volatile acids (formic and/or acetic acids) by (1) natural vaporization and saturation in closed space or by (2) capping brushing. If the first procedure - natural vaporization and saturation in closed space -- represents an improved procedure of the time-concentration parameters, following the researches published by Fries in 1991, and by Calis et al., in 2001 the second one - capped brood brushing -- represents a completely new

procedure, firstly communicated and registered for patent by Siceanu et al. in 2019.

MATERIALS AND METHODS

1. Experimental design

To test the effectiveness of these treatment procedures, an experimental design was established and varroa mite mortality inside the capped brood, found in all the developmental stages, was assessed.

The applied procedures are based on:

(1) the air saturation with highly volatile acids by natural vaporization in a special airtight box, assuming that a high concentration will naturally and rapidly enter the capped cells, and (2) brushing the capped brood combs directly with the highly volatile acids, using the natural properties of capping to absorb the substance and transfer it into cell for a short time interval.

The experiments were carried out in the 2018-2020 active seasons, in an experimental apiary (Băneasa 2) in the frame of Honeybee Genetic and Breeding Laboratory of the Institute for Beekeeping Research and Development - Bucharest (44°29′33″N 26°04′45″E). We included in the experimental apiary a total of 50 honeybee colonies of *Apis mellifera carpatica* subspecies, with young queens (2018, 2019), managed in Dadant hives on 10 frames. The experimental colonies have not been treated since 2018 in order to increase the level of varroa mite infestation for the 2019-2020 experiments. To increase the probability of having as much as possible a high infestation with varroa mite, for a better effectiveness in varroa mite counting, the procedure applications and the measurements were done from July 15th to August 30th, both in 2019 and 2020. At the same time and for the same reason, the donor colonies for capped brood combs were randomly selected from those with the highest level of infestation, being screened by natural mites that had fallen on the bottom boards. The experimental procedures were applied on honeybee capped brood combs, without adult bees (workers, drones, queen). To evaluate the impact of treatments on different categories of mites, the combs were generally selected to have brood of older ages (6-12 days post capping) in order to find as much as possible all the developmental stages of varroa mite.

A number of 10 combs was treated for each experimental variant according to the experimental design in Table 1 and the mite mortality evaluations were done under laboratory conditions.

As natural infestation of capped brood means, generally, varroa mites in a reproducing status and as they can be easily identified by the presence of white faecal deposits on the cell walls, a certain indicator of live mites (Dietemann et al., 2013; Büchler et al., 2017), control variants were not included to assess its natural mortality in the untreated capped brood. In some similar experiments (van Engelsdorp et al., 2008; Fries, 1991), the natural mortality of the varroa mite included in tests as control was extremely low.

Table 1. The experimental design for capped brood treatments by normal vaporization and by brushing the volatile acids

Experimental design and treatment variants	No. of treated combs	Concentration of active substance %	Quantity (ml.)
The experimental group to test the first procedure – The capped brood treatment, for different time intervals, in closed space, saturated with formic or acetic acid vapours by natural vaporization			
Formic acid treatment for 15 minutes (T1-FA 5')	10	85	100
Formic acid treatment for 10 minutes (T2-FA 10')	10	85	100
Formic acid treatment for 5 minutes (T3-FA 15')	10	85	100
Acetic acid treatment for 20 minutes (T4-AA 20')	10	99	100
The second experimental group to test the second procedure - The capped brood treatment by brushing with formic and acetic acids of different concentrations			
Brushing with formic acid 85% (T5-FAB 65%)	10	65	-
Brushing with formic acid 65% (T6-FAB 85%)	10	85	-
Brushing with acetic acid 99% (T7-AAB 80%)	10	80	-
Brushing with acetic acid 80% (T8-AAB 99%)	10	99	-
Brushing with a formula based on formic acid 65% and acetic acids 80% in different proportions* (T9-FAAB 65&80%)	10	65&80	-
*formic acid 65%, acetic acid 80%, plant extracts (*Ocimum basilicum, Thymi vulgaris, Mentha piperita, Mellisa officinalis*) and sugar in proportion of 6:2.5:1:0.5.			

Also, the experiments were designed to include different experimental variants grouped in the two procedures to test the specific variables (substance, time, concentration), so as to be able to perform comparisons, statistical analysis and data interpretation. The plants used in the extract are medicinal and aromatic plants, containing active substances recognized for positive effect on the honeybee digestive system and anti-repellent effect. The sugar role was to assure a good adherence of formula on the comb surface, to better maintain the formula substances in the porous structure of the cap. Thus, the formula based on formic and acetic acid (FAAB 65&80%), as well as some plant extracts and sugar, was specially created to decrease the concentrations of acids, to include the necessary active substances for the best efficacy on varroa mites' mortality, to have a good adherence, as well as to help attract honeybees after treatment to take care of the treated brood in a shorter period of time after treatment.

2. The procedures application.

2.1. The capped brood treatment, for different time intervals, in closed space, saturated with formic or acetic acid vapours by natural vaporization.

Before treatment (at least 10 minutes), an airtight box was prepared, by application of 100 ml formic acid of 85% concentration or acetic acid of 99% concentration on textile elements placed on lateral walls and on the inner cover, so as to sustain a rapid vaporization and air saturation inside the box. As a result of some measurements, the quantity of vaporised formic acid during the treatment of 4 combs, which is the frames capacity of the treatment box in our experiments (including all operations), was between 15 and 30 g at a volume of 33 dm^3.

In order to apply this procedure, irrespective of surface or presence of open brood or food, the worker honeybee capped brood combs to be treated were shaken and brushed off to eliminate the covering bees in the origin colony.

The combs were put into the airtight box, after saturation with formic acid by natural vaporization, they were treated for 5, 10 and 15 minutes. The treated combs were put back into the origin colonies until the next day when the mite mortality was assessed (Figure 3).

Figure 3. The application of treatment in closed space, saturated with organic, volatile acids vapours by natural evaporation

2.2. The application of treatment by brushing the capped brood surfaces with tested substances.

To apply this procedure, irrespective of the capped brood surface or the presence of open brood or food, the worker capped brood combs were shaken and brushed off to eliminate the covering bees in the origin colony. The brood combs were successively treated (brushed) with substances of different concentration or formula (Figure 4), depending on experimental variants and put into a ventilated box placed near the original hive (Figure 5).

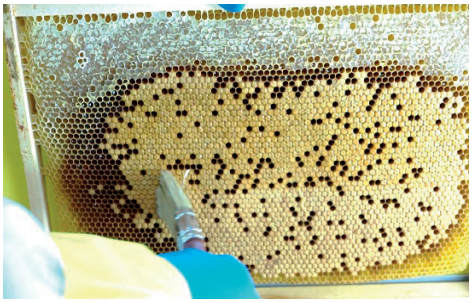


Figure 4. The application of treatment with volatile organic acids by brushing the capped brood

The honeycombs were held so that the treatment solution should not leak into the uncapped cells in which there could be eggs, brood larvae, honey or pollen bread, so as to avoid their contact with acids. To treat the combs with experimental substances, we used a paintbrush with medium stiffness bristles, about 4-10 cm wide. The treatment product was applied and brushed with a light press, to help the cap absorb the tested product. The surface of the capped brood was brushed so that all

cells with capped brood were also covered with the treatment substance. The brushing was done with left-right movements, to avoid the accumulation of drops on the lower edge of the uncapped cells and leakage inside them.
To carry out the treatment, the volatile acids were put into a special plastic box which is strongly fixed by the hive wall (Figure 5). The operation was repeated on all capped brood combs' surfaces from the experimental variants. The treated brood was immediately placed in a well-ventilated box hive type (e.g. frames transport box, swarm box, etc.) as shown in the figure 5. The box was covered with a board, so that the bees could not enter the space (to prevent robbing if there was a risk) and left for 10-15 minutes, during which time, most of the treatment substances evaporated inside and outside the cells.

Figure 5. The application of treatment with volatile organic acids by brushing the capped brood by a simplified variant, near the treated hive

The treated combs were not immediately returned to the colony because the amount of evaporated acids can harm the honeybees or queens in the honeybee colonies, especially in the first minutes. The direct contact of the testing acids with any individual (bees or queen) can kill them. For this reason, it is recommended to keep the treated combs after brushing in separately boxes for at least 10 minutes, depending on the treated surface, until the excess of substances is evaporated.
The treated combs were put back into the origin colonies until the next day when the mite mortality was assessed.
While using the treatment substances, it is mandatory to wear acid-resistant protective gloves, glasses and mask to prevent inhalation of acid vapours or direct contact.
To better understand this procedure, two scientific-technical video-films were developed and openly published (Siceanu et al., 2019; Siceanu, 2020).

3. The measurements on varroa mite mortality inside the capped cells

To give the treatment time for action, and to assess the impact of treatment on different categories of varroa mite which normally is found in the infested cells, the mortality was assessed on the day following the treatment (24 h). For each application procedure specific data about the treatment was registered (concentration, quantity, time), number of checked cells, number of infested cells as well as number of live and dead mites for each category. Thus, treated combs were taken out of the colony and the number of dead and alive varroa mites (including all individuals in a dying state) was assessed, using a stereomicroscope (Olympus SZ61) with 6.7X-45X magnification. To do these evaluations, the cells were opened with a tweezer, cell by cell, in rows, following the standard protocol (Dietemann et al., 2013; Büchler et al., 2017) or in some cases using the artificial decapping method to uncap rapidly a larger portion of cells (Siceanu et al., 2018, 1996). As mentioned above, the infested cells were more easily identified by the presence and white aspect of mite dejection on the cell walls. Each pupa from the infested cells were taken out and carefully put on a slide to be inspected. All the categories of varroa mites that were found and their state (dead or alive) were registered. In the same manner, the emptied cells were inspected. The varroa mites counting was assigned to the following different categories of mites according to their aspect: foundress females (FF), adult males (AM), protonymphs - males and females (P), deutonymphs - females (D), and adult daughters (AD) as shown in figure 6. The adult mites and immature stages (eggs, larvae, protonymphs, deutonymphs) present a sexual dimorphism and a gradual sclerotization of the exoskeleton which help their identification.

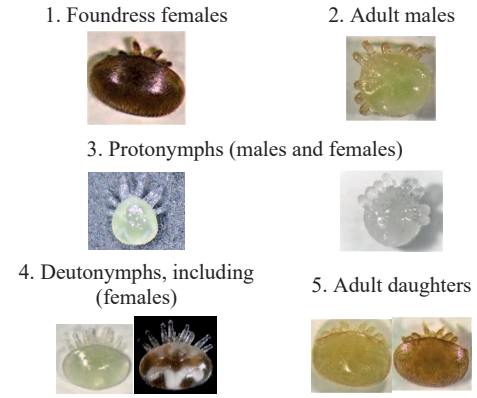


Figure 6. The aspect of different stages of varroa mite development in capped brood (6.7X-45X, stereomicroscope - Olympus SZ 61). Photos© Institute for Beekeeping Research and Development, Bucharest, Honey bee Genetics and Breeding Laboratory

As it is very difficult, confusing and time consuming to distinguish between protonymphs/deutonymphs of males when compared with protonymphs of females, these stages were included into protonymphs category of males and females, and from the treatment perspective they can be similarly affected as they are individuals of similar size with an unsclerotized exoskeleton.

Deutonymphs received a special attention as their immobile phase (which last 48h) (Dietemann et al., 2013), can be assigned to death category, the live individuals presenting an internal specific motility which can be noticed by their transparency. To notice these details, the deutonymphs were placed in a good position and light at a 45X magnification.

To perform statistical analyses on the obtained data, the tests for outlier's data identification (Grubbs test) and normal data distributions (Anderson Darling test) were firstly applied. To apply different statistical tests in order to assess the statistical significance threshold of different treatments' effectiveness, we used a Bartlett test for the variances' homogeneity, calculated in R software followed by specific tests to check the averages' homogeneity assumptions (Free software for statistical analysis). Thus, the homogeneity of the averages within each experimental group was analysed by a Welch's ANOVA test for unequal variance followed by a Games Howell post-hoc test in the frame of the first experimental group, and an ANOVA test followed by a Tukey post-hoc test for equal variance in the second experimental group. Data were calculated in Excel Office 2016 worksheets completed by XRealStats and Sigma XL modules, according to the statistical analysis guidelines presented in the literature (Sandu, 1995; Pirk et al., 2013). Additionally, a set of boxplots histograms on different treatments and categories of mites in the frame of the two groups of treatments were presented. It is important to mention that the percentage of varroa mite mortality 24 hours later, following the treatment application, was the response variable in all the statistical analyses.

RESULTS AND DISCUSSIONS

The obtained results regarding the average of varroa mite mortality in the cells (%), assessed at 24 hours after treatments application, in different treatments, are shown in tables 2, 3, and 4. The results were obtained by evaluating an average of 26.4 single or multiple infested cells per comb, out of 139.3 checked cells per comb in average, per total experiment. The general infestation level of brood combs on average was 19.5% (Table 3). According to these data, a high percentage of varroa mortality (>85%) was registered in more treatments performed by the two types of procedures: FA 10 min, FA 15 min, FAB 65%, FAB 85%, AAB 99%, and FAAB 65&80%. Analysing the averages, in the first experimental group (T1-T4), the best effectiveness of brood treatment (Ave. = 97.96%, St err. ± 0.56) was registered after keeping the capped brood combs in the saturated space with formic acid vapours for 15 minutes. A lower effectiveness (Ave. = 85.74%, St err. ± 1.89) was registered at a 10 minutes interval, while a low effectiveness (Ave. = 26.22%, St err. ± 1.44) was registered after 5 minutes of treatment. These data show an increasing effectiveness of the formic acid combating the varroa mite in a saturated space, in a certain time interval (5-15 minutes), with maximum effectiveness at 15 minutes treatment. The effectiveness of acetic acid 99% (Ave = 68.24%, St err. ± 1.27) when used to saturate a treatment space for 20 minutes was lower than that of the formic acid used for 10 minutes.

In the second experimental group (T5-T9) regarding the brushing of capped brood with volatile acids of different concentrations, a high effectiveness (over 90%) of treatments on varroa mite mortality inside the cells was registered in the experimental variants in which formic acid was used: FAB 65% (Ave. = 90.48%, St err. ± 1.29), FAB 85% (Ave. = 92.64%, St err. ± 1.38), and FAAB 65&80% (Ave. = 96.36%, St err. ± 0.84). Acetic acid of 99% and 80%, when used alone in brood brushing, showed a lower effectiveness (AAB 99%: Ave. = 89.68%, St err. ± 0.89, respectively AAB 99%: Ave. = 74.46%, St err. ± 1.88), but a better one than in the treatment in saturated box (AA 20'). For a better overview, the results of each experimental variant were plotted in figure 7, highlighting the quartiles repartition and averages of varroa mite mortality as percentage. Thus, one can easily remark the best treatments, also by values repartition on quartiles (75th, 50th and 25th) and overall average of each treatment.

Table 2. The varroa mite mortality percentage in average per each comb, in different experimental variants

Treated brood combs	*The 1st experimental group*				*The 2nd experimental group*				
	T1 FA 5'	**T2 FA 10'**	**T3 FA 15'**	**T4 AA 20'**	**T5 FAB 65%**	**T6 FAB 85%**	**T7 AAB 80%**	**T8 AAB 99%**	**T9 FAAB 65&80%**
C1	22.54	80.90	94.00	64.79	89.63	90.85	81.03	90.91	100.00
C2	32.65	78.43	98.08	64.29	88.27	95.83	82.76	91.85	93.33
C3	29.23	90.14	96.97	72.22	90.00	85.99	70.23	88.71	100.00
C4	25.53	86.61	99.07	71.43	96.10	92.12	78.70	90.72	98.55
C5	17.46	80.43	100.00	76.12	96.23	97.45	75.84	95.05	97.50
C6	26.09	86.67	96.77	68.14	88.24	95.92	66.67	90.00	92.55
C7	27.85	87.37	98.89	65.31	83.33	86.73	67.42	87.39	96.15
C8	22.22	92.50	97.83	67.09	87.95	89.11	78.38	89.47	96.05
C9	27.37	95.65	97.96	63.95	90.43	94.25	70.69	84.42	95.45
C10	31.25	78.69	100.00	69.09	94.64	98.17	72.86	88.30	94.00
Ave.	**26.22**	**85.74**	**97.96**	**68.24**	**90.48**	**92.64**	**74.46**	**89.68**	**96.36**
St. Err. ±	**1.44**	**1.89**	**0.56**	**1.27**	**1.29**	**1.38**	**1.80**	**0.89**	**0.84**

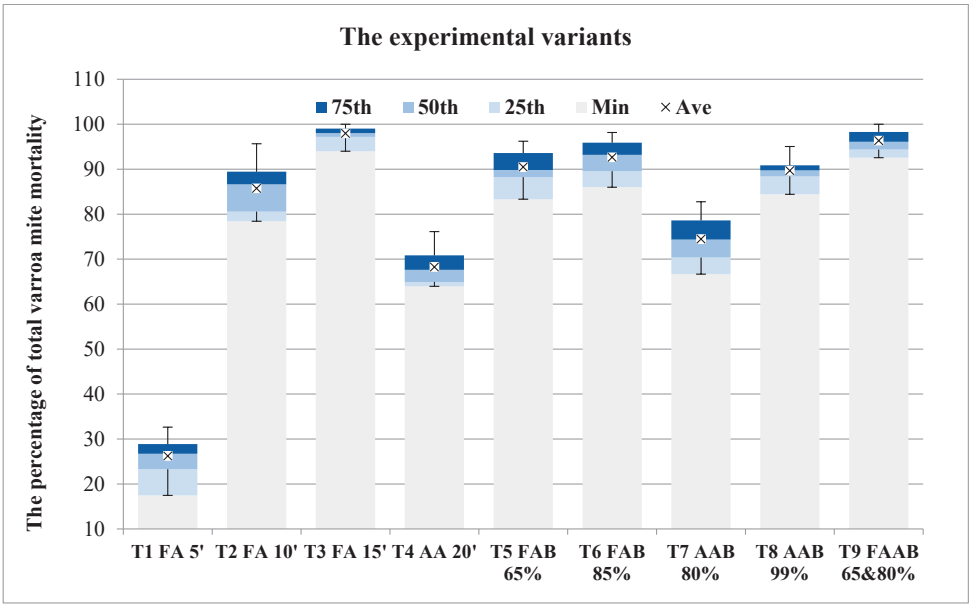


Figure 7. A box plot presentation of varroa mite mortality data (%) in capped brood treated with formic and acetic acids by experimentally tested procedures

Table 3. The obtained results regarding the number of checked cells, infested cells, varroa mites and the average of mortality on each treatment

Treatments	Number of combs	No. of checked cells	No. of evaluated infested cells	Infestation level %	The varroa mite's evaluation		
					Total (dead & alive) (T)	Dead (D)	Mortality % (M)
T1 - FA 5 min.	10	1356	178	13.13	606	158	26.07
T2 - FA 10 min.	10	1866	230	12.33	734	633	86.24
T3 - FA 15 min.	10	1228	168	13.68	763	749	98.17
T4 - AA 20 min.	10	1164	232	19.93	826	560	67.80
T5 - FAB 65%	10	1548	415	26.81	1609	1457	90.55
T6 - FAB 85%	10	1308	420	32.11	1632	1499	91.85
T7 - AAB 80%	10	1394	251	18.01	1189	890	74.85
T8 - AAB 99%	10	861	221	25.67	931	837	89.90
T9 - FAAB 65&80	10	1824	259	14.20	1030	988	95.92
Total	90	12549	2374	18.92	9320	7771	-
Ave.	10	1394.3	263.8	19.5	1035.6	863.4	83.38
St. Err. ± T1-T4	-	*89.85*	*9.52*	*0.98*	-	-	*15.79*
St. Err. ± T5-T9	-	*74.44*	*26.62*	*1.47*	-	-	*3.60*

Table 4. The obtained results regarding the number of varroa mites found in brood (total and dead) as well as its mortality in average (%) on different categories of mites and each treatment

Treatments	***The number of varroa mites found in brood and its mortality on different categories after treatments (at 24 h)***														
	Foundress			**Males**			**Protonymphs**			**Deutonymphs**			**Daughters**		
	T	**D**	**M %**	**T**	**D**	**M %**	**T**	**D**	**M %**	**T**	**D**	**M %**	**T**	**D**	**M %**
T1 FA 5 min.	188	37	**19.6**	82	26	**31.7**	147	42	**28.5**	115	32	**27.8**	74	21	**28.3**
T2 FA 10 min.	235	198	**84.2**	90	80	**88.8**	164	145	**88.4**	151	130	**86.0**	94	80	**85.1**
T3 FA 15 min.	168	164	**97.6**	84	82	**97.6**	215	215	**100.0**	198	193	**97.4**	98	95	**96.9**
T4 AA 20 min.	258	101	**39.1**	93	81	**87.1**	137	124	**90.5**	172	152	**88.3**	166	102	**61.4**
T5 FAB 65%	474	428	**90.3**	223	206	**92.3**	238	230	**96.6**	441	390	**88.4**	233	203	**87.1**
T6 FAB 85%	486	454	**93.4**	215	205	**95.3**	211	209	**99.0**	461	392	**85.0**	259	239	**92.2**
T7 AAB 80%	278	182	**65.4**	169	108	**63.9**	222	196	**88.2**	289	229	**79.2**	231	175	**75.7**
T8 AAB 99%	310	277	**89.3**	145	131	**90.3**	184	175	**95.1**	175	149	**85.1**	117	105	**89.74**
T9 FAAB 65&80	274	261	**95.2**	88	80	**90.9**	297	297	**100.0**	257	240	**93.3**	114	110	**96.4**
Total	**2671**	**2102**	**-**	**1189**	**999**	**-**	**1815**	**1633**	**-**	**2259**	**1907**	**-**	**1386**	**1130**	**-**
Ave.	**296.8**	**233.6**	**78.7**	**132.1**	**111.0**	**84.0**	**201.7**	**181.4**	**89.9**	**251.0**	**211.9**	**84.4**	**154.0**	**125.6**	**81.5**
St. Err. ± T1-T4	-	-	*18.4*	-	-	*15.0*	-	-	*16.3*	-	-	*15.8*	-	-	*15.1*
St. Err. ± T5-T9	-	-	*5.4*	-	-	*5.7*	-	-	*2.0*	-	-	*2.3*	-	-	*3.4*

In order to perform statistical analyses, the data were checked out for outliers' values, using the Grubbs test in Excel Office 2016 worksheets, checked out also by XRealStats software, the obtained results showing the lack of these data. Further on, the data normality was checked out using the Anderson-Darling test performed in Excel Office 2016 completed by Sigma XL

module. The all obtained p-values were greater than the level of confidence (α=0.05) which validate the assumption that the data sampled are from a normal distribution.
To establish the homogeneity of variances of the tested samples, in a normal distribution of data, a Bartlett test performed in R software was performed for equal samples, all treatments and by groups of treatments. The results are presented in the table 5.

Table 5. The results on variances homogeneity – Bartlett test, equal samples

Treatments	**Bartlett's K-squared**	df	p-value	X^2 critic α=0.05	The results
T1-T9 (all treatments)	17.618	8	0.02428	15.51	unequal variance
T1-T4 (the 1st group of treatments)	10.543	3	0.01447	7.81	unequal variance
T5-T9 (the 2nd group of treatments)	6.8424	4	0.1445	9.49	equal variance

The obtained values and their probability show a heterogenic variance in the tested treatments which is generated by the first group of treatments, as by subsequently testing an unequal variance in the first group of treatments (K-squared > X2 critic, at α=0.05) and an equal variance in the second group of treatments was found.
To continue with the statistical analysis on the first group of treatments, a Welch's ANOVA test assuming unequal variance was applied to establish if the differences would be identified also concerning the treatments' averages.

Table 6. The Welch's ANOVA test of averages assuming unequal variances for the 1st group of treatments T1-T4

Welch's ANOVA test	Numerator df	Denominator df	F-calc.	Probability level
Between Groups	3	17.87	735.4	6.59E-19
F-critic (df 3; 18; α= 0.05) =3.16 F-critic (df 3; 18; α= 0.001) = 8.49 The result. F calc> F crit. The null hypothesis of equal averages is rejected				

The summarised results in the table 6 show highly significant differences between the averages of the 1st group of treatment.
As a result, a Games-Howell post-hoc test was applied further on to establish the statistical significance of differences between the averages of treatments, grouped two by two. The results are presented in table 7.

Table 7. The pair-wise comparison assuming unequal variances and equal samples (Games-Howell post-hoc test) for the first group of treatments T1-T4 (XRealStats)

Games-Howell test		Ave. diff.	q-calc.	df	q-crit α=0.05	p-val.
T1 FA 5'	T2 FA 10'	59.5	35.4	17	4.02	1.14E-13
T1 FA 5'	T3 FA 15'	71.7	65.6	12	4.20	-4.4E-13
T1 FA 5'	T4 AA 20'	42.0	30.9	18	4.00	1.66E-13
T2 FA 10'	T3 FA 15'	12.2	8.7	11	4.26	0.00039
T2 FA 10'	T4 AA 20'	17.5	10.8	16	4.05	5.72E-06
T3 FA 15'	T4 AA 20'	29.7	30.2	12	4.2	1.96E-10

As it can be easily noticed, there are highly significant differences between all treatments when compared two by two, highlighted by the pairwise average difference where q-calculated is higher than q-critic at a confidence level α=0.05. The lowest difference can be remarked between the 10 and 15 minutes treatments when formic acid was used.

Table 8. The results on averages' homogeneity (ANOVA single factor test), used for test the equal samples and equal variances for the 2nd group of treatments T5-T9

ANOVA single factor test					
Source of Variation	*SS*	*df*	*MS*	*F calc.*	*P-value*
Between treatments	2811.7	4	702.95	42.19	1.11E-14
Within treatments	749.73	45	16.66	-	-
Total	3561.5	49	-	-	-
F-critic (df 4; 45; α= 0.05) =2.61 F-critic (df 4; 45; α=0.001) = 5.70 F calc > F crit. The null hypothesis of equal averages is rejected					

The results of ANOVA single factor test, presented in table 8, show highly significant differences between all treatments as F calculated is higher than F critic (α = 0.001).
To statistically compare the treatments in the second group of treatments we used a one-way ANOVA test followed by a Tukey post-hoc test. Comparing the different brushing treatments by Tukey post-hoc test, to determine if at least one group of averages is different from the others, the following results (Table 9) were obtained:

Table 9. The pair-wise comparison assuming equal variances and equal samples (Tukey post-hoc test), for the second group of treatments T5-T9

Tukey test	T5-T9	T5-FAB 65%	T6-FAB 85%	T7-AAB 80%	T8-AAB 99%	T9-FAAB 65&80%
T5-T9	Ave.	**90.48**	**92.64**	**74.46**	**89.68**	**96.36**
T5-FAB 65%	**90.48**	0	*2.16 (NS*)*	*-16.02 (HS)*	*-0.80 (NS)*	*5.88 (S)*
T6-FAB 85%	**92.64**	0.761	0	*-18.18 (HS)*	*-2.96 (NS)*	*3.72 (NS)*
T7-AAB 80%	**74.46**	2.58E-10	5.84E-12	0	*15.22 (HS)*	*21.90 (HS)*
T8-AAB 99%	**89.68**	0.992	0.491	1.09E-09	0	*6.68 (HS)*
T9-FAAB 65&80%	**96.36**	0.019	0.265	2.46E-14	0.005	0
w-critic (tab) = q (df 5; 45; α=0.05) = 5.21 w-critic (tab) = q (df 5; 45; α=0.01) = 6.36						
**NS – Non-significant differences; S - Significant differences; HS - Highly significant differences.*						

This statistical test shows us that the varroa mite mortality registered non-significant differences (NS, w calculated < w critic, at α = 0.01) between the following brushing treatments:

- formic acid 85% and formic acid 65% concentration;
- formic acid 85% and formula based on formic and acetic acid (65&80%);
- formic acid 85% and acetic acid 99%;
- formic acid 65% and acetic acid 99%.

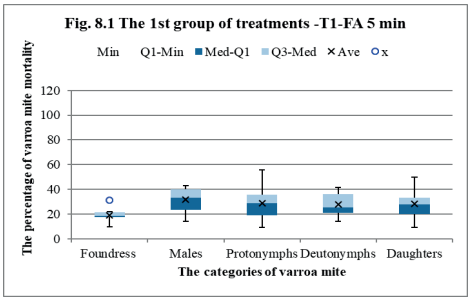
Comparing the treatments based on formic acid 65% with the formulation based on formic and acetic acids we registered significant differences (S) in varroa mortality at the level of confidence α = 0.05, but no differences at α = 0.01.

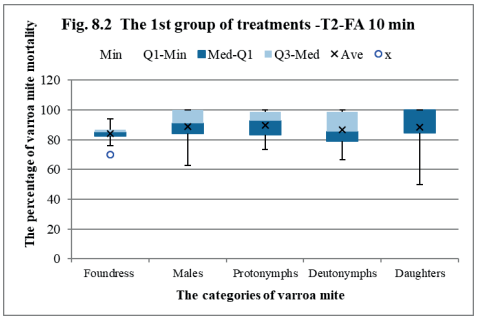
Highly significant differences in varroa mite mortality were found when the treatment formula was compared with acetic acids-based treatments, but important differences were found also between the two acetic acid-based treatments.

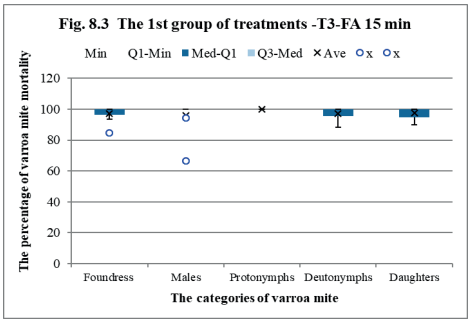
Highly significant differences were found also when acetic acid 99% was compared with formula based on formic and acetic acid, but at a lower level (w = 6.68, w calc at α at 0.01 = 6.36).

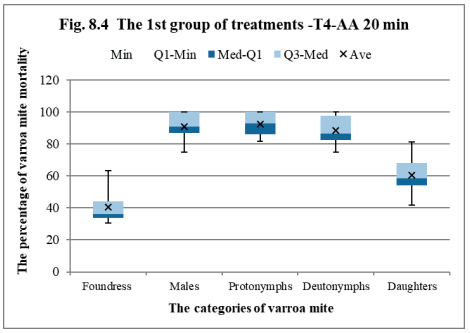
Regarding the different categories of varroa mite mortality in the brood cells (at 24 h) following the two procedures of treatment, the results on their mortality and standard error (±) for each treatment are presented in table S2.

For a better image of the data obtained on each treatment (n = 10 combs), box plots with quartiles, medians and averages as well as their limits of variation are presented in Figures 8.1 - 8.9.





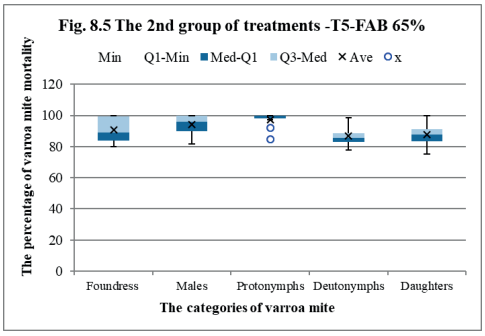


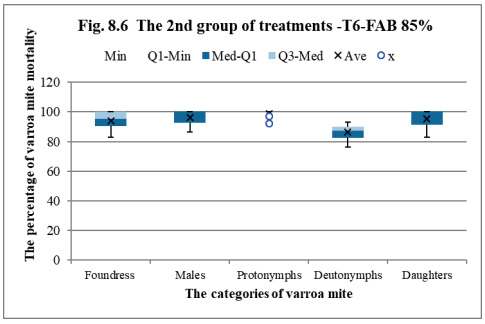


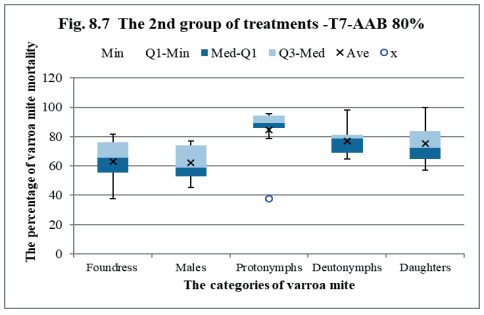
Figures 8.1-8.4. Boxplots on different categories of varroa mite mortality in honeybee brood treated with formic and acetic acids by natural vaporization in closed space procedure

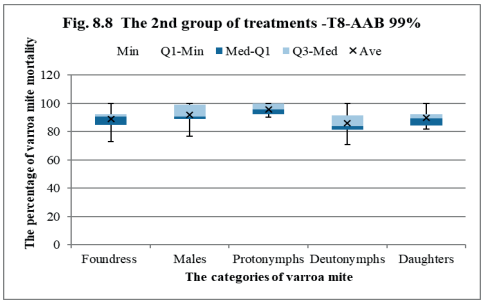
These figures show that in the 1st group of treatments, the formic acid act almost equal on different varroa categories, but the treatment duration is very important on the mortality level, while acetic acid act better on immature

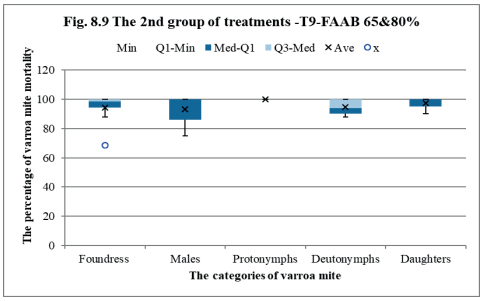
and unsclerotised varroa individuals. In the 2nd group of treatments one can notice that there is a better and more similar effectiveness on all varroa categories in using both active substances (formic and acetic acid) with lower values when using acetic acid alone and in lower concentration.











Figures 8.5-8.9. Boxplots on different categories of varroa mite mortality in honeybee brood treated with formic and acetic acids by brushing procedure

The results we have obtained validate the hypothesis that the new tested treatment procedures are very effective (up to 100%) in treating *Varroa destructor* mite in the capped brood of the honeybee colonies, in short applications (minutes), severely interrupting the reproductive phase of varroa. However, the heterogenic variances and averages in the 1st group of treatments shows that the time parameter as well as the different volatilization properties of the two substances are very important in performing capped brood treatments in acid-saturated spaces, influencing the percentage of varroa mite mortality in the capped brood. Thus, the obtained results show us the importance of a minimal treatment duration, for acid molecules to penetrate the caps and make contact with the different categories of mites to have an immediate high mortality. This experiment shows us that, when the formic acid is used, it is important to keep the combs in the saturated boxes for a minimum of 10 minutes to have at least an 85% immediate mortality of varroa mite inside cells. In the second group of treatments, all the experimental brushing treatments having formic acid in their composition registered very good results on mortality of varroa mites. The best effectiveness was obtained with the formic acid of an 85% concentration or when the formula based on formic and acetic acid was used, but insignificant differences were registered between all treatments based on formic acid (65%, 85% and formula). Good results (on average an 89% mortality) were registered also when the acetic acid of 99% concentration was used and insignificant differences were found when it was compared

with the formic acid 65% and 85%. The obtained results are better in the case of brushing procedures as once the capping is imbued, a part of the substance will immediately penetrate the cap and will fill the space of cells. As in the first group of treatments, the formic acid used by brushing procedure was proved to be more effective than acetic acid in order to obtain an immediate mortality, evaluated at 24 h after treatments.

According to the mortality level of different categories of varroa mite, the obtained results in the first group of treatments, where acid concentration varies with the treatment time (minutes) and the substance used (formic acid as compared with acetic acid), one can notice that adult females are the most resistant category to the treatment, especially when acetic acid is used, while the immature mites (protonymphs and deutonymphs) are more sensitive, especially protonymphs. This sensitivity depends most probably on the level of vapours (acid concentration) entering the cells and sclerotization degree of their body. The lack of sclerotization in immature stages of varroa brood is an important advantage in these treatments, especially if we want to decrease the time-concentration-dose parameters in the different treatment formula of current procedures.

Deutonymphs stages registered lower values because of the immobile phase which shows a greater resistance to volatiles, as in the case of the pupal stage in honeybees. This resistance can be noticed by observations done on the following stage - the freshly transformed daughters, which could be found live at the evaluation moment, on the next day after treatment. Being very effective in rapidly killing the mites, even the most resistant individuals (adult females), the use of formic and acetic acids in honeybee brood treatments can be considered safe for risks of resistance that these mites could develop, the organic volatile acids being recognized to pose minimal risks (Rosenkranz et al., 2010).

It is important also to mention different observations done during the evaluations:

- the most part of live varroa mites at the evaluation moment looked to be affected by these treatments, as a lower vitality was noticed during the evaluations.
- in some re-evaluations done two or three days after treatment, in the case of effective and very effective treatments (over 70% mortality), the adult females of varroa which remained alive were not capable to continue reproduction; they were found in a dying state, and the eggs were not present inside the cells anymore.

Consequently, from the varroa mite mortality evaluation perspective, we consider that the best moment for the evaluation of the treatments' effectiveness should be done at 2-3 days after the treatment, if there is no purpose to identify the different categories of mite progeny. After this period, the dead protonymphs and deutonymphs are in a decomposing stage and sometimes cannot be identified anymore, while the apparently live varroa mites on the first day after treatment as well as its reproduction activity can be clearly evaluated.

The life cycle of varroa mite would be seriously affected if the foundress is dead or in a dying state and its reproduction and offspring care (e.g., preparing the feeding site) will be affected, too. The same situation would be if the male is dead because the daughters, in case of survival (resulted from immobile deutonymphs) will not have been mated. Even if the viability of honeybee brood was not the purpose of this research, specific experiments being necessary, it was obvious to notice during the experiments that the pupal period was not affected by treatments, continuing its normal development. In these experiments, all the honeybees that emerged from the treated brood were found active and healthy, the hive population and activity being normal during the whole period of experiments. As we noticed, only the mobile stages found in the cocooning, pupation and emerging moments were found to be affected and only the individuals that passed through these stages in the interval of time that the brood was exposed to the substances, and these observations have already been documented even on a longer exposure – 1-2 hours (Calis, 2001; Fries, 1991). According to our observations as well as from older research (Siceanu, 1996), the honeybee pupal stages are more resistant to different factors than larval stages, especially when compared to open brood that requires regular feeding. In the capped brood period, only the nest temperature

and humidity are important to the whole transformation from prepupa to adult honeybee. The scientific literature (Ruttner, 1980) shows that the honeybee brood, both larval and capped brood, if put outside the hive (not in sunlight) for a couple of hours or even more, is relative highly resistant. Thus, in the brood protection perspective, the brushing procedure can be considered superior to the treatment in a closed space as the volatile substances will come in contact only with the capped stages of honeybee brood and the mites inside cells, while in treatment boxes all brood, including larvae and eggs are treated and open brood is clearly affected. Having an immediate result and being targeted only on the capped brood frames, the effect of any external temperature and humidity do not influence the results and procedures' effectiveness as in the classical treatments with formic acids. More than this, by these new treatments we can avoid exposing the adult honeybees which are very sensitive to these substances, as their volatility is very high, increasing rapidly at high, external and nest temperatures.

Currently, at an international level, the treatment of capped brood with organic volatile acids is not practically used, the only method discussed in the literature and proposed in practice being the treatment in closed space (airtight box) for 1-2 hours (Guido, 2018; Calis, 2001; Fries, 1991). Shortening the time of treatment in boxes and developing totally new, minimally invasive and practical procedures such as brushing capped brood with effective volatile substance, would help beekeepers maintain a better control of varroosis. By enlarging the application period and choosing the key moments in the season, especially at the beginning of the season and before "winter bee" rearing, when the surface of capped brood is smaller, to minimize the workload or to combine with different local techniques whenever nest management is necessary (Siceanu, 2020), it is possible to increase substantially the benefit of this application and its effectiveness in combating varroa mite. For example, in the temperate season, the treatment may be done at any moment of the active season, when there is an intervention in the brood nest, even just before or during honey flows, as these substances do not contaminate the honey as well as all the other bee products, especially when applied by these procedures.

Actually, the majority of these treatments are done at the end of the summer season (e.g., August-October for the northern hemisphere, in temperate climate) when the honeybee colony population decreases and the mites' population increases and concentrates itself on the last brood and winter honeybee.

However, to drastically reduce the infestation level and disturb the population dynamic of the mite, the following key moments for applying these treatment procedures would be:

1. Apply early in the spring when there are small areas of capped brood, and the beekeeper performs some inspections or operations for reorganizing the nest (reduction or enlargement). Preferably, the treatment should be done before the beginning of drone rearing if the weather allows the interventions into the hive.
2. Apply when the artificial swarms are established using capped brood, usually with 1-3 frames of capped brood. This is an important treatment in order to give a clean start to the new colony, as usually a lot of varroa mites are taken out together with the capped brood.
3. Apply in the summer, just before the period of "winter bees" rearing, to produce healthy bees under a very low infestation. This can be done easily in the periods when there is a honey flow and the brood surfaces are reduced because the honeybees block the nest with honey, usually the beekeepers are forced to make room for egg laying to obtain bees for wintering.

Taking into consideration the 8-10-fold higher infestation rates of drone brood compared to worker brood, the treatment could be applied on all drone brood surfaces, which highly increases the effectiveness of overall treatment as well as the health of drones and reproduction biology.

In this concept of treatment, in order to kill also the phoretic varroa mites, two options could be available:

1) a classical treatment of honeybee colony with a rapid effect in the same period with brood treatment (e.g., the day before or after a brood treatment)

2) a second brood treatment with formic or acetic acids can be applied after 9 -12 days from the first treatment, a necessary interval of time to allow most part of phoretic mites (foundress females) enter the brood (before capping).

Decreasing the treatment duration and the concentration in active substances as well as the optimization of application procedures during normal inspections, are objectives for further investigations, in order to stimulate beekeepers to apply the capped brood treatment as well as to better protect the honeybee colony, brood and hive products.

Going further with the application possibilities, the new approach could be an effective treatment tool also in combating *Tropilaelaps* sp., taking into account the similarities regarding the reproductive and phoretic phases of these parasites, with a much shorter phoretic phase which contributes to the ineffectiveness of other treatments used in varroa mite control (Pettis et al., 2017; Raffique et al, 2012).

CONCLUSIONS

The two procedures using short time treatments with organic volatile acids are very effective in combating *Varroa destructor* mite in the reproductive phase, interrupting its life cycle.

According to the obtained data, a very high effectiveness of treatments (>90% mortality) was registered in four out of the nine experimental variants, at 24 h evaluation:

(1) the 15 minutes treatment of caped brood in saturated boxes with formic acid;
(2) the treatment of caped brood by brushing with formic acid of 85% concentration;
(3) the treatment of caped brood by brushing with formic acid of 65% concentration;
(4) the treatment of caped brood by brushing with a formula based on formic acid of 65% concentration and acetic acid of 80% concentration.

A good effectiveness (>85% mortality) was also registered in other two experimental variants:

(1) the 10 minutes treatment of caped brood in saturated boxes with formic acid;
(2) the treatment of caped brood by brushing with acetic acid of 99% concentration.

Both formic and acetic acids proved to be effective in saturated space, but their concentration is an important factor when used.

For the first group of treatments, a 10 minutes treatment with formic acid in closed boxes should be sufficient, but further studies could better establish the optimum time-concentration variables. The new procedure of targeted capped brood treatment by brushing could be appreciated as better as compared with saturated space procedure as it does not affect the larval open brood, being a minimally invasive procedure especially with an optimised acid concentration formula. It valorises the natural property of caps to absorb and transfer the volatile organic substances into the cells, transforming its barrier role in a support for substances.

The effectiveness of new, optimal treatment formula for interrupting the life cycle of mite could be better evaluated after several days, when the reproductive success, live status and resistance of individuals can be better evaluated.

By applying the brood treatments in the key moments of the season, even earlier in the active season, and understanding the varroa mite-honeybee colony population dynamic, the level of infestation will decrease substantially, as well as the risks of colony collapsing in the inactive season.

By using the brood treatment and having in view the formic and acetic acids' property of rapid vaporization, the honey bee colony and by-products are not exposed to contamination substances, their impact being limited only to treated combs for a very short time period.

The present approach of brood treatment could open new ways to practical, flexible, organic and cost-efficient treatments in combating varroa mite in the world-wide beekeeping, in obtaining clean hive products for daily consume or apitherapeutic use, as well as in the multifactorial studies which aim to better study and explain the honeybee colony losses.

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CORRELATION BETWEEN TOTAL PHENOL AND FLAVONOID CONTENT WITH SOME PHISICO-CHEMICAL PARAMETERS OF MONOFLORAL ROMANIAN HONEY

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Abstract

Twenty-four samples of monofloral Romanian honey (acacia, linden, rapeseed and sunflower) were analysed for their total phenolic and flavonoid contents, pH, free acidity, ash, electrical conductivity and color intensity. The analyses were performed in accordance with Romanian and EU standards and according to the methods in the literature. The results for color varied between 0.9-69.1 mm Pfund, for pH and free acidity between 3.54-4.44 and 4.7-15.7 meq kg^{-1}, respectively. The values of ash and electrical conductivity were between 0.043-0.291% and 0.12-0.55 mS cm^{-1}. The total phenolic content ranged from 14.50 mg GAE/100 g to 30.13 mg GAE/100 g while total flavonoid content ranged from 0.59 mg Q/100 g to 2.84 mg Q/100 g. The Pearson correlation analysis indicates positive significant correlations between color and total flavonoid content, ash and electrical conductivity, ash and total polyphenols content.

Key words*: correlation, honey, total flavonoid, total phenol.*

INTRODUCTION

The sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of plants or excretions of plant-sucking insects on the living parts of plants it is called honey (European Commission, 2002).
This viscous liquid is considered a complete food because contains many substances: carbohydrates, water, protein, minerals, vitamins and antioxidants (Halouzka, 2016).
The organic acids of honey give it an acidic character specific important for its preservation.
The diversity of minerals from honey contribute to the nutritional value. Many studies showed that in darker honeys there is a greater amount of minerals (De-Melo et al., 2017; Karabagias et al., 2017).
The amount of the component substances from honey depends on the environment from which it is harvested, therefore, honey is considered an environmental bioindicator (da Silva et al., 2016; De-Melo et al., 2017).
Phenolic compounds (flavonoids, phenolic acids), are responsible for the antioxidant activity of honey with favorable effects for human health (Alvarez-Suarez et al., 2010; Bogdanov, 2015).
For the antioxidant, antimicrobial and other beneficial properties in human health, honey is consumed as food or used as an ingredient (da Silva et al., 2016; De-Melo et al., 2017).
The aim of this study was to evaluate some quality parameters of honey samples and to assess the correlation between the investigated parameters.

MATERIALS AND METHODS

Twenty-four samples of monofloral honey were collected in 2017 from beekeepers at different sites in Iasi county. The six samples of each acacia (AH), linden (LH), rapeseed (RH) and sunflower (SFH) were analysed for their total phenolic and flavonoid content, pH, free acidity, ash, electrical conductivity and color intensity.
Samples were stored in laboratory at 20 ± 3°C in the dark. All analyses were done in accordance with Romanian, EU standards or according to the methods in the literature.
Shimadzu UV-mini-1240 spectrophotometer was used to determine the color of honey

samples. A 50% honey aqueous solution (w/v) was measured at 635 nm. The color of honey samples, was established after conversion of the absorbance values in mm Pfund (Table 1) (Ferreira et al., 2009; Pontis et al., 2014; Sant'ana et al., 2014).

Table 1. Pfund scale for determining color*

Color	Pfund scale (mm)
Water white	1 to 8
Extra white	8-17
White	17-34
Extra light amber	34-50
Light amber	50-85
Amber	85-114
Dark amber	More than 114

*Sereia et al. (2017)

A 10% (w/v) honey solution was prepared to determine the pH with WTW MULTI 3320 multiparameter (Bogdanov, 2009).

Free acidity was determined on a 10% (w/v) honey solution titrated with 0.1 N NaOH using TITRONIC universal-SCHOTT Instruments (Popescu & Meica, 1997; Standard Roman, 2009). The results were expressed in meq kg^{-1}.

The mineral content of honey samples was determined by calcination of samples in a muffle furnace (SUPERTHERM) at 550°C (Cantarelli et al., 2008; Popescu & Meica, 1997). The results were expressed in percentages (g/100 g).

The electrical conductivity was measured on a 20% (w/w) honey solution (dry matter basis) with WTW MULTI 3320 multiparameter (Bogdanov, 2009; Popescu & Meica, 1997). The results were expressed in mS cm^{-1}.

The total phenolic content (TPC) was determined by using Folin-Ciocalteu method, modified from Bobiș et al. (2008). The absorbance was measured at 742 nm against a blank with UV-1400 SHIMADZU Spectrophotometer. The calibration curve was made in 5 calibration points (y=0.0967x+0.083; R^2=0.9972) with gallic acid. The results were expressed in mg of gallic acid equivalents (GAE)/100 g.

Total flavonoid content was determined using aluminum chloride. The absorbance was measured at 430 nm (UV-1400 SHIMADZU Spectrophotometer). A standard calibration curve of quercetin was obtained in 5 calibration points (y=0.1326x-0.0123; R^2=0.9990). The results were expressed in mg of quercetin (Q)/100 g (Özkök et al., 2010; Pontis et al., 2014).

Analyses were made in triplicate. The data obtained were processed statistically with SPSS Statistical version 26.0. Correlations were tested by using Pearson's correlation coefficient (r) between the investigated parameters.

RESULTS AND DISCUSSIONS

Honey impressed consumers with its color, flavour and taste. The variety of colors is related to floral and geographical origin, content of polyphenols and flavonoids, storage time (Cimpoiu et al., 2013; De-Melo et al., 2017; Sereia et al., 2017).

The color intensity of all investigated honey samples is shown in Figure 2.

The results showed that the acacia samples have the lightest color-water white, with a maximum of 3.5 mm Pfund at AH1 sample (Table 2).

The most varied colors were observed for the sunflower honey samples, from white to light amber (Figure 1).

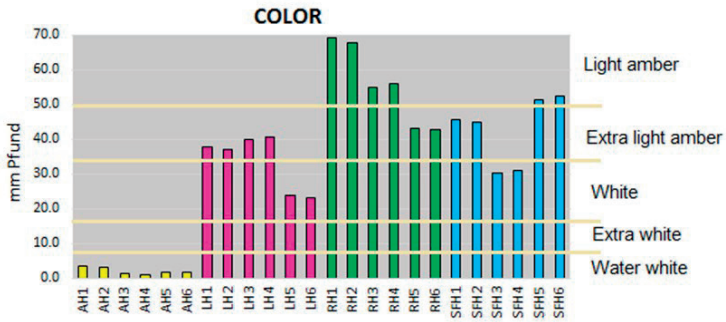

Figure 1. Color of honey samples

Table 2. Descriptive statistics of mm Pfund, pH and Free Acidity of honey samples

Type	n	Descriptive statistics	mm Pfund	Color	pH	Free Acidity (meq kg^{-1})
AH	6	Min.-Max.	0.9-3.5	water white	3.54-3.75	4.7-7.1
		Mean±SD	2.0±1.08		3.65±0.09	6.1±0.92
		CV	54.59		2.57	15.05
LH	6	Min.-Max.	23.1-40.8	white-extra light amber	3.83-4.44	11.4-13.5
		Mean±SD	33.7±8.14		4.14±0.11	12.4±0.84
		CV	24.13		6.56	6.77
RH	6	Min.-Max.	42.8-69.1	extra light amber-light amber	3.59-3.96	9.2-11.2
		Mean±SD	55.7±20.51		3.82±0.18	10.1±0.83
		CV	9.15		4.69	8.22
SFH	6	Min.-Max.	30.3-52.3	white-light amber	4.00-4.07	14.4-15.7
		Mean±SD	44.8±12.06		4.04±0.04	15.00±0.42
		CV	26.92		0.95	2.78

n - no. samples; SD - standard deviation; CV - coefficient of variation

The color in honey samples varied from 0.9 mm Pfund in acacia sample (AH1) to 69.1 mm Pfund in rapeseed sample (RH1) (Figure 1). The highest mean value of intensity of color was found for the rapeseed honey type, of 55.7 mm Pfund (Table 2).

Honey is an acidic food. pH values are found in the range 3.54-4.44. Acacia honey samples recorded the lowest pH value (Table 2). The high values of free acidity indicate a fermentation process. Maximum allowed value accepted and request in legislation is 50 milliequivalents acid per 1000 g (European Commission, 2002). The highest mean value of free acidity of 15.0 meq kg^{-1} was found for the sunflower honey (Table. 2).

Low values of pH and free acidity inhibit the growth of microorganisms, ensure product stability and a longer storage time (Pascual-Maté et al., 2018; Popescu & Meica, 1997).

The lowest mean value of ash of 0.060% was found for acacia honey samples and the highest mean value of ash was found for linden honey samples, 0.240% (Table 3).

Table 3. Descriptive statistics of ash, electrical conductivity, total polyphenols content and total flavonoids content of honey samples

Type	n	Descriptive statistics	Ash (%)	EC (mS cm^{-1})	TPC (mg GAE/100g)	TFC (mg Q/100g)
AH	6	Min.-Max.	0.043-0.076	0.12-0.16	14.50-16.80	0.59-0.79
		Mean±SD	0.060±0.01	0.15±0.02	15.65±0.93	0.66±0.07
		CV	20.94	10.64	5.96	10.68
LH	6	Min.-Max.	0.176-0.291	0.43-0.55	26.12-30.13	1.93-2.59
		Mean±SD	0.240±0.04	0.50±0.05	28.41±1.77	2.32±0.28
		CV	18.71	10.54	6.24	12.09
RH	6	Min.-Max.	0.046-0.100	0.18-0.21	17.02-25.51	1.85-2.52
		Mean±SD	0.074±0.02	0.19±0.02	21.26±3.72	2.20±0.27
		CV	26.05	8.43	17.51	12.39
SFH	6	Min.-Max.	0.155-0.278	0.40-0.47	19.24-28.05	1.83-2.84
		Mean±SD	0.213±0.05	0.42±0.043	24.02±3.95	2.39±0.45
		CV	24.62	6.08	16.46	18.66

n - no. samples; EC - electrical conductivity; TPC - total polyphenols content: TFC - total flavonoids content; SD - standard deviation; CV - coefficient of variation

Acacia samples registered the minimum mean value of electrical conductivity of 0.15 mS cm^{-1} and the maximum mean value of 0.50 mS cm^{-1}. All the values do not exceed the recommended limit value (0.8 mS cm^{-1}) (European Commission, 2002) and indicate the floral origin of investigated honey samples.

The climatic conditions, the botanical origin of honey, the season determined the content of polyphenolic compounds that give antioxidant properties of honey (Soares et al., 2017).

Polyphenolic compounds are mainly responsible for the antioxidant properties of honey. The content of these compounds depends on season, climatic conditions and mostly on the botanical origin of honey (Soares et al., 2017). The lowest value of total phenolic content was 14.50 mg GAE/100 g for one acacia honey sample and the highest value of 30.13 mg GAE/100 g was found for a linden honey sample (Table 3). The total flavonoid content ranged from 0.59 mg Q/100 g to 2.84 mg Q/100 g, with the highest value for sunflower honey (Table 3).

Similar studies on honey samples on the same parameters, from different countries, showed various values (Tables 4 and 5).

Table 4. Some parameters of acacia, linden, and polyfloral honey in literature

Country	pH	Free acidity (meq kg^{-1})	Ash	EC (mS cm^{-1})	References
Acacia					
Serbia	3.49-5.85	7.8-29.6	-	0.1-0.68	Lazarević et al., 2012
Romania	3.65-4.63	1.84-10.87	-	0.097-0.35	Stihi et al., 2016, Mărghitaș et al., 2010; Popescu et al., 2015, Scripcă et al., 2019
Poland	3.79	25.6	-	0.42	Tomczyk et al., 2019
Slovakia	3.71	16.1	-	0.20	Tomczyk et al., 2019
Linden					
Poland	3.81-4.13	14.5-34.2	-	0.53-0.579	Kędzierska-Matysek et al., 2018; Tomczyk et al., 2019
Serbia	3.98-5.40	8.2-26.2	-	0,3-0,76	Lazarević et al., 2012
România	3.6-4.7	-	0.186	0.20-0.73	Stihi et al., 2016; Popescu et al., 2015; Purcarea et al., 2016
Slovakia	3.90	21.6	-	0.23	Tomczyk et al., 2019
Rapeseed					
Slovakia	-	19	0.516	-	Kasperová et al., 2012
Romania	3,91-3,93	-	0.159-0.163	-	Stihi et al., 2016
Romania	4,22	16	0.162	-	Pauliuc et al., 2020
Sunflower					
Portugal	3,84	25.5	0,235	0.15	Aazza și colab., 2013
Poland	3,96	18.5	0.361	-	Kędzierska-Matysek et al., 2018
Serbia	3,17-4,14	11-42.7	0.19-0.55	-	Lazarević et al., 2012

EC=electrical conductivity

Table 5. Total polyphenols and flavonoids content of acacia, linden, rapeseed and sunflower honey in literature

Country	TPC (mg GAE kg^{-1})	TFC (mg QE kg^{-1})	References
Linden			
Czech Republic	450.37-730.09	18.79-35.61	Halouzka et al., 2016
Romania	160-380	47-69.8	Mărghitaș et al., 2009
Slovakia	350	2.57	Tomczyk et al., 2019
Acacia			
Czech Republica	238.36	8.73	Halouzka et al., 2016
Romania	120-260	8.4-32	Mărghitaș et al., 2010
Slovakia	200	1.37	Tomczyk et al., 2019
Rapeseed			
Czech Republic	94.3-119.2	5.8-6.1	Lachman et al., 2010
Romania	199	202	Pauliuc et al., 2020
Slovakia	210	2.16	Tomczyk et al., 2019
Sunflower			
Portugal	366.9	19.3	Aazza et al., 2013
Turkey	776.4	-	Gül & Pehlivan, 2018
Romania	200.0-450.0	115.3-153.3	Mărghitaș et al., 2009
Turkey	309.2-690.8	-	Özkök & Silici, 2018
Romania	8-16	-	Cimpoiu et al., 2013
Romania	211	228	Pauliuc et al., 2020

TPC= total polyphenols content, TFC= total flavonoids content

Principal Component Analysis of honey samples is presented in Figure 2. The samples of honey bee grouping on the type according to the studied parameters is highlighted. The best visual is the acacia honey samples group (Figure 3) by forming a well-highlighted cluster.

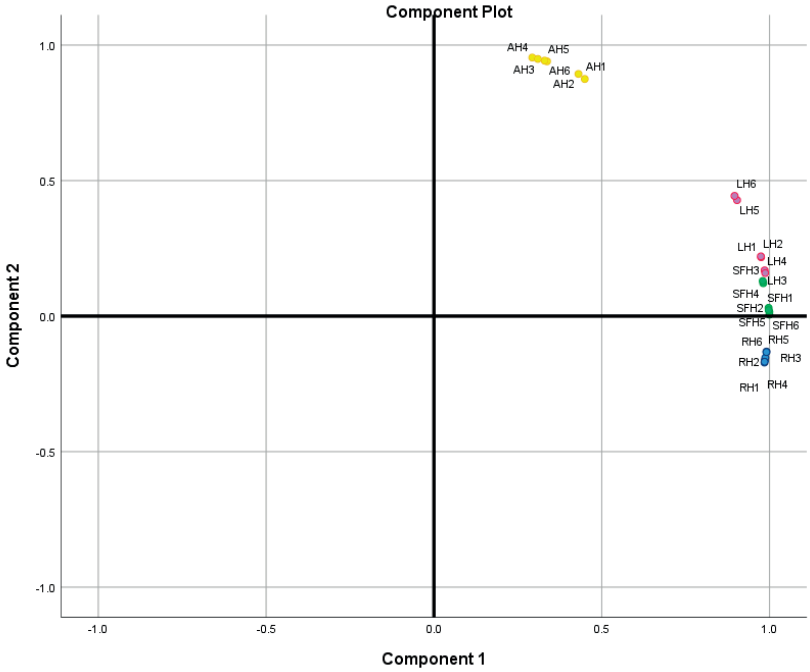

Figure 2. Principal Component Analysis of honey samples

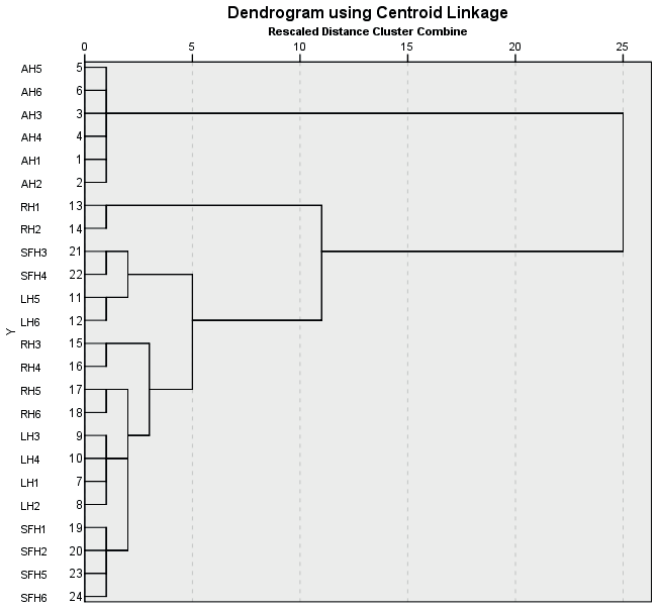

Figure 3. Hierarchical cluster analysis based studied honey parameters

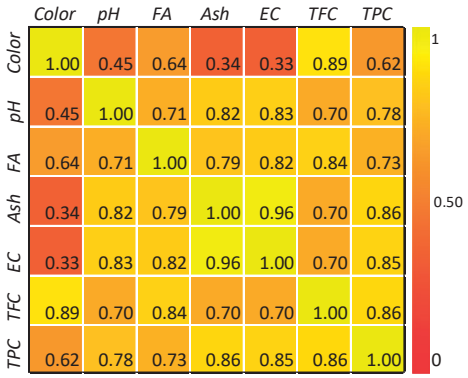

Figure 4. Pearson correlation coefficients of honey samples parameters

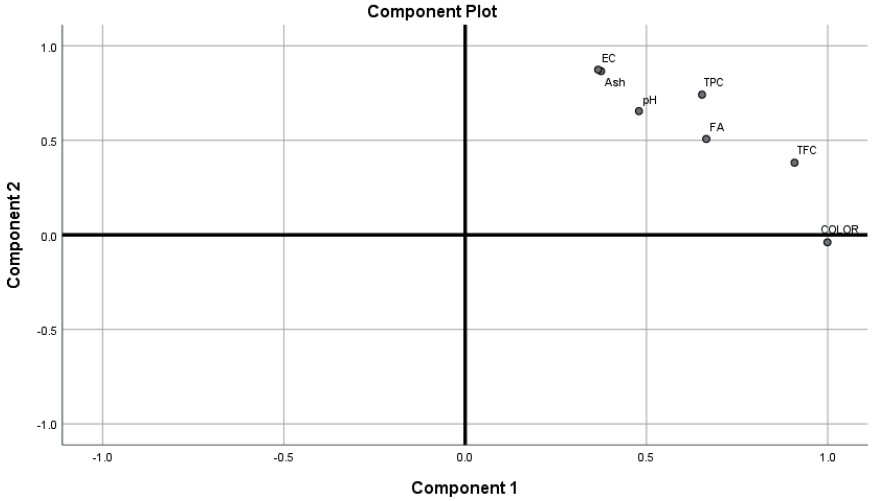

Figure 5. Principal Component Analysis of TPC, TFC and some physicochemical parameters of honey samples

Pearson correlation coefficients between the investigated parameters of twenty four honey samples is shown in Figure 4. The results of Pearson coefficient show strong positive linear correlations between ash content and electrical conductivity (r = 0.96) (Figure 5). A strong positive linear correlation (r = 0.89) was found between mm Pfund and total flavonoids content, between ash and total phenolic content, between total phenolic content and total flavonoids content, respectively (r = 0.86). Correlation Pearson value of 0.62 indicate a medium positive correlation between color and total phenolic content, of 0.64 between color and free acidity. Low positive correlation was found between pH and color (r = 0.45) and color and electrical conductivity (r = 0.33) (Figure 4) as represented in Figure 5.

Several investigations on different honey samples showed correlations between physicochemical parameters. Pontis et al. (2014) showed strong correlations between total phenolic content and color (r = 0.967), total flavonoids content and color (r = 0.924) and between total phenolic content and total flavonoids content (r = 0.926). Al Farsi et al. (2018) found strong correlation between same honey parameters, with Pearson coefficient values of 0.974, 0.999 and 0.977, respectively. Pearson coefficient of 0.75 between ash and electrical conductivity was reported by Ahmida et al. (2013); total flavonoids content showed significant correlation with color (r = 0.82) as shown by Almeida et al. (2016). High linear correlation value of 0.8569 and of 0.963 was obtained by Cimpoiu et al. (2013) between total

phenolic content and the color intensity in Romanian honey and by Kek et al. (2014) in malaysian honey, respectively.

CONCLUSIONS

Strong positive correlations are noticed between ash and electrical conductivity, color and total flavonoid content, between total phenolic content and total flavonoid content.
Medium positive correlations were found between color and free acidity, color and total flavonoid content.
Some parameters are not significantly correlated, with Pearson coefficients below 0,30: color with pH, ash, and with electrical conductivity, respectively.
The quality of honey bee depends on the geographical area, the floral origin, the climatic conditions but also on the health of the bee family.

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FUNGI AND MYCOTOXINS CONTROL OF WHEAT GRAINS USING ESSENTIAL OILS

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Abstract

The paper aims to study the antifungal and antimycotoxigenic effect of some essential oils (garden thyme, oregano, coriander, dill and fennel) on wheat seeds. In order to test the protective effect associated with treatment with essential oils (EOs), wheat seeds naturally contaminated with deoxynivalenol (DON) were sprayed with different concentrations of EOs, and after 7 days and 14 days the seed contamination index (SCI), fungal genera and DON content were determined. The obtained results showed that the seed contamination index (SCI), after one week of treatment, is higher than control in case of fumigation of wheat seeds with oregano and fennel essential oil and lower than control in case of coriander, thyme and dill essential oils. The predominant fungal species in this phase are: Fusarium, Cladosporium and Rhizopus. Two weeks after treatment, it is observed that the treatment with essential oils provides fungal protection. SCI is maximum in the case of control and the potential to inhibit micellar colonization increases in the order: fennel <dill = oregano <coriander <thyme. With the exception of dill oil, which did not reduce Fusarium contamination, the other essential oils provide a significant reduction in the number of seeds contaminated with this type of fungus. Regarding the antimycotoxigenic effect, the level of DON decrease after treatment with essential oils, in all experimental variants tested, the decrease being more pronounced after 14 days after treatment, compared to 7 days.

Key words*: Coriander, Deoxynivalenol, Dill, Fennel, Oregano, Thyme.*

INTRODUCTION

Infection of cereal grains by fungi is a serious problem worldwide, phenomenons that reduces yield, quality and nutritional value of cereals and develop the production of mycotoxins that are harmful to both humans and animals (Petcu et al., 2019). Preventing contamination with pathogens through the use of essential oils is considered a viable non-polluting strategy to reduce the risks associated with mycotoxin contamination of processed food and feed.

Previous studies draw attention to the functionality of essential oils (EOs) of medicinal plants in term of fungal contamination of cereals studied and proven by researches in this regard (Naeini et al., 2010; Isman et al., 2000; Quiroga et al., 2001; Sumalan et al., 2013). The inhibition potential of some EOs against natural mycoflora and *Fusarium* mycotoxins production has been associated with the chemical composition, monoterpenic phenols, especially thymol, carvacrol and eugenol in the oils (Soliman et al, 2002; Lee et al., 2007).

The EOs of *Thymus vulgaris* (TEO) and *Coriandrum sativum* (CEO) inhibited the growth both for filamentous fungi and yeasts that are involved in the postharvest spoilage of wheat seeds. After 5 days of treatment CEO, SCI were maximum (33.33%) for a level of 500 ppm and decreases (30%) for a level of 1000 ppm, respectively at 20% for a level of 2000 ppm (Sumalan et al., 2009).

Regarding the *Thymus vulgaris* oil (TEO) the SCI values were in the range 36.67-20% depending on the applied level (Alexa et al., 2018). Similar results on the antifungal effect and complete inhibition of the growth of *Aspergillus flavus, Fusarium osysporum, Curvularia lunata, Aspergillus terreus, Aspergillus fumigantus, Alternaria and*

Cladosporium sp. of TEO have been reported previously (Dambolena et al.; 2008, Kumar et al., 2008).
Other in vitro study (Negrea et al., 2018a) highlighted the fungistatic and fungicidal effect of oregano essential oil (OEO) against *Fusarium graminearum* at lower concentrations: minimum concentration with fungistatic effect - 0.06%, minimum concentration whit fungicidal effect - 0.1% and fungicidal concentration - 0.2%.
High volatility, absence of toxicity and especially their antimicrobial effects recommend the use of natural preparations based on EOs in organic agriculture and horticulture.
The aim of this study is to analyze the antifungal and antimycotoxignic effect of different essential oils: garden thyme (TEO), oregano (OEO), coriander (CEO), dill (DEO) and fennel (FEO) on wheat seeds in order to use these natural compounds as protective agents in grain warehouses. Also, the effect of treatment on wheat germination was tested.

MATERIALS AND METHODS

Sample preparation

For the experiment Antille variety wheat obtained by ecological technology was used. In order to test the protective effect associated with the treatment with EOs, 50 g of wheat sample (with a known DON concentration-0.464 ppm) were sprayed with different concentrations of EOs and after 7 days and 14 days were analyzed to detect the seed contamination and DON content.
The moisture content of the sample was 12.41% and the water activity index (a_w) was 0.9.
The oils used were: garden thyme (TEO) - 0.03%; oregano (OEO) - 0.03%; coriander (CEO) - 0.2%; dill (DEO) - 0.2%; fennel (FEO) - 0.2%. The concentrations were selected after the in vitro test regarding minimum inhibitory concentration (MIC) of each EOs (Negrea et al., 2018a; Negrea et al., 2018b).

Determination of seed contamination index (SCI) and identification of fungal genera

The observations were made after 7 and 14 days by estimating the degree of seed germination and the seed contamination index (SCI) for each treatment variant following the procedure of Doolotkeldieva et al. (2010).

$$\text{SCI}\ (\%) = \frac{\text{contaminated seeds}}{\text{total seeds}} \cdot 100$$

Identification of fungal genera

The identification of fungus genera has been performed according to Hocking et al. (2006).
The frequency of occurrence of the fungal genera (Fr) was calculated using formula (Doolotkeldieva, 2010):

$$\text{Fr}\ (\%) = \frac{\text{number of seeds with fungal genus}}{\text{total number of seeds}} \cdot 100$$

Determination of mycotoxins:

The method used was the enzyme-linked immunosorbent assay (ELISA), and sample preparation was performed in accordance with the manufacturer's instructions for analysis of deoxynivalenol (DON) in cereals (R-Biopharm). The ground sample (5 g) was extracted with 100 ml of distilled water and homogenized using a stirrer 20 min for extraction. The extract was filtered, and 1 ml of the filtrate was used directly for enzymatic analysis. Standard solutions and samples (50 µl) are mixed with 50 µl enzyme conjugate in individual cells from the ELISA kit plates. 50 µl of antibody solution are added and incubated for 10 minutes at room temperature. The cells were washed three times with 250 µl of distilled water, then the substrate (100 µl) was added and incubated for another 5 minutes at room temperature. The stop solution (100 µl) was added to each cell and the yellow color intensity was measured at 450 nm using an ELISA 96 reader (PR-1100, Bio-Rad Laboratories, USA).

Determination of wheat germination

In order to study the effect of treatment with EOs against wheat germination, wheat samples were sterilized by using sodium hypochlorite solution (1:9), and washed two times with distilled water. A total of 10 seeds were placed in a Petri dish. Then, the seeds were treated with each EOs in the same concentrations used for antifungal experiment. The Petri dishes were kept at 25◦C into the dark. After seven days, the number of germinated wheat seeds was recorded. A seed was considered

germinated when the radicles was elongated up to 3 mm (Alexa et al., 2018). The number of germinated seeds was divided to the number of seeds used (10) and that multiplied with 100, resulting the percentage of germinated seeds (GS).

RESULTS AND DISCUSSIONS

Seed contamination index (SCI) and identification of fungal genera

Fungal seed colonization index (SCI) after 1 week and after 2 weeks of fumigation with essential oils (EOs) is presented in Figure 1.

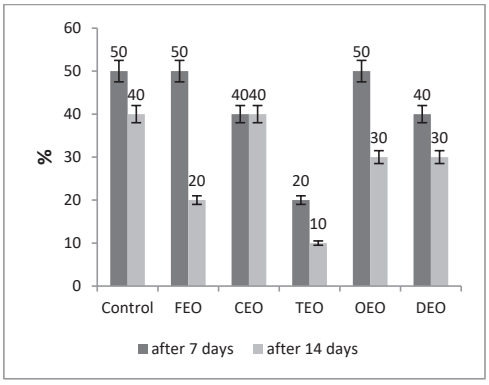

Figure 1. SCI (%) after 7 days and 14 days after fumigation with EOs

Figure 1 shows that SCI after one week of treatment is as the control in case of fumigation of wheat seeds with OEO and FEO and lower than the control in case of use of CEO, DEO and TEO. The lowest SCI (20%) was recorded in the case of TEO treatment. The inhibition potential of micellar colonization increases in the order: FEO < DEO = OEO < CEO < TEO. After 14 days, the SCI decrease for all samples, excepting CEO. The SCI was 30% when DEO and OEO was applied, 20% for FEO and 10% for TEO. The results highlighted the maximal antifungal potential of TEO after 14 days of treatment.

The inhibition capacity of TEO was proven even after 5 days of treatment when the growth of molds in wheat samples was inhibited in the range of 36.67-20% depending on the applied level (Sumalan et al., 2013). Similar results on the antifungal effect of TEO have been reported by Dambolena et al. (2008) and Kumar et al. (2008).

The inhibition rate depends on applied concentration of EOs. In the case of treatment with CEO, after 5 days, SCI values were 33.33% for a level of 500 ppm, 30% for a level of 1000 ppm and 20% for a level of 2000 ppm (Sumalan et al., 2013).

The predominant fungal species after 7 days of treatment with EOs are: *Fusarium, Cladosporium and Rhizopus* (Figure 2). Two weeks after the treatment, it was observed that the treatment with EOs provides fungal protection. At this stage, the predominant fungal species is *Alternaria* and fungal strains *Aspergillius, Penicillium and Fusarium* are also found in control. With the exception of DEO, which did not reduce *Fusarium* contamination, the other analyzed EOs provides a significant reduction in the number of seeds contaminated with this type of fungus.

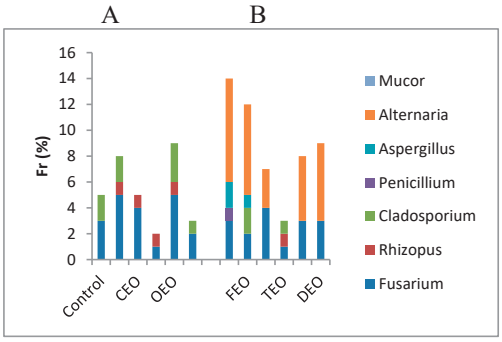

Figure 2. The frequency of occurrence of the fungal genera (Fr) after 1 week (A) and after 2 weeks (B) of fumigation with essential oils (EOs)

TEO was proven its maximal antifungal potential after 14 days of treatment, followed by CEO, OEO, DEO and FEO.

The antifungal potential of TEO has been previously demonstrated by our research team against *Fusarium graminaerum* (Alexa et al., 2018). Also, the antifungal effect of TEO against *Verticillium dahliae, Fusarium* sp., *Penicillium* sp., *and Aspergillius* sp. was reported (Arslan et al., 2010; Kocic-Tanackov et al., 2013).

The results are in accord with previously data reported in literature. In this regard, other study highlighted that the treatment with CEO doesn't inhibit the occurrence of fungi such as *Alternaria, Fusarium, Aspergillus and Hyphopichia*. Only at high concentration

(2000 ppm) it was noticed to decrease the fungus frequency (Sumalan et al., 2013). Opposite, TEO exhibited a broad spectrum fungitoxicity against: *Fusarium, Cladosporium and Aspergillus* (Sumalan et al., 2013), *Aspergillus flavus, Fusarium osysporum, Curvularia lunata, Aspergillus terreus, Aspergillus fumigantus, Alternaria and Cladosporium s*p. (Kumar et al., 2008) and *Aspergillus niger, A. ochraceus and A. flavus* (Bluma et al., 2008) by treatment with different concentrations of TEO.

DON inhibition

Figure 3 shows the level of DON contamination after 7 and 14 days of treatment. It can be noted that the DON concentration is lower as in Control for all analyzed samples. The initial level of DON was 0.464 ppm and decreased in control, after 7 days at 0.282 ppm, while in the wheat samples with EOs treatment the concentration of DON is lower (between 0.141-0.199 ppm). The higher antimicotoxigenic potential was proven by DEO, followed by FEO, TEO, CEO and OEO. After 14 days of treatment the inhibition effect of EOs against DON increased. The profile of DON was same as after 7 days of treatment and highlights the maximal antimicotoxigenic potential of DEO (0.027 ppm) and de minimal effect when OEO was applied (0.118 ppm). The results shown that the EO with the highest antifungal effect is not the one with the highest antimicotoxigenic effect. TEO was proven to inhibit the fungal activity but had a moderate action in terms of DON inhibition.

Responsible for the antifungal effect of EOs can be the changes induced on the fungal morphogenesis and fungus growth produced by the chemical compounds of EOs (Rassoli et al., 2006). The effect of major chemical compounds on antifungal activity varies as follow: phenols > alcohols > aldehydes > ketones > ethers > hydrocarbons (Singh et al., 2012).

Figure 3 shows that the level of DON contamination decreases after treatment with EOs, in all experimental variants tested, the decrease being more pronounced after 14 days of treatment, compared to 7 days. The maximum efficiency is observed in the case of treatment with EOs from the *Umbelifaere* family (DEO> FEO> CEO), followed by oils from the *Lamiaceae* family (TEO> OEO). The reduction of DON contamination of wheat seeds is 29.43-50.52% after 7 days of treatment, respectively between 55.47-89.81% after 14 days of treatment.

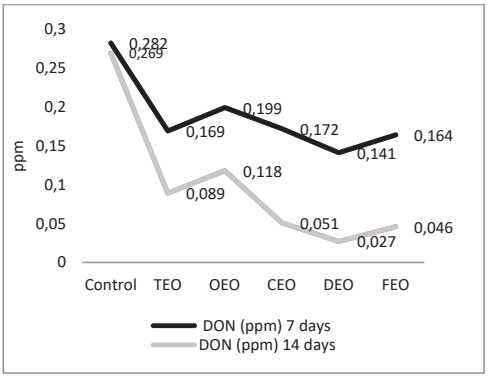

Figure 3. DON content (ppm) after 7 days and 14 days of treatment with EOs

Seeds germination

The effect of EOs on seed germination is presented in the Figure 4. It can be seen 10% non-geminated seeds in the case of treatment with CEO, OEO or FEO and 20% when DEO was applied on wheat control.

The effect of TEO on wheat germination reported in previous study (Alexa et al., 2018), highlighted that 0.3-1% TEO inhibited 40% of seed germination. The use of lower concentration of TEO (0.03%), concentration that was proved with antifungal effect, don`t affect the wheat germination. This aspect is useful in choosing the concentrations used in the field in the protection of agricultural crops, so that the antifungal effect is maximum and the germination of seeds is not affected. Regarding other EOs analyzed, the tested concentrations with antifungal effect, leads to a germination seed coefficient (GS) over 80%, without significant effect on wheat germination.

Previous study regarding the effect of FEO on wheat seeds germination shows that the rate of inhibition varies between 88.88-61.11%, depending on the concentration level (1-0.3%) (Negrea et al., 2018b). The lower concentration used in our study (0.2%), that proven a fungicidal effect, inhibited just 10% of seed in term of germination.

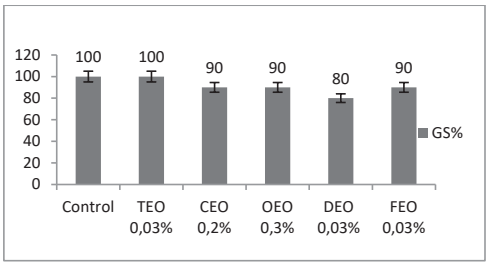

Figure 4. The effect of EOs treatment on seeds germination

CONCLUSIONS

Our results highlight the antifungal potential of EOs, especially those of the *Lamiaceae* family (garden thyme and oregano), and the antifungal effects against DON exhibited by EOs from *Umbeliferae* family (dill and coriander).
Wheat germination capacity is not affected by treatment with EOs, that recommends them as potential natural antifungal and antimycotoxigenic agents with applicability in agriculture and protection of cereals and plant raw materials used in the food industry.

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PRODUCT DEVELOPMENT OF ORGANIC MACARONS ENRICHED WITH FREEZE DRIED APPLE POWDER

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Abstract

The object of the study is to realize on and test organic apple powder in unconventional foods, in order to increase the nutritional and sensory quality. The innovative product is a macarons reformulated, with high nutritional value, with addition of husked hemp seeds and organic freeze dried apple powder. Organic freeze dried apple powder obtained from apples Gala variety and is characterized by a large amount of ascorbic acid and polyphenols, with high antioxidant potential. The purposes of this study were to study the novel recipe of macarons with hemp seeds husked and freeze dried apple powder, to study the consumer acceptance, the nutritional values of macaron and the production cost of the standard recipes. The results showed that the sensory quality evaluation of the recipe shows a very good appreciation of 3 attributes (appearance, flavour and smell, taste), which had average score of 4.43. The product is a sweet, fragrant and aerated dessert, which can be easily associated with different foams (mousse, creams, ice cream, etc.). The calculation of the nutritional value it was realised with a specialised soft.

Key words*: antioxidant activity, freeze dried, organic powder, sensory analyses, stability test.*

INTRODUCTION

The consumer has become more and more attentive to the quality and functional role of the food they consume. Prefer products from sustainable or organic farming and processing so as to respect the environment. Fruits and vegetables from organic farming are very important source of ingredients for obtaining healthy foods with high nutritional value (Dragomir et al., 2019b).

Apple is one of the important fruit crops known to mankind and is produced all over the world in the temperate climate (FAO, 1989). Most of the production of the fruit is used for snack lunch, purpose but a portion is being processed into various products of which apple juice is processed a preponderance or other food products (Nakov et al., 2020). Should be given more importance apple peels, a by-product of the food industry, is rich in fiber, polyphenols and minerals and is a potentially attractive ingredient for bakery products (Nakov et al., 2020). Organic apples have a high content in polyphenols, compounds which are recognized to have multiple biological activities and various health benefits as potential agents for preventing and treating many oxidative stress-related diseases, such as cardiovascular diseases, cancer, ageing, diabetes mellitus and neurodegenerative diseases (Bădulescu et al., 2019; Bujdei et al., 2019).

Organic apples are high quality and could be used to obtain natural value added powders by lyophilization, in order to preserve most of these valuable compounds inside. Drying of apple is an effective method to reduce its bulk and to extend the shelf life. Low moisture content of apple powders is important for maintaining good storage stability by preventing deteriorative reactions because of high water activity (Sahni & Shere, 2017; Sahni & Shere, 2018). Owing to the pleasant fruity odor, Sudha et al. (2007) also regarded apple powders as a potential flavoring ingredient in cake products, which needs more experiments to implement at the commercial scale (Fengzhi et al., 2020).

Bakery products are liked by people of all age groups and include a wide variety of products like cakes, breads, biscuits etc. Since demand and acceptability for bakery products is more,

they can be used as a vehicle for fortification and enhancing the nutritional quality. Bakery products like cakes are rich in starch, fat and energy but depleted of fiber (Singh, 2016; Dragomir et al., 2020a; Dragomir et al., 2020b). All these new products obtained from organic apples show a high potential to be used as functional ingredient and can be used to fortify organic food products in order to increase their nutritional and their antioxidant potential (Badulescu et al., 2019; Dragomir et al., 2019a).

MATERIALS AND METHODS

In this paper we want to use and teste of organic freeze dried apple powder, like a additive, in novel pastry foods, in order to increase the sensory and nutritional value. The innovative product is macarons reformulated.
Organic apples powder is naturally sweet, high in fiber, and a rich source of antioxidant compounds known as polyphenols. Also, it contains high levels of magnesium, potassium, zinc, and Vitamin C, and its high fiber content is beneficial for optimal digestive health. The compatibility of organic apple powders for replacement of wheat flour or like a new ingredient, coupled with the consumer acceptability of sensory characteristics provide new insight for use of apple powders as a value-added food ingredient for muffins, other bakery products or selected functional foods and nutraceuticals (Rupasinghe et al., 2009; Lauková, 2011).

Materials

Organic ingredient used in study is *organic apple powder*, obtained organic Gala variety, which was dehydrated by the lyophilisation process. The powder was obtained from peel, pulp, and mixture of both and their characterization is comprised most the antioxidant ability and free radical scavenging capacity, with correlation with content of polyphenolics and ascorbic acid, according to (Li et al., 2014; Bădulescu et al., 2019). Drying using low temperatures represent a simple and easy way for minimally processing of organic fruits, moreover this procedure is accepted in organic agriculture (Stan et al., 2020).

Organic freeze dried apple powder is an excellent ingredient to add natural sweetness, flavor, acidity and fiber in each recipes ranging from breakfast cereals, baked goods, cake mixes, pastries, savory dishes, and fruit sauces to desserts.
In order to highlight the sensory changes of the addition of organic apple powder formed two samples, respectively:

Table 1. Codes used for samples analysis

Code	Sample
S0	Macarons with peeled hemp seeds;
S1	Macarons with peeled hemp seeds and enriched with 1% organic freeze dried apple powder.

The organic ingredients used were obtained at the Research Center for Studies of Food Quality and Agricultural Products from USAMV Bucharest, within the SusOrgPlus project: Intelligent food processing chains, natural additives and colourants, which aims to develop advanced processing technologies for organic products and by-products.

Methods

The innovative product is macarons reformulated, with high nutritional value, with husked hemp seeds and enriched with organic freeze dried apple powder.
The methods used in order to develop and characterized the products analysed were:

- Recipe and product development;
- Determining consumer acceptance - 5-point Hedonic evaluation scale;
- Sensory determination during storage;
- Determination of the total content of polyphenolic compounds by the Folin-Ciocâlteu method;
- Determination of antioxidant activity using the DPPH method;
- Nutrient Content

RESULTS AND DISCUSSIONS

1. Recipe and product development

The macarons reformulated, with husked hemp seeds and enriched with organic freeze dried apple powder, called BIO PRICONELA, a name used in the following.

The recipe for macarons with hemp seeds peeled and enriched with freeze-dried organic apple powder is as follows: unrefined brown sugar 49.3%, egg white 37%, peeled hemp seeds 12.4%, salt 0.3%, 1% freeze dried apple powder (Table 2).

Table 2. Recipe used for samples analysis

Code	Sample
S0	Unrefined brown sugar 49.3%, egg white 37%, peeled hemp seeds 12.4%, salt 0.3.
S1	Unrefined brown sugar 49.3%, egg white 37%, peeled hemp seeds 12.4%, salt 0.3%, 1% freeze dried apple powder.

All ingredients, except the organic apple powders, were purchased from retail specialty stores with organic products. Organic apple powders were obtained in the framework of the SusOrgPlus project at the Research Centre for Studies of Food Quality and Agricultural Products, from USAMV Bucharest. The BIO PRICONELA product was obtained in the Bakery Pilot Station of the Faculty of Animal Productions Engineering and Management, from USAMV Bucharest, within the SusOrgPlus project support.

Technological information. The technology is based on the use of heat-treated egg white and processed in the form of meringue, to which has been added hemp seeds husked and freeze dried organic apple powder.
Organic apple powder, used in the study, is a fine, special and aromatic powder. It has a high tendency to hydrate and form agglomerations if stored in unsuitable conditions.
The powder was added before foaming and after foaming. It has been observed that it solubilizes and incorporates better during the processing of egg whites.

Products develop. The technological process of obtaining the product includes the following steps:

- *Thermic treatment*: The egg white, unrefined brown sugar and freeze dried apple powder are heated under continuous mixing to a temperature of 70°C. Swiss meringue is product obtained of egg white, which together with the sugar, is heat treated at temperature of 70°C and then frothing. The foam is firm, stable and can include various other ingredients (example: seeds, nuts) that favor the obtaining of a varied assortment range.
- *Foaming*: The mixture obtained is placed in the planetary mixer tank and foamed for 20 minutes, foam must be: white, consistent, stable and shiny;
- The peeled hemp seeds are introduced in the egg white foam, by light mixing with a spatula;
- *Forming*: Macarons are forming using a cream steel nozzle with star form;
- *Bake* at 100°C for 150 minutes
- The pieces are cooled to room temperature

The pieces lost their volume very easily, after being taken out of the oven (Figure 1).

Figure 1. BIO PRICONELA before baking (left) and after baking (right) (Original photo)

The product has special sensory characteristics, noting the high palatability, due to the final note of nuts, combined with the sweet-sour taste of apple. The pieces are brown, crispy on the outside and slightly gummy on the inside (specifically for this type of dessert), airy / frothy, dry, fine and light (typical for Swiss meringue).
The brown color of macarons is due to the use of brown sugar and husked seeds from the recipe. The taste is sweet, sour aromatic due to the dehydrated apple powder and towards the end with a note of nutmeg, taste given by the presence of hulled hemp seeds.
BIO PRICONELA enriched with dehydrated apple powder is sweet, airy, flavored dessert that can be easily associated with different foams (mousse, cream, ice cream, etc.).
It is recommended to store in hermetically sealed packaging and kept at room temperature, without high humidity fluctuations.

2. Consumer acceptance

The sensorial evaluation of product was carried out in order to observe the impact of organic

apple powders incorporation, on its sensory characteristics.

The sensory properties (appearance, taste, color, flavor and smell, texture and overall acceptability) of fresh prepared were evaluated by 30 panellists. The group panellists, with different ages, were chosen to determine the level of acceptance of macarons enriched with organic apple powder.

The members of the group of evaluators were asked to evaluate the sensory characteristics and to rate the products using a 5-point Hedonic evaluation scale, with appropriate descriptive terms ranging from "1 - Dislike Very Much" to "5 - Like Very Much" to indicate their preference. Sensory tests were performed taking into account: appearance, taste, color, flavor and smell, texture and overall acceptability

To achieve the sensory profile, the evaluator completed a form for each test. After scaling the average values of the 5 attributes and their representation on a spider diagram, the following representation was obtained.

Consumer acceptance testing was performed in the Sensory Analysis Laboratory of the Research Center for Studies of Food Quality and Agricultural Products, USAMV Bucharest.

Following analysis, consumer acceptance was very good in all age segments, the new product being to the liking of consumers and consumer acceptability. The score obtained for each attribute was processed, and the average values are presented in Figure 2.

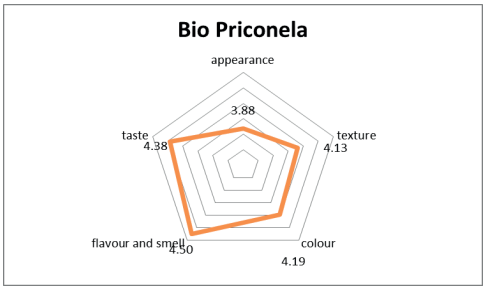

Figure 2. Consumer acceptability scores on a 5-point Hedonic scale for BIO PRICONELA

After establishing consumer acceptability, the product was especially appreciated for its flavor and smell attributes. For the general acceptability, an arithmetic mean of 4.04 was obtained.

3. Sensory determination during storage

The influence of organic ingredients on the sensory quality of the tested products was evaluated by the intensity of followed attributes: appearance, taste, color, flavor and smell (aroma and retronasal), texture and overall acceptability, regarding its behavior in keeping and storage conditions.

In order to highlight the sensory changes of the addition of organic apple powder, 2 batch from which two samples were formed 2 samples (taken at random), respectively:

- S0 - control sample;
- S1 - BIO PRICONELA.

The BIO PRICONELA products were packed in bags with a watertight closure system, and stored in rooms with room's temperature and humidity constant. The samples tested during the sensory analysis were randomly selected samples from each of the 2 batch of products obtained in the Pilot Station.

The samples were evaluated with a time interval of 7 days, for 60 days. Averages were made of the values recorded on the attributes of each evaluator, for each product. Finally, they were obtained for each relevant attribute in assessing the sensory differences between the products.

3.1. *Sensory analysis*

Prior to sensory analysis sessions, samples were tempered for 30 minutes at a sensory analysis room temperature (25°C).

After the sensory evaluation, carried out at the laboratory level, it showed that the products with apple powder was characterized by fruity and sweet taste, with fruits smell, S1 firmer than the S0, samples without apple powde

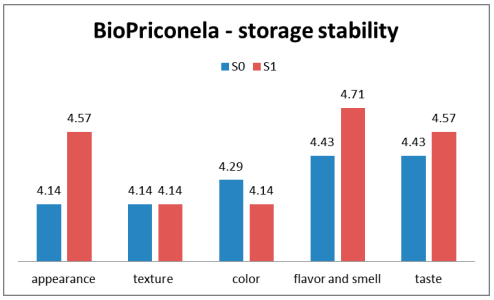

Figure 3. Graphical representation of the results obtained from the sensory analysis for the two samples: control (S0) and sample- Bio Priconela (S1)

According to the obtained results, there is a very good appreciation for 3 attributes (appearance, aroma, taste). The analyses sample S1 test, presented a better preservation of the characteristics over time was observed compared to S0, and taste and flavor were present throughout the analysis (Figure 3).

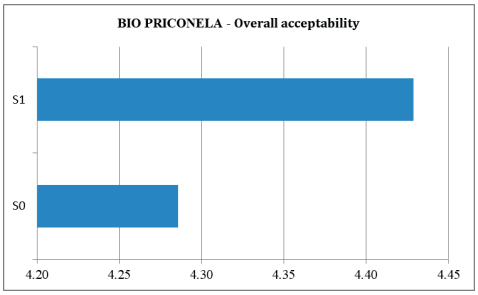

Figure 4. Graphic representation of the total score for the overall acceptability of the BIO PRICONELA product

At the stability evaluation, the overall acceptability in the case of the sample S1 received an average value of 4.43 and S0 a average value of 4.29 (Figure 4).
It can be concluded that BIO PROCINELA - macarons enriched with organic freeze dried apple powder - behaved very well during the study period. Obtained results indicated that control and tested products showed detectable differences in their sensory parameters. The sensory analysis highlighted the important effect of the enriched with freeze dried apple powders in improving product flavor and smell and appearance.

3.2. Total polyphenol content (TPC)

For total polyphenol compounds in the extract of BIO PRICONELA sample, were determined according to the Folin-Ciocalteu method following a protocol adapted by Georgé et al., 2005 and results were expressed as mg/g gallic acid equivalents (GAE). Concentration of 1 mg/mL of each Bio Priconela sample, extracts were prepared in their own solvents and 0.5 mL of each sample mixed with 2.5 mL of a 10-fold diluted Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate solution. Then the samples were kept for 30 min at room temperature and at the end the absorbance was read spectrometrically (T80 + UV/VIS spectrophotometer) at 760 nm.

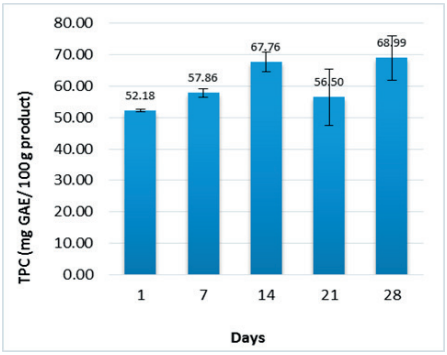

Figure 5. Total polyphenol content of BIO PRICONELA during the products storage stability

Small variation of TPC and DPPH scavenging activity was observed through time for the final product BIO PRICONELA, both with tendency of increasing in time

3.3. *Antioxidant activity*

Determination of antioxidant activity using the DPPH method. The antioxidant activity of the samples is determined based on the DPPH test, using the stable free radical 2,2-diphenyl-1-picrilhydrazyl - DPPH, according to a method Bujor et al. (2016).
Antioxidant activity is expressed as a percentage (%) of inhibition of DPPH radicals relative to the reference solution using the equation:

$$\%I = \frac{A_0 - A_c}{A_0}$$

where:
A_0 – absorbance of the reference sample at t = 0 minutes
A_c – absorbance of samples (with polyphenolic extract) after 30 minutes of rest (t = 30 minutes)

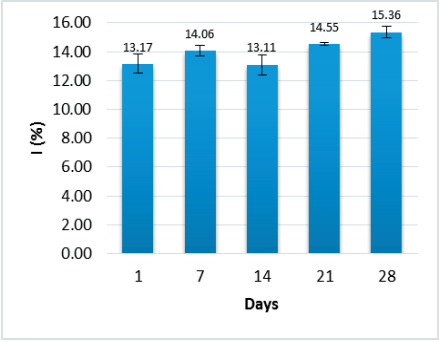

Figure 6. Total antioxidant activity of BIO PRICONELA content of during the products storage stability

Both results provide that BIO PRICONELA bioactive compounds are stable for a 28 days storage period.

4. Nutrient Content

Nutrient content it was calculated using a program nutritional development tool, *Softfedima programme* (http://softfedima.ro/). This programme makes it easy to prepare a nutrition facts panel, nutrition data sheet, ingredient statement for any food product. Formulas can be adjusted for moisture and/or fat content. Information can be printed, saved as a PDF document.

For BIO PRICONELA, the calculation of the nutritional value, baking and cooling losses of average 40% were taken into account, so that the energy value 489.6 kcal per 100g of BioPriconela product (Table 3).

Table 3. Nutritional declaration for macarons

Nutritional value for 100 g produs		
S0		S1 - BIO PRICONELA
Energy	2065,5 KJ	2066,8 KJ
	489,3 kcal	489,6 kcal
Total fat	10.5 g	10.4 g
Saturated fat	1 g	1 g
Carbohydrates	86.3 g	86.6 g
Sugar	82.8 g	82.9 g
Fiber	1.8 g	2 g
Protein	11.5 g	11.4 g
Salt	0.8 g	0.8 g
Allergens: the product contains avidin		

It is found that the addition of organic powder causes a change in the content of carbohydrates (carbohydrates: S0 - 86.3 g, S1 - 86.6 g), dietary fiber (fiber: S0 - 1.8 g, S1 - 2 g) also protein but in smaller quantities. These values show the influence of the addition of dehydrated apple powder. In terms of energy value, the values have not changed much, but there is a significant increase in energy value with the increase in the percentage of dehydrated apple powder added.

These values show the influence of the addition of dehydrated apple powder. In terms of energy value, the changes were insignificant, which recommends the consumption of this innovative product especially due to its nutraceutical properties than its energy value.

CONCLUSIONS

The use of organic products in the food industry for the consumption of healthy foods is a global recommendation. In this sense, it is expected to capitalize on organic products throughout the year, not only in season, in the form of food additives. The organic products obtained benefit from an increased shelf life and prevent food waste.

As consumers become more conscious about the ingredients and origin of the purchased products, organic and sustainable food and drink options are increasing in prevalence.

At the organoleptic test macarons with organic apples powders BIO PRICONELA obtained high scores in sensorial quality and overall acceptability.

In general, it can be stated that enrichment of cookies with apple powders is advantageous due to the increased nutritional value, as apple fiber is rich source of dietary fibers, as well as with bioactive compounds with antioxidant activity.

In generally, it can be stated that enrichment of cookies with apple powder is advantageous due to the increased nutritional value, as apple fibre is rich source of dietary fibre. For S1 sample nutritional value, baking and cooling losses of average 40% were taken into account, so that the energy value 489.6 kcal per 100g of BioPriconela product.

Sensory attributes like flavor and taste are important in the consumer's purchasing decision of minimally processed organic apple products.

Macarons enriched with freeze dried apple powder BIO PRICONELA can be an excellent sweet dessert, flavored, with aerated structure, brittle at biting and a good palatability. It can be easily associated with different foams (mousse, creams, ice cream, etc.).

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THE INFLUENCE OF SOME USUAL PRESERVATION METHODS ON THE CONTENT OF VITAMIN C, CHLOROPHYLLS AND CAROTENOIDS FROM BASIL (*OCIMUM BASILICUM*), LOVAGE (*LEVISTICUM OFFICINALE*) AND THYME (*THYMUS VULGARIS*) LEAVES

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Abstract

Basil (Ocimum basilicum), lovage (Levisticum officinale) and thyme (Thymus vulgaris) are aromatic plants highly valued and used by consumers around the world for the flavor they give to the foods in which are added. Because these plants are not always available fresh on the market, in order to be able to enjoy them longer, many consumers choose to either dry or freeze them at home. The purpose of this paper was to study the influence of traditional drying (for 7 days in dark room, at a temperature of 20-22°C) and freezing (at -18°C) on the content of vitamin C, chlorophylls and carotenoids from basil, lovage and thyme leaves. Vitamin C content was determined by iodometric method, and concentration of chlorophylls and carotenoids by a spectrophotometric assay. The determinations were performed on fresh plants, immediately after the drying process and at 2 months of storage in a dry state, respectively after 7days of freezing and after 2 months of storage in the freezer. In fresh samples, the highest concentration of vitamin C was found in lovage, followed by thyme. Fresh lovage also showed the highest content of chlorophyll and carotenoid. The experimental data showed that in the preserved plants the content of vitamin C, chlorophylls and carotenoids decreases, compared to the fresh ones, the losses being higher after the traditional air drying, than after freezing process. For both dried and frozen plants, a decrease in the concentration of the compounds mentioned above was found after 2 months of storage under specific conditions, but the losses were higher during the storage of dried plants.

Key words*: basil, carotenoids, chlorophylls, lovage, thyme, vitamin C.*

INTRODUCTION

Although aromatic herbs have been used for medicinal purposes for thousands of years and have also played a major role in cooking, especially due to their very pleasant aromas, they have only recently been studied in many scientific papers (Rosłon et al., 2013; Reda et al., 2007; Politeo et al., 2007). Basil (*Ocimum basilicum*) - *Lamiaceae* family- is native to areas from Asia and Africa and was brought to Europe from India in the sixteenth century, and later to America in the seventeenth century. Basil is one of the most important aromatic herbs in many cultures and cuisines, including the Mediterranean, Thai, Vietnamese (Stobart, 1982). In terms of chemical composition, it contains essential oils (0.10-0.20%), triterpene saponosides, tanoids, chlorophyll, vitamin C, carotenoids, a wide range of phenolic compounds, having different antioxidant activities, depending on the species and varieties of basil (Savu et al., 2002; Politeo et al., 2006; Nurzyńska-Wierdak, 2011). Lovage (*Levisticum officinale*) - *Apiaceae* family - perennial plant known and cultivated since antiquity worldwide as an aromatic plant, the leaves and petiole being used in the

aromatization of culinary preparations, and as a medicinal plant in Europe, the seeds and root rich in active principles being used for therapeutic qualities (Złotek et al., 2020; Kemzūraitė et al., 2014). Lovage leaves are rich in essential oil, vitamin C, carotenoids, chlorophyll pigments, polyphenolic compounds, minerals (Złotek et al., 2019; Złotek et al., 2020; Miran et al., 2018). Thyme (*Thymus vulgaris*) –*Lamiaceae* family has its origins on the European shores of the Mediterranean Sea and is cultivated today throughout Europe (De Martino et al., 2009). The chemical composition of thyme leaves includes essential oil, flavonic derivatives, polyphenolcarboxylic acids, waxes and triterpenes, a bitter principle, dietary fiber, vitamin C, vitamin B6, chlorophylls and carotenoids, mineral elements (especially iron, calcium, magnesium, manganese) (Reda et al., 2007; El-Qudah, 2014). Vitamin C (also called ascorbic acid) is a water-soluble vitamin, with a strong antioxidant activity, particularly important for the life of living organisms, it intervenes in a multitude of biochemical and metabolic processes and is found in high concentrations in green plants and in various fruits and vegetables (Devaki & Raveendran, 2017). Chlorophylls are a class of natural green compounds, found in almost all green parts of plants, such as leaves and stems and are also present in some algae and cyanobacteria, these pigments being involved with carotenoids in the process of photosynthesis (Pareek et al., 2017). So far, six different types of chlorophyll have been discovered and studied: chlorophyll *a*, chlorophyll *b*, chlorophyll *c*, chlorophyll *d*, chlorophyll *e* and chlorophyll *f.* Chlorophyll *a* is the most common in green plant tissues, followed by chlorophyll *b* (Vernon and Seely, 1966; Eugene and Govindjee, 1969; Chen et al., 2010). The human body cannot synthesize chlorophylls but is able to deposit dietary chlorophyll. The importance of this pigments for human health is not to be neglected: interrupting diverse diseases such as cancer, cardiovascular, and other chronic diseases, also helps solve pancreatic problems (pancreatitis) (Sangeetha & Baskaran, 2010). Carotenoids are non-nitrogenous natural pigments with a polyisoprene structure that give yellow, orange or red color to the tissues in which they are found. More than 700 different types of carotenoids have been discovered so far. Carotenoid pigments are synthesized only by the plant kingdom. Animals, both vertebrates and invertebrates, as well as humans, do not have the ability to synthesize carotenoids, which must be brought into these organisms through food (Rodriguez Amaya & Kimura, 2004). Without carotenoids, photosynthesis in an oxygenated atmosphere would be impossible. Also, carotenoid compounds intervene in the process of vision (provitamins A), in growth and reproduction, protection against cancer and heart disease, as antioxidants and regulators of the immune system (Krinsky, 1993; Olson, 1999). As aromatic plants are not available fresh all over the world, throughout the year, different preservation techniques have been developed over time, aiming to ensure a better protection of their bioactive compounds and sensory properties (Petcu C.D et al., 2014; Bhatta et al., 2020; Calín-Sánchez et al., 2020). However, two traditional techniques of preserving aromatic plants in their own households are still used to a large extent among the population: traditional drying and freezing. The aim of this paper was to study the effects of traditional drying (for 7 days in dark rooms, at 20-22°C) and freezing (at -18°C) on the content of ascorbic acid, chlorophyll *a*, chlorophyll *b*, total chlorophylls and carotenoids in basil, lovage and thyme leaves.

MATERIALS AND METHODS

The raw materials were purchased fresh from the local market in Timișoara, Romania (supermarket), respectively leaves of: basil (*Ocimum basilicum*), lovage (*Levisticum officinale*) and thyme (*Thymus vulgaris*). Samples were taken from fresh leaves and were preserved by traditional drying for 7 days at 20-22°C, in a dark room as well as by freezing at -18°C. For the analysis of vitamin C, chlorophylls and carotenoids content, samples were taken both from fresh and preserved plants and also immediately after the drying process and after 2 months of dry storage (in the dark room, at 20-22°C), as well as after 7 days, respectively after 2 months of freezing. The fresh, dried and frozen samples

were weighed to determine the water loss relative to the fresh samples and to be able to express the results by reference to fresh weight (FW) of plant tissue. The vitamin C content of the samples was determined by the adapted iodometric method, using the same working methodology as the one presented by Dumbrava et al. (2016). Analysis of the of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids content was performed by a spectrophotometric method (Lichtenthaler, 1987; Porra et al., 1989). Weighed 1 g of each sample of fresh and processed aromatic plant and then crushed it in a mortar with a little quartz sand and acetone 80%. The obtained homogenate was then centrifuged at 5000 rpm for 5 minutes, and the supernatant was collected in brown glass containers. The precipitate was taken up in solvent and centrifuged until the colourless. The combined supernatants were analyzed at 646 nm, 663 nm and 470 nm on a UV-VIS spectrophotometer (Analytic Jena Specord 205, Jena, Germany). The chlorophylls and carotenoids content was quantified according to the formulas of Lichtenthaler and Wellburn (1983):

Chl a=12.21·(A_{663})-2.81·(A_{646})

Chl b=20.13·(A_{646})-5.03·(A_{663})

Chl_{total} =17.32·(A_{646})+7.18·(A_{663})

Carotenoids=[(1000·A_{470})-(3,27·Chl a)

-(104· Chl b)]/229

where:

Chl a - chlorophyll *a*, in mg/l,

Chl b - chlorophyll *b*, in mg/l,

Chl_{total} - total chlorophyll content, in mg/l,

A_{663} - absorbance of the sample at 663 nm,

A_{646} - sample absorbance at 646 nm

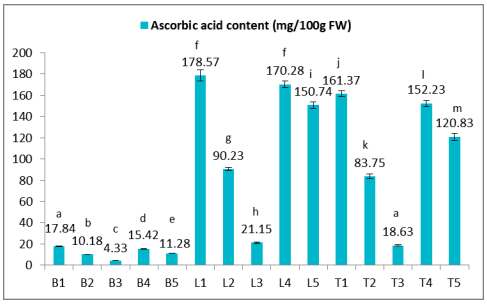
A_{470} - sample absorbance at 470 nm

All determinations were made in duplicate or triplicate and the results are reported as mean values ± standard deviation (SD). t-Test: Two-Sample Assuming Equal Variances were applied to evaluate the statistical significance ($p<0.05$). Statistical processing data was performed using the Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

In all experimental determinations we noted the samples with the following codes which will be used in the following figures and tables: B1-basil leaves fresh, B2-basil leaves immediately after traditional drying (7 days at room temperature in dark room), B3-basil leaves after 2 months of dried storage, B4-basil leaves after 7 days of freezing, B5-basil leaves after 2 months of freezing, L1-lovage leaves fresh, L2-lovage leaves immediately after traditional drying (7 days at room temperature in dark room), L3-lovage leaves after 2 months of dried storage, L4-lovage leaves after 7 days of freezing, L5-lovage leaves after 2 mounts of freezing, T1-thyme leaves fresh, T2-thyme leaves immediately after traditional drying (7 days at room temperature in dark room), T3-thyme leaves after 2 months of dried storage, T4-thyme leaves after 7 days of freezing, T5-thyme leaves after 2 months of freezing.

The ascorbic acid content of the analyzed samples is presented in Figure 1.



Each value was the mean of triplicate measurements; a-m Different letter indicate significant difference within samples ($p<0.05$)

Figure 1. Ascorbic acid content of fresh and preserved samples

Among the fresh plants, the highest content of ascorbic acid was found in the lovage leaves (178.57±5.43 mg/100 g FW), followed by the thyme leaves (161.37±3.06 mg/100 g FW), while the basil leaves were about 10 times poorer in vitamin C (17.84±0.83 mg/100 g FW) than those of lovage. As it can be seen from Figure 1, the preservation of these aromatic herbs by traditional drying has led to a more pronounced decrease in vitamin C content than preservation by freezing, especially after a period of 2 months of plant storage in the dry state, when values of 4.33±0.07 mg/100 g FW for dry basil, 21.15±0.41 mg/100 g FW for dry lovage and 18.63±0.48 mg/100 g FW for dry thyme were found. After one week of freezing, the ascorbic acid content of studied plants was only slightly lower than that of fresh plants

(15.42±0.33 mg/100 g FW - for basil, 170.28±3.05 mg/100 g FW - for lovage, 152.23±2.94 mg/100 g FW - for thyme), however, after 2 months of freezing there is a more significant reduction in this content (11.28±0.30 mg/100 g FW for basil, 150.74±3.12 mg/100 g FW for lovage, 120.83±3.44 mg/100 g FW for thyme).

In Figure 2 is presented the average losses (%) of vitamin C in the samples of preserved plants, compared to fresh plants

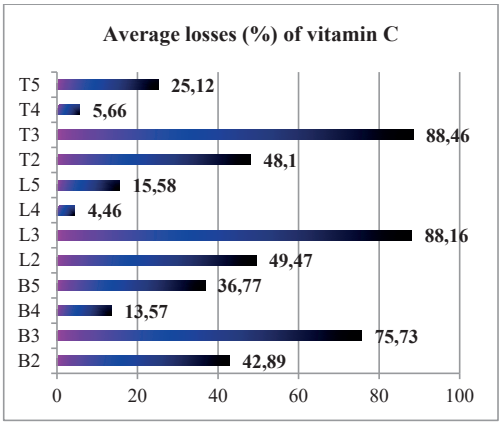


Figure 2. Average losses of vitamin C (%) in the samples of preserved aromatic plants

For the samples stored in the dry state for 2 months, the highest losses of ascorbic acid were reported: 88.46% in the samples of dried thyme, 88.16% for dried lovage and 75.73% for dried basil. Much less vitamin C was lost during freezing than during traditional drying and dry storage, with the lowest losses in the case of lovage leaves (4.46% after 7 days of freezing and 15.58% after 2 months) and the highest in the case of basil (13.57% after 7 days of freezing and 36.77% after 2 months). In the literature there are very different data for the content of vitamin C in the leaves of basil, lovage and thyme, which vary greatly depending on the plant variety, growing conditions, geographical area, method of processing etc. Cătunescu et al. (2017) reported a vitamin C content for fresh lovage leaves of 173.49±3.37 mg/100 g FW, and for those stored at cold (4°C) for 8 days: 171.33±5.48 mg/100 g FW. Złotek et al. (2020) found a smaller content in the fresh lovage leaves, of only 49.13±0.63 mg/100 g FW, and after traditional drying: 4.84±0.11 mg/100 g FW. For fresh basil Holland et al. (1991) reported 26 mg ascorbic acid/100 g and for fresh thyme, Dauqan & Abdullah (2017) found 160.1 mg ascorbic acid/100 g.

In Table 1 are presented the concentrations of chlorophyll *a*, chlorophyll *b* and total chlorophyll in the fresh and preserved aromatic plants. In all samples, both fresh and processed, chlorophyll *a* was much higher concentration than chlorophyll *b.* Among the raw materials, the highest chlorophylls content was determined in fresh lovage leaves (chlorophyll *a*: 945.10±14.24 µg/g FW. chlorophyll *b*: 315.21±11.22 µg/g FW, total chlorophylls: 1260.31±25.46 µg/g FW) and the lowest in basil leaves (chlorophyll *a*: 287.42±8.32 µg/g FW, chlorophyll *b*: 94.70±2.04 µg/g FW, total chlorophylls: 382.12±10.35 µg/g FW). As in the case of vitamin C, it was found that in samples preserved by drying the concentration of chlorophyll pigments is lower than in samples of plants preserved by freezing.

Table 1. Chlorophyll a, chlorophyll b and total chlorophyll content of the fresh and preserved aromatic plants

Sample	Chl a (µg/g FW)	Chl b (µg/g FW)	Chl total (µg/g FW)
B1	287.42±8.32^{a}	94.70±2.04^{a}	382.12±10.35^{a}
B2	134.72±4.99^{b}	40.28±0.99^{b}	175±5.98^{b}
B3	88.92±1.98^{c}	25.41±0.50^{c}	114.33±2.47^{c}
B4	270.24±7.17^{a,d}	91.25±2.12^{a,d}	361.49±9.29^{a,d}
B5	250.61±6.96^{d}	82.32±2.97^{d}	332.93±9.92^{c,d}
L1	945.10±14.24^{e}	315.21±11.22^{e}	1260.31±25.46^{e}
L2	402.44±10.49^{f}	145.92±2.26^{f}	548.36±8.23^{f}
L3	215.36±6.35^{g}	80.41±2.84^{d}	296.77±9.19^{g}
L4	928.64±15.08^{e}	306.98±7.10^{e}	1235.62±22.17^{e}
L5	860.45±12.19^{h}	296.41±6.70^{e}	1156.86±5.49^{h}
T1	420.45±7.65^{f,i}	151.30±3.90^{f}	571.75±11.55^{f,i}
T2	207.63±3.37^{g}	75.72±2.25^{d}	283.35±5.61^{g}
T3	101.23±2.63^{k}	42.64±2.14^{b}	143.87±4.77^{k}
T4	411,78±7.55^{f,i}	147.33±2.90^{f}	559.11±10.45^{f}
T5	382.21±5.05^{f,j}	130.14±2.94^{g}	512.35±7.99^{f,j}

Each value was the mean of triplicate measurements; a-j Different letter indicate significant difference within samples ($p<0.05$)

Figure 3 shows the average chlorophyll losses from the samples of preserved aromatic plants, compared to the fresh ones. As it can be seen, traditional drying causes significant losses of chlorophyll pigments in all studied herbs, even after the end of the drying process (7 days) losses exceeding 50% (for basil: 53.13%, 57.46% and 54.20%; for lovage: 57.42%, 53.71%, and 54.49%; for thyme: 50.61%,

59.95% and 50.44% losses of chlorophyll *a*, chlorophyll *b* and total chlorophylls respectively) and after 2 months of dry storage reaching over 70% (for basil: 69.06%, 73.16% and 70.08%; for lovage: 78.03%, 74.49%, and 76.45%; for thyme: 75.92%, 71.82% and 74.84% losses of chlorophyll *a*, chlorophyll *b* and total chlorophylls respectively). Instead, freezing at -18 C of these plants causes much lower losses of chlorophyll, which are very small after 7 days of freezing and increasing slightly after two 2 months of freezing. Lovage leaves had the lowest chlorophyll losses during freezing (after 7 days: 1.74%, 2.61% and 1.96% losses of chlorophyll *a*, chlorophyll *b* and total chlorophylls respectively; after 2 months: 8.96%, 5.96% and 8.21% losses of chlorophyll *a*, chlorophyll *b* and total chlorophylls respectively).

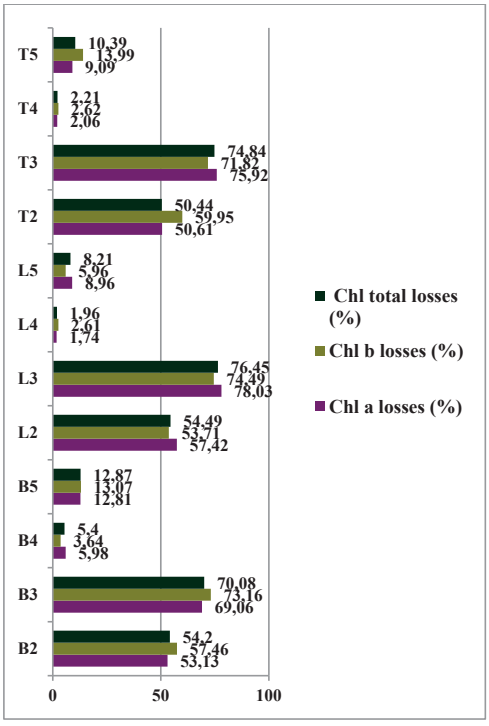
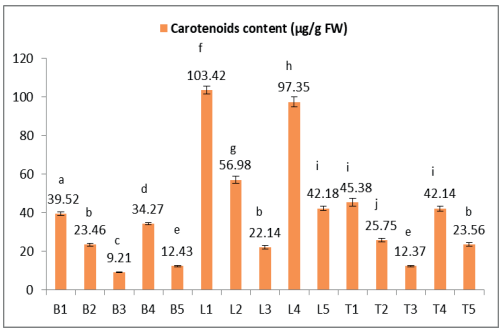


Figure 3. Average losses of chlorophylls (%) in the samples of preserved aromatic plants

Because the amount of chlorophylls accumulated in plant leaves matrix depends on a multitude of factors, including plant variety, pedoclimatic conditions, the degree of plant development (Vernon & Seely, 1966; Limantara et al., 2015), literature data for basil, lovage and thyme report quite different values of the chlorophylls concentration both in the fresh and processed plant material. Thus, Arunrangsi et al. (2013) reported for fresh basil leaves values of chlorophyll content quite close to what we found (chlorophyll *a*: 263.0±0.6 µg/g, chlorophyll *b*: 94.7±0.8 µg/g), but, also for basil Taie et al. (2010) report a higher chlorophyll content (chlorophyll *a*: 680±0.55 µg/g, chlorophyll *b*: 58±0.007 µg/g). Sledz and Witrowa-Rajchert (2012) studied the effect of microwave-convective drying on the chlorophyll content of several aromatic plants, including basil and lovage. They observed that the chlorophyll *a* content decreased after drying, compared to fresh plants, from 15.20 mg/g DW to 14.20 mg/g DW for lovage, respectively from 14.10 mg/g DW to 13.04 mg/g DW for basil. El-Qudah (2014) found an average contents of chlorophyll *a*, chlorophyll *b* and total chlorophylls in fresh thyme leaves of 2.82, 1.31 and 4.13 (mg/g DW), respectively.

The carotenoids content of the analyzed samples of fresh and preserved aromatic plants is presented in Figure 4. Among the fresh plants analyzed, lovage had a much higher concentration of carotenoids (103.42±1.95 µg/g FW) than thyme (45.38±1.90 µg/g FW) and basil (39.52±1.00 µg/g FW). It was also found that all samples subjected to preservation suffered losses of this compounds, those preserved by traditional drying had lower concentrations of carotenoidic pigments than samples preserved by freezing. Thus, the lowest concentrations of carotenoids were determined in the samples of dried basil, then in those of dried thyme, after two months of dry storage (9.21±0.14 µg/g FW, respectively 12.37±0.37 µg/g FW). The highest carotenoid content of all samples preserved by drying was found in lovage samples (56.98±1.77 µg/g FW after completing the traditional drying process and respectively 22.14±0.81 µg/g FW after 2 months of dry storage). Also, among the samples of frozen plants, lovage had the highest content of carotenoid compounds (97.35±2.45 µg/g FW after 7 days of freezing and 42.18±1.17 µg/g FW after 2 months of freezing



Each value was the mean of triplicate measurements; a-j Different letter indicate significant difference within samples ($p<0.05$)

Figure 4. Carotenoids content of the fresh and preserved aromatic plants

Figure 5 shows the losses in carotenoids in the preserved samples of aromatic plants compared to the fresh plants.

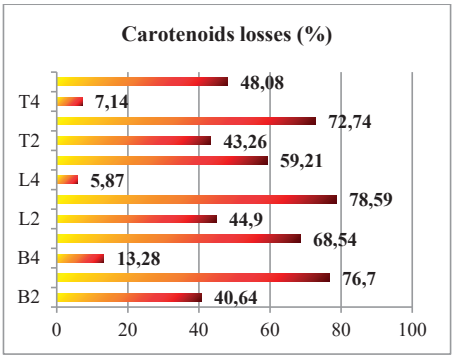


Figure 5. Average losses of carotenoids (%) in the samples of preserved aromatic plants

In the samples of aromatic plants preserved by drying, the greatest losses of carotenoids were reported for lovage (44,9% after completing the traditional drying process and 78.59% after 2 months of dry storage). However, lovage recorded the lowest carotenoids losses after 7 days of freezing (5.84%) and thyme had the lowest carotenoids losses both after 2 months of freezing (48.08%) and after 2 months of dry storage (72.74%). After completing the drying process (7 days) the lowest carotenoids losses were reported in basil (40.64%).

As for carotenoid compounds, the concentrations determined in plants vary depending on the growing conditions, geographical area, variety, extraction method, etc., the literature indicates quite different values for the concentrations of these compounds in fresh basil, lovage and thyme leaves. For these plants subjected to freezing or to traditional drying, there are relatively few data. Nazin et al. (2019) found for fresh basil grown in different lighting conditions, values of carotenoids concentration between 171.42±8.1 and 316.67±9.1 µg/g DW; also, Taie et al. (2010) reported very high carotenoid values for fresh basil between 184±0.014 and 516±0.02 µg/g FW. For fresh lovage leaves, Złotek et al. (2020) determined very low values for carotenoids: 1.37±0.28 µg/g FW and after the traditional drying process they found 0.22±0.01 µg/g FW (meaning losses of 83.94% carotenoids following the traditional drying process, higher than those obtained by us). Sharafzadeh & Alizadeh, (2011) studying the carotenoid compounds in thyme leaves, determined values between 560 and 920 µg/g DW (depending on the type of fertilizer used).

CONCLUSIONS

The application of classical preservation techniques (freezing at -18°C and respectively traditional drying at 20-22°C for 7 days) of aromatic plants in households causes different losses of vitamin C, chlorophylls and carotenoids. Of the two preservation methods studied, traditional drying has been shown to produce much greater losses than freezing. Thus, if after 7 days of freezing the losses of ascorbic acid, chlorophylls and carotenoids were relatively small for lovage and thyme and slightly higher in the case of basil, in the case of traditional drying, immediately after the completion of the process there were large losses of vitamin C (over 40% in all plants), chlorophylls (over 50% in all plants) and carotenoids (over 40% in all plants). For plants stored in the dry state for 2 months in specific conditions (20-22°C in the dark room) the losses of vitamin C and carotenoids in all plants were about twice as high and the losses of total chlorophylls reached over 70%. Also, the freezing for 2 months of the plants determined a more pronounced decrease of the concentration of the analyzed active principles, but the losses were still significantly lower than those found in the case of dried plants stored for 2 months. Thus, the present study shows that freezing, because it protects better the

content of vitamin C, chlorophylls and carotenoids in the studied plants, than traditional drying, is the most recommended classic method for preserving them in households.

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ASSESMENT OF BYOLOGICALY ACTIVE AND TRANS FATTY ACIDS IN FAT FRACTION ON THE COW'S YOGURT

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Abstract

Natural sources of trans isomers of fatty acids are primarily the milk fat and other fats from animal origin. The study was conducted with yogurt containing 2, 3 and 4.5% for the determination of biologically active and trans fatty acids and qualitative assessment of the fat fraction. Yogurt with 4.5% fat have a highest content of saturated fatty acids SFA- 3.13 g/100 g product, monounsaturated fatty acids MUFA- 1.18 g/100 g product, polyunsaturated fatty acids PUFA- 0.15 g/100 g product, oleic- 0.98 g/100 g product, linoleic-0.08 g/100 g product, trans fatty acids- 0.14 g/100 g product, conjugated linoleic acid CLA- 0.03 g/100 g product as long as 2% yogurt is the poor of biologically active fatty acids- oleic acid- 0.43 g/100 g product, linoleic- 0.04 g/100 g product, trans fatty acids- 0.09 g/100 g product, CLA-0.01 g/100 g product. Lipid preventive score, index of atherogenicity and thrombogenicity is highest at 4.5% yogurt- 9.50 g/100 g product, 3.02 and 2.82. The analyzed yoghurts are characterized as a food product with a low content of trans fatty acids- 0.06 to 0.14 g/100 g product and a low content of saturated fatty acids in the yogurt with 2% fat- 1.45.

Key words*: conjugated linoleic acid (CLA), trans fatty acids, yogurt.*

INTRODUCTION

Yogurt was a dairy product produced by bacterial fermentation of milk using *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. It was a widespread food product on the Balkans and the Middle East and was produced from cows, sheep's, goat's, buffalo's milk or a mixture and is one of the healthiest and most nutritious foods (Serafeimidou et al., 2012; Gahruie et al., 2015). Florence et al. (2012) found that the use of organic cow's milk compared to conventional cow's milk production has more health benefits because the content of saturated fatty acids was lower due to an increase in biologically active trans fatty acids: trans octadecene acid-1.6 times, CLA-1.4 times and alpha linolenic-1.6 times compared to conventional yogurt. Serafeimidou et al. (2013), in their research on the shelf life of yogurt from cows and sheep, found that saturated fatty acids in cow's yogurt increase, while in sheep decrease, omega-3 fatty acids increase the both types in yogurt at the end of the shelf life, while the conjugated linoleic acid decreases with the storage of cow's yoghurt, but increases with sheep's. Diets rich by saturated fatty acids such as lauric (C12:0), myristic (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) were strongly associated with an increased risk of atherosclerosis, obesity and coronary heart disease (Pilarczyk et al., 2015). According to the indices proposed by Ulbricht and Southgate (1991), lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) were atherogenic, and myristic (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) had a thrombogenic by nature, while omega-3, omega-6 and monounsaturated fatty acids were antiatherogenic and antithrombogenic pattern. De Souza et al. (2015) were found in cow's milk atherogenic index- 4.10 and thrombogenic index- 5.17. The ratio between hyper- and hypocholesterolemic fatty acids, found by Fernandez et al. (2007) in Iberian ham below 2.5, which was defined as favorable compared to the other types of ham studied by them. Tonial et al. (2014), in two species of fish, received values for AI-0.55-0.60; TI- 0.82-0.87 and hH- 1.56-1.63.

The daily intake of trans fatty acids should not exceed 0.5% of energy intake. According to EU and Council Regulation (EC) No 1924/2006 on 20 December 2006, the content of saturated

fatty acids and trans fatty acids in solid products shall not exceed 1.5 g/100 g product or 0.75 g/100 ml liquid, as in both cases the content of saturated fatty acids and trans fatty acids does not exceed 10% of the daily energy intake and these foods were labeled as foods with low SFA content. The claim that a food does not contain SFA may be indicated only if the content of SFA and TFA does not exceed 0.1 g/100 g product or 0.1 g/100 ml liquid (Regulation (EC) No 1924/2006).

The study was conducted with yogurt containing 2, 3 and 4.5% for the determination of biologically active and trans fatty acids and qualitative assessment of the fat fraction.

MATERIALS AND METHODS

Yogurts produced by Research Institute of Mountain Stockbreeding and Agriculture-Smolyan with a milk fat content, respectively 2% (4 pieces), 3% (4 pieces) and 4.5% (4 pieces) for fatty acid composition and for establishment of the content of trans fatty acids, biologically active and anticancer substances in the fatty fraction were studied. The extraction of total lipids was carried out by the Roese-Gottlieb method, using diethyl ether and petroleum ether and subsequent methylation with sodium methylate (CH_3ONa, Merck, Darmstadt) and drying with $NaHSO4.H_2O$. Fatty acid methyl esters (FAME) were analyzed using a Shimadzu-2010 gas chromatograph (Kioto, Japan) equipped with a flame ionization detector and an automatic injection system (AOC-2010i). The analysis was performed on a CP 7420 capillary column (100 m x 0.25 mm i.d., 0.2 μm film, Varian Inc., Palo Alto, CA). Hydrogen is used as the carrier gas, and as a make-up gas - nitrogen. Four-step furnace mode is programmed - the column's initial temperature is 80°C/min, maintained for 15 minutes, then increased by 12ºC/min to 170ºC and maintained for 20 minutes, followed by a further increase of 4ºC/min to 186ºC for 19 minutes and up to 220ºC with 4ºC/min until the process is complete.

The qualitative evaluation of the fat fraction of the yoghurts obtained includes the following indicators: lipid preventive score, atherogenic and thrombogenic index (Ulbricht & Southgate, 1991), the ratio of hyper- and hypocholesterolemic fatty acids (Ivanova & Hadzhinikolova, 2015), trans fatty acids and the amount of saturated fatty acids (Regulation (EC) No 1924/2006).

LPS= FAT +2x SFA- MUFA- 0.5 PUFA

AI= 12:0+ 4×14:0 +16:0 /[ΣMUFAs+PUFA n6+PUFA n3]

TI = (14:0+16:0+18:0)/[0.5×ΣMUFAs+0.5× ×PUFAn6+3×PUFAn−3+PUFA n3/ PUFA n6]

h/H=(C18:1n-9+C18:1n-7+C18:2n-6+C18:3n-3+C18:3n-6+C20:3n-6+C20:4n-6+C20:5n-3+C22:4n-6+C22:5n-3+C22:6n-3)/ (C14:0+C16:0)

where:
LPS - lipopolysaccharide;
AI - atherogenic index;
TI - thrombogenic index;
h/H - hypocholesterolemic/ hypercholesterolemic ratio.

The data were processed using the variation statistics methods using the statistical package of the EXCEL 2013 computer program. The reliability of the differences between the analyzed milks was established by Student's t-test.

RESULTS AND DISCUSSIONS

The fatty acid composition was an important characteristic of the fat fraction for determining the content of trans fatty acids and biologically active components. Milk fat contains mainly saturated fatty acids. The amount of saturated fatty acids in the studied yoghurts varies from 68.54 to 69.85 g/100 g fat, monounsaturated fatty acids range from 26.30 to 27.43 g/100 g fat and polyunsaturated from 3.12 to 3. 29 g/100 g fat. The biologically active fatty acids in yoghurt were from 2.86 to 3.01 for trans fatty acids, from 0.43 to 0.47 g/100 g fat for ω-3, from 2.39 to 2.50 g/100 g fat for ω-6 and from 0.49 to 0.56 g/100g fat by conjugated linoleic acid. The ratio between ω-6 and ω-3 fatty acids was from 5.19 to 5.54 in the analyzed yogurts (Table 1). Yogurt produced

from cow, sheep and goat milk yogurt contain 0.128-1.501, 0.405-1.250 and 0.433-0.976 g conjugated linoleic acid or CLA per 100 g fat, respectively (Serafeimidou et al., 2012; Serafeimidou et al., 2013; Sumarmono et al., 2015; Kalinova, 2020). Determined that the trans fatty acids in pasteurised milk and yogurt were in small amounts (about 0.50%) and did not show fluctuations. Paszczyk et al. (2020) was established the storage effect in yogurt and changes in fatty acids, especially CLA and trans isomers in cow milk yogurts which decrease by shelf life. Gutiérrez (2016) establishes CLA content in cow's milk varies between 2 and 37 mg/g fat and is mainly influenced by the diet offered to animals, as technological processes may cause slight changes in CLA concentration, but the mechanisms leading to these changes have not yet been established, but the increase of the concentration of CLA by lactic fermentation depends on the strain due to the different linoleate isomerase activity of the species.

Table 1. Fatty acid composition of yoghurt with different milk fat content (g/100 g fat)

FA	2%		3%		4.5%	
	x	sd	x	sd	X	Sd
SFA	**68.54**	1.86	**69.85**	1.77	**69.58**	3.62
MUFA	**27.43**	1.73	**26.21**	0.93	**26.30**	2.65
PUFA	**3.28**	0.23	**3.12**	0.34	**3.29**	0.57
Σ *trans* FA	**2.97**	0.16	**2.86**	0.91	**3.01**	1.07
Σ *cis* FA	**23.55**	1.61	**22.41**	0.05	**22.38**	1.66
Σ CLA	**0.56**	0.04	**0.54**	0.12	**0.60**	0.15
C-16:0/C-18:1cis9	**1.37**	0.14	**1.47**	0.08	**1.48**	0.22
C-16:0/C-18:1total	**1.18**	0.12	**1.26**	0.12	**1.27**	0.23
Σ n-3	**0.45**	0.04	**0.43**	0.02	**0.47**	0.06
Σ n-6	**2.50**	0.15	**2.39**	0.33	**2.46**	0.48
Σ n-6/ Σ n-3	**5.54**	0.10	**5.61**	0.37	**5.19**	0.27
Σ SCT (C-4>C-8)	**7.10**	0.22	**6.95**	1.21	**6.60**	1.00
Σ MCT (C-10>C-14)	**16.23**	1.14	**17.04**	0.03	**17.80**	1.11
CLA 9c,11t	**0.51**	0.03	**0.49**	0.12	**0.56**	0.15

Table 2. Fatty acid composition of yoghurt with different fat content (g/100 g product)

	2%		3%		4.5%	
FA	x	sd	x	sd	x	sd
C12:0	**0.06**	0.02	**0.09**	0.03	**0.14**	0.05
C14:0	**0.22**	0.03	**0.32**	0.05	**0.49**	0.07
C16:0	**0.62**	0.06	**0.93**	0.09	**1.40**	0.14
C18:0	**0.23**	0.04	**0.34**	0.05	**0.51**	0.08
C18:1n-9	**0.43**	0.06	**0.65**	0.09	**0.98**	0.14
C18:2n-6	**0.04**	0.01	**0.05**	0.01	**0.08**	0.02
SFA	**1.39**	0.10	**2.09**	0.15	**3.13**	0.23
MUFA	**0.53**	0.08	**0.79**	0.13	**1.18**	0.19
PUFA	**0.07**	0.01	**0.10**	0.02	**0.15**	0.02
TFA	**0.06**	0.02	**0.09**	0.03	**0.14**	0.05
ω-3	**0.01**	0.00	**0.01**	0.00	**0.02**	0.00
ω-6	**0.05**	0.01	**0.07**	0.02	**0.11**	0.02
CLA 9c,11t	**0.01**	0.00	**0.02**	0.00	**0.03**	0.00

Based on the obtained fatty acid profile the amount of saturated fatty acids in the final product was highest at 4.5% fat content and represents 3.13 g/100 g product, while in low-fat 2% yogurt they were 1.39 g/100 g product. The content of mono- and polyunsaturated fatty acids, 0.53 and 0.07 g/100 g product in 2% yoghurt and 1.18 and 0.15 g/100 g of product in 4.5% yoghurt, respectively (Table 2).

The main representatives of saturated fatty acids that were related to human nutrition were lauric (C12:0), myristic (C14:0) acid, palmitic (C16:0) and stearic acid (C18:0). Yogurt with 4.5% fat content has the highest content of these four representatives, respectively 0.14, 0.49, 1.40 and 0.51 g/100 g product. The oleic acid in the tested samples was in the highest concentration by 4.5%- 0.98 g/100 g products, the lowest by 2% yogurt- 0.43 g/100 g product. The linoleic acid in the tested yoghurts was range from 0.04 to 0.08 g/100 g product.

Serhan et al. (2016) in the production of concentrated yoghurt from goats, has a fat content of 9.25%, protein 9.12%, ash 1.16%, saturated fatty acids 69.1 g/100 g fat, polyunsaturated 27.2 g/100 g fat and monounsaturated fatty acids 3.4 g/100 g fat and atherogenic index 2.84. TFA was an indicator of food safety, because it has a negative effect on many vital functions (Chen & Liu, 2020).

The total content of trans fatty acids in the analyses yoghurts was from 0.09 g/100 g product in 2% yoghurt to 0.14 g/100 g product in 4.5% yoghurt. Omega-3 fatty acids in yogurt with a fat content of 2 and 3% were 0.01 g/ 100 g product, while in 4.5%- 0.02 g/100 g product. Omega-6 fatty acids range from 0.05 to 0.11 g/100 g product. Another important biologically active component of milk fat was CLA, whose content was highest in yogurt with 4.5% fat content- 0.03 g/100 g product. The statistical reliability of the results for fatty acids is presented in Table 3.

Table 3. Statistical reliability of the results for the fatty acid composition of yoghurt with fat content of 2, 3 and 4.5%

Fatty acids	2% / 3%	2% / 4.5%	3% / 4.5%
C12:0		*	
C14:0	**	**	**
C16:0	**	***	**
C18:0	**	**	**
C18:1n-9	**	***	**
C18:2n-6	*	**	*
SFA	***	***	***
MUFA	*	**	**
PUFA	**	***	**
TFA		*	
ω-3	***	***	***
ω-6	*	**	*
CLA 9c,11t	***	***	***

P< 0.001- ***; P< 0.01- **; P< 0.05- *

Table 4. Qualitative indicators of the fat fraction in yoghurt with different fat content (g/100 g product)

Показател	2%		3%		4.5%	
	X	SD	X	SD	X	SD
LPS	4.22 a***,b***,c***	0.29	6.34	0.44	9.50	0.66
AI	2.72	0.88	2.43	0.88	3.02	0.88
TI	2.61	0.64	2.41	0.64	2.82	0.64
h/H	0.59	0.14	0.62	0.14	0.56	0.14
TFA	0.06	0.02	0.09	0.03	0.14	0.05
SFA+TFA	1.45 a***,b***,c***	0.08	2.18	0.12	3.27	0.18

a-2%/ 3% yogurt, b- 2%/ 4.5% yogurt, c- 3%/4.5% yogurt, *P<0.05, ** P<0.01, ***P<0.001

High reliability of the results was found for saturated fatty acids and conjugated linoleic acid in the three types of yoghurt.

The qualitative assessment of the fat fraction was made on the basis of the following indicators: lipid preventive score, atherogenic and thrombogenic index and the ratio between hyper- and hypocholesterolemic fatty acids (Table 4). All indices have their advantages and disadvantages; therefore, the rational choice to

be used is crucial. Of these nutritional indices, IA and IT are the most commonly used to assess the composition of fatty acids as they outline significant implications and provide clear evidence (Chen & Liu, 2020).

The lipid preventive score in yogurts with different fat content was the highest at 4.5% and reaches a value of 9.50 g/100 g product and was lowest at 2%- 4.22 g/100 g product. The atherogenic index gives the relationship between the sum of basic saturated fatty acids and unsaturated fatty acids, the former being considered pro-atherogenic (favoring the adhesion of lipids in immune and circulatory cells) and the latter anti-atherogenic (inhibiting plaque aggregation and reducing plaque aggregation and levels of esterified fatty acids, cholesterol, and phospholipids, thus preventing the onset of micro- and macrocoronary heart disease). The thrombogenic index gives the tendency to form clots in blood vessels and is defined as the ratio between prothrombogenic (saturated fatty acids) and antithrombogenic (monounsaturated and polyunsaturated omega-3 and omega-6 fatty acids) fatty acids (Gahruie et al., 2015). The atherogenic index was the lowest in yogurt 3% - 2.43, while in 4.5% it reaches - 3.02, the results were similar for the thrombogenic index- the lowest in 3% yogurt - 2.41 and the most-high at 4.5% - 2.82. The studied yoghurts were characterized as a food product with low content of trans fatty acids from 0.06 to 0.14 g/100g product and low content of saturated fatty acids in yogurt with 2% fat content- 1.45, while in 3 and 4.5% fat content of yoghurt, the content of saturated and trans fatty acids exceeds 1.5 g/100 g product, therefore exceeding 10% of the daily energy intake was defined as a food with a high content of saturated fatty acids.

CONCLUSIONS

Of the studied yoghurts with different fat content, the poorest of biologically active fatty acids was 2% yoghurt, but according to quality indicators for assessment of the fat fraction it was most suitable for healthy human nutrition and with the lowest content of saturated and trans fatty acids, while 4.5% yogurt was richest in natural biologically active fatty acids.

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COMPARATIVE STUDY ON THE VARIATION OF CORTISOL LEVEL IN BLOOD SERUM DEPENDING ON SWINE SLAUGHTERING METHOD

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Abstract

Stress is defined as a complex chain of events, consisting in a stimulus that causes a subsequent reaction in the brain and activates physiological reactions. It is important to adopt good practices during slaughter. In assessing the level of stress, the following variables should be taken into account: the means of transport, the way of slaughter (with or without stunning), accidental fall of animals, refusal of animals to enter the containment box, excessive movement of animals during containment. In the living organism, a series of biochemical and energetic transformations take place, which are in close interdependence, and are subjected to the mechanisms of regulation and metabolic control, which cease with the suppression of animal life, therefore, after slaughtering animals, a series of transformations appear in the muscle tissue. The study was conducted between 2019-2020, on two batches of conventionally slaughtered pigs (with stunning) in slaughterhouses and on a batch of traditionally slaughtered pigs (without stunning). In the slaughterhouses, the technological flow of pigs slaughtering was monitored and blood samples were collected in order to extract the serum and measure the cortisol level. Cortisol was measured in a specialized laboratory by the immunoenzymatic method by chemiluminescence detection. Determination of cortisol levels in blood samples taken from conventional pig slaughter revealed different values, exceeding the established reference values, compared with blood samples collected from households following traditional slaughter, the level of which is lower, sometimes falling within the reference values. The growth and handling of pigs before slaughter induces their stress, so special attention must be paid to the slaughter process in order to minimize stress levels and improve the quality of the meat.

Key words: *cortisol, pigs, stress, stunning.*

INTRODUCTION

Meat has played a crucial role in human evolution and is an important component of a healthy and balanced diet due to its nutritional richness (Savu et al., 2002; Williamson et al, 2005; McNeill & Van Elswyk, 2012; Pereira & Vicente, 2012; Petcu, 2013; Predescu et al, 2018). The nutritional composition of meat varies depending on the animal's breed, age, sex, diet, body weight, fattening status, rational feeding, animal health, animal movement, season, but also on the way of slaughter (with or without stunning) (Williams, 2007; Banu et al., 2009).

In pigs, in particular, there are a number of growth and fattening factors that affect the quality of the meat, such as: type of shelter, shelter size, microclimate in the shelter, animal density, feed, animal sex, age at slaughter, health, genetic factors, stressors and last but not least the weight at slaughter (Banu et al., 2009; Tăpăloagă, 2012).

In the living organism there are a series of biochemical and energetic transformations, which are in close interdependence, as they are subjected to the mechanisms of regulation and metabolic control, mechanisms that end with the suppression of animal life (Ionescu & Diaconescu, 2010).

After the slaughter of the animals, a series of transformations appear in the muscles tissue, as the blood pressure decreases, a peripheral vasoconstriction occurs, the thermoregulatory mechanisms no longer work, disturbances appear at the level of all homeostatic mechanisms and the susceptibility to microbial attacks increases (Ionescu & Diaconescu, 2010; Papuc et al., 2013; Petcu, 2015).

When the blood flow is interrupted, the oxygen supply is suppressed. Tissue respiration continues for a short time, until oxygen depletion. The absence of oxygen leads to the cessation of aerobic processes, and so the formation of lactic acid takes place through the anaerobic degradation of glucose. The accumulation of lactic acid in the muscles has the effect of decreasing the pH value, leading to its acidification (Ionescu & Diaconescu, 2010).

In order to obtain meat with physico-chemical characteristics corresponding to human consumption, animals are slaughtered, by different methods, depending on the species, religious precepts or geographical area. The process of sacrifice entails a series of consequences that have attracted the attention of the scientific world.

Stress is defined as a complex cascade of events, consisting of a stimulus (stressor), which causes a subsequent reaction in the brain (stress perception) and activates physiological reactions (stress response) (Dhabhar & McEwen, 1997; Ciliberti et al., 2017).

A stressor that lasts for a few minutes to hours is defined as acute stress, while a stressor that persists for several hours a day for weeks or months is defined as chronic stress (Dhabhar, 2002; Ciliberti et al., 2017).

Determination of cortisol is one of the most widely used methods of stress assessment in animals, because it provides information about the activity of the hypothalamic-pituitary-adrenal axis. The most frequently collected biological samples for cortisol dosing are: blood (serum, plasma), saliva, urine, feces, milk and hair (Casal et al., 2017).

MATERIALS AND METHODS

The study was conducted in 2019-2020 on three batches of pigs. The pig slaughtering technological flow was monitored in the slaughterhouses and blood samples were collected.

- Batch 1: 8 blood samples collected from a batch of 150 pigs, the Great White breed with an approximate body weight of 110-120 kg and the age of 8-9 months, slaughtered in a slaughterhouse, using stunning.
- Batch 2: 10 blood samples collected from a batch of 390 Metis breed pigs with an approximate body weight of 120-130 kg and the age of 7 months after slaughter in a slaughterhouse, using stunning.
- Batch 3: 12 blood samples collected from traditionally slaughtered pigs in the household of the population, the Metis breed, about one year old.

In the case of slaughter in the slaughterhouse, the pigs enter the adduction corridor and are electrically stunned, by positioning two electrodes at the level of the head. Immediately after stunning, hanging on the airline takes place and the next stage is bleeding.

In the case of traditional slaughtering, the pigs are slaughtered without stunning, by stabbing.

The aim of this study is to perform laboratory tests aimed at dosing cortisol from blood samples collected at the time of bleeding (approximately 9 ml of blood collected in a BD Vacutainer - Clot Activator Tube) (Figure 1). Blood samples were immediately transported to a specialized laboratory, and the cortisol level was dosed by the immunoenzymatic method by chemiluminescence detection.

In order to determine these parameters, specialized training and laboratory equipment, as well as specific materials and reagents are required.

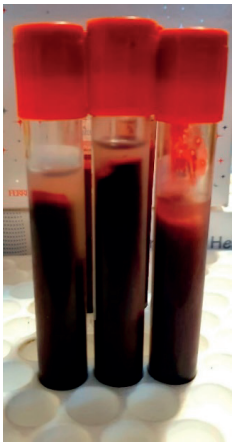

Figure 1. Blood samples

Animal welfare during transport and slaughtering is a matter of concern for consumers. It is necessary to pay attention to animals during transport, before slaughtering and during slaughtering (Petcu, 2015; Small & Hewitt, 2017).

People who understand the behaviour of animals will be able to board them properly into the means of transportation meant to carry them to the slaughterhouses, although stress is inevitable during the transport of animals from farm to slaughterhouse (Ferguson & Warner, 2009). Behavioural principles are recommended for the transport of animals, because this contributes to their welfare (Grandin, 2010; Grandin, 2019).

It is important to adopt good practices during slaughter, including systematic checks to determine when the animal begins to lose consciousness and when it loses it completely (Velarde & Dalmau, 2018).

In assessing the level of stress, the following variables should be taken into account: accidental fall of animals, refusal of animals to enter the containment box, excessive movement of animals during containment (Grandin, 2018).

Animals that become stressed before slaughter will have high levels of blood lactate concentration and will be more likely to have harder muscles. A calm animal that did not became restless and frightened will be more easily manipulated and will also be safer for the slaughterhouse staff (Grandin, 2010; Grandin, 2019).

The slaughtering without stunning is performed mainly for the purpose of religious sacrifice (Halal and Kosher), but also for the traditional sacrifice practiced in Romania for many years. If the animals are conscious during slaughtering, the risk of suffering increases. Immobilization of conscious animals for the purpose of cutting the neck causes stress. The incision made in the neck to cut the blood vessels, involves substantial damage to tissues in areas well represented by nociceptors (activation of the nociceptive system of protection induces suffering, pain in the animal). Death is not immediate and there is a period when the animal is still conscious and can feel anxiety, pain, suffering (Velarde & Dalmau, 2018).

Electrical stunning in pigs

Proper handling of animals during the slaughter process in well-designed units will minimize stress levels, improve efficiency and maintain good meat quality (www.grandin.com).

Handling in the last five to ten minutes before stunning the animals will have a significant effect on the blood lactate concentration. Studies have shown that high levels of lactate are associated with intense handling of animals that leads to stress. Also, improper electrical stunning of pigs and imposition of a second stunning leads to animal stress (Benjamin et al., 2001; Hambrecht et al., 2004; Hambrecht et al., 2005).

Slaughtered animals are stunned in order to enter a state of unconsciousness, insensitivity and immobility before bleeding. This state of unconsciousness should last long enough to ensure that the animal does not feel pain during bleeding (Wormuth et al., 1981; Schutt-Abraham, 1982; Gregory & Wotton, 1990; Hillebrand et al., 1996).

The effectiveness of the stunning process induces a state of instant unconsciousness and insensitivity to pain, which lasts until the death of the animal and has no negative effect on meat quality (Savenije et al., 2002; Joseph et al., 2013).

Animal welfare during slaughter was one of the major criteria that led to the formation of legislative requirements on stunning animals worldwide (Joseph et al., 2013).

Presently, the emphasis is on improving the animal's slaughtering process and new slaughtering procedures are followed, thus implementing various handling, stunning or monitoring techniques. The effectiveness and efficiency of stunning are of the utmost importance in facilitating the slaughter of animals, both for welfare and legislative reasons (Grandin, 2002; Atkinson et al., 2013; Grandin, 2019; Wagner et al., 2019).

Electrical stunning or electronarcosis is the passage through the brain of an electric current with voltage, amperage and frequency related to the species, which causes a disruption of normal brain activity, so that there is an immediate loss of consciousness and sensitivity. The efficiency of electronarcosis results from the interaction that is established between current, application time and the body's resistance. Practically, from the moment the two electrodes (positive and negative) are applied on the surface of the animal's body (Figure 2), the potential difference leads to the appearance of a current flow, with a certain

force, which will be counteracted by the resistance offered by skin (as a first obstacle) and the internal environment of the body (muscles, bones, blood vessels, etc.) (Guide on the protection of animals during slaughter, 2010; Petcu, 2015).

Figure 2. Electrical stunning in pigs

In all cases, the current level must be reached within one second from the start of the stunning and must be maintained for at least 1 to 3 seconds, according to the manufacturer's instructions. The electrodes must be placed so that they enclose the cranial box, allowing current to pass through it. The operator must ensure that there is a good electrical contact. In the case of pigs, the electrodes are located at the base of the ears, between the ears and the eyes. The alternating electric current, with low voltage is applied bilaterally, in the upper region of the skull, with the help of two electrodes of different shapes (Petcu, 2015).
Electrical stunning is based on the short-term action of electric current of a certain intensity and voltage on the central nervous system, causing paresis and loss of consciousness during the time in which the bleeding occurs (Petcu, 2015).
It is undeniable that an insufficient amperage or a current that after touching the animal's head takes it in another direction, without actually crossing the brain, will not induce the necessary state of unconsciousness, but pain caused by electric shock (Petcu, 2015).

RESULTS AND DISCUSSIONS

The period and method of slaughter are very complex and can represent different types of stress for the animal. How animals react to these stressors depends on their individual emotional reactivity (Deiss et al., 2009).
It has been shown that there is a direct correlation between meat quality and how animals are slaughtered (with or without stunning).

Results and discussions about the cortisol level in blood serum

Stress before slaughter has a negative impact on animal welfare and meat quality (D'Eath et al., 2010).
Determination of cortisol level is one of the most widely used methods for assessing stress in animals, as it provides information about the activity of the hypothalamic-pituitary-adrenal axis (Casal et al., 2017).
Deiss et al. has shown that the highest levels of cortisol (measured from blood samples) were observed in isolated animals. In general, young animals showed higher cortisol values (Linares et al., 2008; Deiss et al., 2009).
Determination of cortisol level in blood serum samples collected from conventionally slaughtered pigs revealed different values, exceeding the reference interval set by Jackson et al in 2002.

Study 1 - Determination of cortisol level from blood serum samples harvested from conventionally slaughtered pigs in June 2019

Following the analysis of the cortisol level from the 8 blood serum samples harvested from conventionally slaughtered pigs in June 2019, it was observed that 7 of the total samples had higher values compared to the reference interval (2.6-3.3 μg/dL), a single sample recording an optimal cortisol level, namely 2.80 μg/dL.
The results obtained from the dosing of cortisol level in the samples of group 1 are presented in Table 1.

Table 1. Results of cortisol level dosing in conventionally slaughtered pigs in batch 1

No.	Species	Breed	Age	Sex	Slaughtering date	Method	Cortisol level µg/dL	Reference interval
1.	swine	Large White	8 months	M	06.06.2019	Immunological	2.80 µg/dL	2.6-3.3 µg/dL
2.	swine	Large White	9 months	M	06.06.2019	Immunological	**3.73 µg/dL**	2.6-3.3 µg/dL
3.	swine	Large White	8 months	M	06.06.2019	Immunological	**4.39 µg/dL**	2.6-3.3 µg/dL
4.	swine	Large White	8 months	M	06.06.2019	Immunological	**4.89 µg/dL**	2.6-3.3 µg/dL
5.	swine	Large White	8 months	M	06.06.2019	Immunological	**5.51 µg/dL**	2.6-3.3 µg/dL
6.	swine	Large White	9 months	M	06.06.2019	Immunological	**6.21 µg/dL**	2.6-3.3 µg/dL
7.	swine	Large White	8 months	M	06.06.2019	Immunological	**7.10 µg/dL**	2.6-3.3 µg/dL
8.	swine	Large White	7 months	M	06.06.2019	Immunological	**7.99 µg/dL**	2.6-3.3 µg/dL

Study 2 - Determination of cortisol level from blood serum samples harvested from conventionally slaughtered pigs in November 2019

In November 2019, 10 blood samples collected from conventionally slaughtered pigs in a slaughterhouse were analysed in a specialized laboratory. All cortisol values obtained by analysis using immunological examination exceeded the reference interval. The lowest value recorded was 4.63 µg/dL and the highest value 16.0 µg/dL. The accepted reference interval is 2.6-3.3 µg/dL.

Sample number 7 registered a value 4 times higher compared to the reference interval, and sample number 8 registered a value 5 times higher. The results are presented in Table 2.

Table 2. Results of cortisol level dosing in conventionally slaughtered pigs in batch 2

No.	Species	Breed	Age	Sex	Slaughtering date	Method	Cortisol level µg/dL	Reference interval
1.	swine	half-breed	7 months	M	18.11.2019	Immunological	**7.23 µg/dL**	2.6-3.3 µg/dL
2.	swine	half-breed	7 months	M	18.11.2019	Immunological	**4.63 µg/dL**	2.6-3.3 µg/dL
3.	swine	half-breed	7 months	M	18.11.2019	Immunological	**6.83 µg/dL**	2.6-3.3 µg/dL
4.	swine	half-breed	7 months	M	18.11.2019	Immunological	**7.23 µg/dL**	2.6-3.3 µg/dL
5.	swine	half-breed	7 months	M	18.11.2019	Immunological	**8.15 µg/dL**	2.6-3.3 µg/dL
6.	swine	half-breed	7 months	M	18.11.2019	Immunological	**9.67 µg/dL**	2.6-3.3 µg/dL
7.	swine	half-breed	7 months	M	18.11.2019	Immunological	**12.7 µg/dL**	2.6-3.3 µg/dL
8.	swine	half-breed	7 months	M	18.11.2019	Immunological	**16.0 µg/dL**	2.6-3.3 µg/dL
9.	swine	half-breed	7 months	M	18.11.2019	Immunological	**9.34 µg/dL**	2.6-3.3 µg/dL
10.	swine	half-breed	7 months	M	18.11.2019	Immunological	**7.56 µg/dL**	2.6-3.3 µg/dL

Comparing the two groups analyzed, it can be seen that the highest values of cortisol levels were recorded in the group slaughtered in November, compared to the group slaughtered in June. This most likely correlates with the low temperatures to which the animals were exposed in the cold season, as temperature is, according to numerous studies, an important factor influencing the stress level of animals.

Guerrini and Bertchinger showed that the lowest plasma cortisol values were recorded during exposure of animals in a warm environment, and the highest values were recorded at the time of their exposure in a cool and moist environment. These results suggest that exposure of animals in a moist and low temperature environment causes an increase in cortisol concentration (Guerrini & Bertchinger, 1982).

Study 3 - Determination of cortisol level from blood serum samples harvested from traditionally slaughtered pigs in December 2020

The samples of study 3 were collected, following the traditional slaughter of pigs, in December 2020 in the period before Christmas, from the households of the population from Dâmboviţa county.

12 blood samples were studied, five of them obtaining an optimal cortisol level, and the other seven exceeding the values of the reference interval, but not as much as in the case of the results obtained from pigs slaughtered with stunning in the slaughterhouse, which most likely correlates with the growth method practiced, with the fact that the animals do not suffer from transport

stress and with the fact that the animals do not sit in crowded lots and do not feel the reactions of those slaughtered before them. The results obtained are presented in Table 3.

Table 3. Results of cortisol level dosing in traditionally slaughtered pigs in batch 3

No.	Species	Breed	Sex	Age	Weight	Slaughtering date	Growth system	Method	Cortisol level µg/dL	Reference interval
1.	swine	half-breed	M	12 months	160 kg	12.12.2020	Household	Immunological	3.00 µg/dL	2.6-3.3 µg/dL
2.	swine	half-breed	M	12 months	180 kg	13.12.2020	Household	Immunological	2.49 µg/dL	2.6-3.3 µg/dL
3.	swine	half-breed	M	12 months	200 kg	13.12.2020	Household	Immunological	2.37 µg/dL	2.6-3.3 µg/dL
„4.	swine	half-breed	M	12 months	160 kg	14.12.2020	Household	Immunological	2.12 µg/dL	2.6-3.3 µg/dL
5.	swine	half-breed	M	12 months	190 kg	14.12.2020	Household	Immunological	**6.12 µg/dL**	2.6-3.3 µg/dL
6.	swine	half-breed	M	12 months	160 kg	18.12.2020	Household	Immunological	**5.43 µg/dL**	2.6-3.3 µg/dL
7.	swine	half-breed	F	12 months	130 kg	18.12.2020	Household	Immunological	**6.24 µg/dL**	2.6-3.3 µg/dL
8.	swine	half-breed	M	18 months	350 kg	19.12.2020	Household	Immunological	**3.74 µg/dL**	2.6-3.3 µg/dL
9.	swine	half-breed	M	12 months	140 kg	19.12.2020	Household	Immunological	**5.14 µg/dL**	2.6-3.3 µg/dL
10.	swine	half-breed	M	12 months	220 kg	20.12.2020	Household	Immunological	**7.26 µg/dL**	2.6-3.3 µg/dL
11.	swine	half-breed	M	12 months	160 kg	21.12.2020	Household	Immunological	**4.20 µg/dL**	2.6-3.3 µg/dL
12.	swine	half-breed	M	12 months	200 kg	21.12.2020	Household	Immunological	2.41 µg/dL	2.6-3.3 µg/dL

Śmiecińska et al. in 2011 conducted a study on a batch of 24 pigs slaughtered immediately after transport and a batch of 20 pigs slaughtered after a 24 hour rest period. The cortisol level recorded an average value of 26.54 µg/dL in pigs slaughtered immediately after transport and an average value of 15.44 µg/dL in the group of pigs slaughtered after a rest period of 24 hours.

Batches 1 and 2 of the present study were slaughter after a rest period and had a mean cortisol value of 7.33 µg/dL, which is lower than the results of the above study, but which exceeds the reference interval. of 2.6-3.3 µg/dL.

Batch 3 represented by blood samples from pigs slaughtered in the traditional system, recorded an average cortisol level of 4.21 µg/dL, this being a value close to the maximum limit of the reference interval (2.6-3.3 µg/dL).

Increased cortisol levels are an indicator of the stress response of animals, resulting from the stimulation of the sympathetic and parasympathetic nervous system and the hypothalamic-pituitary-adrenal axis (Śmiecińska et al., 2011).

The above stimulates the adrenergic system to produce catecholamines and improves the secretion of steroid hormones, mainly cortisol, from the adrenal cortex (Zavy et al., 1992). Handling operations before slaughter induce an intense response to stress (Śmiecińska et al., 2011). At the same time, rest before slaughter physiologically balances the body and alleviates the stress induced by pre-slaughter manipulation (Gispert et al. 2000; Fischer, 2001; Śmiecińska et al., 2011).

The results obtained from the summary statistics (mean values and standard deviation) of blood samples collected are shown in Table 4.

Tabel 4. Summary statistics of cortisol level in blood serum samples (mean values and standard deviation) harvested from slaughtered pigs

Batch number	Cortisol (mean values and standard deviation)	Samples number
1	5.3275±1.73425	8
2	8.9340±3.267324	10
3	4.2100±1.785344	12

CONCLUSIONS

In the slaughterhouses from the study, all technological stages of animal slaughter are observed. No accidental fall of the animals on the supply corridor was observed, nor was their refusal to enter the containment box. The stunning method practiced is electric stunning.

Excessive handling of pigs before slaughter induces their stress, therefore special attention must be paid to the slaughter process in order to minimize stress levels and improve meat quality.

Respecting the rest period before slaughter physiologically balances the body and alleviates the stress induced by animals handling.

The highest values of cortisol levels were recorded in the batch slaughtered in November, compared to the batch slaughtered in June, which most likely correlates with the low temperatures to which the animals were exposed, as temperature is an important factor that influences the stress level of the animals.

Samples collected from traditionally slaughtered pigs obtained lower cortisol levels compared to blood samples collected from conventionally slaughtered pigs, which most likely correlated with the way the animals were grown, with the fact that they did not suffer from transport stress and the fact that the animals do not live in crowded batches.

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A PRELIMINARY STUDY ON THE POTENTIAL OF ROMANIAN NATIVE FLORA TO OBTAIN MILK-CLOTTING PLANT PROTEASES

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Abstract

The increase in cheese consumption worldwide, in symbiosis with the promotion and consolidation of lacto-vegetarian diets, as well as with the support of foods with religious restrictions (Halal, Kosher), has imposed a sustained trend of identifying plant proteases with milk-clotting activity. In the spirit of this trend, appeared the necessity to scan the native flora of Romania, to find the local resources of vegetal milk-clotting enzymes. This article presents a series of tests performed on indigenous plants like Taraxacum officinale, Rumex acetosa, Lactuca sativa, Urtica dioica, to identify and evaluate the potential for milk coagulation, knowing that they are sources of plant protease. The research involved the use of plant tissues as such, but also in order to obtain extracts that were later used in milk coagulation. The analysis consisted in establishing the milk-clotting activity (according with Soxhlet method), but also physic-chemical and sensory analyses. The obtained results revealed milk-clotting activity on almost all plants researched. A more accurate characterization of the purified plant milk-clotting enzyme would be interesting to be performed in the future.

Key words*: cheesemaking, milk-clotting activity, plant rennet.*

INTRODUCTION

Milk is a natural product of mammals with the role of feeding and providing immune protection for newborns. It has also been a valuable source of food for humans since prehistoric times. The domestication of animals took place more than 6,000 years ago. Sanskrit writings attest to the fact that milk was already an important food at that time. It was so valuable that the ancient Hindus valued welfare according to the number of cattle. Over time, cows have become sacred animals, and are still considered a part of India's population (Ciocârlie, 2002).

Among animal foods, milk is the most complete and most easily assimilated food by the body, being one of the staple foods in human nutrition, especially during growth, because it contains all the substances necessary for normal growth and development of the body. Milk is the food strictly necessary to feed the sick, the elderly and all those who work in a toxic environment or in difficult working conditions. Being an excellent food product, a very varied assortment of food products can be prepared from it. Historical documents show that the pieces of cheese were brought as a gift to the kings of that time and also served as objects of exchange for nomadic peoples (Guzun, 2001). Enzymatic coagulation of milk is an important step in the cheese-making process. In general, coagulation of milk with calf rennet is the most used procedure. Research over time has shown that several plant proteases have the ability to coagulate milk (Liburdi et al., 2019). They are generally extracted from the leaves and stems of plants.

According Abebe et al. (2020), the high prices of calf rennet, refusal to accept cheese made from animal rennet in general and porcine rennet by vegetarians and Muslims, respectively, requested the need to substitute animal rennet with easily available, relatively cheap, and acceptable source of rennet for cheese preparation.

Milk-clotting is the main stage of cheese production. It is made with milk-clotting enzymes that are prepared by proteolytic enzymes, this being the oldest application of enzymes, known for thousands of years. Historically, clot extracted from ruminants' stomachs is the most widely used source of enzymes in the manufacture of cheese (Bornaz et al., 2010), although there is evidence of early use of coagulation enzymes in microorganisms

and plants. Genetically engineered clot substitutes produced by microorganisms have been shown to be suitable substitutes for animal rennet, but growing interest has been directed at plants rennet, it means, enzymes for coagulating milk extracted from plants (Manzoor et al., 2014).

Animal rennet is a complex of enzymes produced in the stomach of any mammal, including proteases that coagulate milk, producing its separation into a solid part (curd) and a liquid part (whey). The presence of these enzymes is very important in the stomachs of young mammals for digesting the breast milk with which they are breastfed. The main active enzyme in the clot is called chymosin or renin (EC 3.4.23.4), along with pepsin and lipase. The natural calf rennet is extracted from the inner mucosa of the fourth chamber of the stomach of young, unweaned calves. The rennet extracted from older calves, fed on grass or cereals, contains less or no chymosin, but more pepsin. This rennet can only be used for some special types of milk and cheese. Each ruminant species produces a special type of rennet to digest the milk of its own species, so there is, for example, a goat's rennet for coagulating goat's milk or a lamb's rennet for coagulating sheep's milk.

The researches targeting the clotting of milk with the aid of plant coagulants has shown a growing interest in the industry of milk and dairy products, due to both the easy availability of raw materials and simple extraction processes (Shah et al., 2014). Another argument in the use of clots obtained from plant sources is that the use of vegetable proteases in the process of obtaining cheeses promotes greater acceptability of this range of products from people with a vegetarian diet, to which are added certain benefits represented the fact that they can improve their nutritional intake with various bioactive compounds from plant sources used in the process of milk coagulation.

In view of the aspects mentioned above, in our study it has been established as objective the evaluation of the coagulation potential of some vegetal extracts coming from plants harvested from the spontaneous flora, from our country, in the processing of cow's milk. The targeted plants were *Taraxacum officinale* (dandelion), *Rumex acetosa* (sorrel), *Lactuca sativa* (lettuce) and *Urtica dioica* (nettle).

Taraxacum officinale is an herbaceous, perennial plant, with a height of 20-50 cm, with truncate leaves, arranged in a basal rosette and with yellow flowers grouped in calatids. The parts used for medicinal use are: roots that contain bitter principles, young leaves and juice. The plant is rich in triterpenoids that have anti-inflammatory properties (Oroian, 2018). More recent research has shown that in addition to salicylic acid, the leaves also contain vitamins A, B, C and D, and a substance called choline, which is also found in the gallbladder (Hoffmann, 2016).

Rumex acetosa it is a perennial plant found in spontaneous flora in almost all regions of the globe. It is a very popular crop plant in some regions. It is cultivated for its leaves rich in iron, vitamins and oxalic acid, having the advantage that it appears very early (Gescher et al., 2011). It has the highest content of oxalic acid (approximately 5.27%) in dry matter. From a medical point of view, it is considered a vegetable with emollient action due to the high content of oxalic acid, which gives it its specific sour taste (Drăghici, 2009).

Lactuca sativa has been cultivated since ancient times by the Egyptians, Greeks and Romans and is also popularly called lettuce. Lettuce was brought to the new world by Christopher Columbus. It is an annual plant with a short vegetation period. It is cultivated for its leaves and heads, which are eaten mainly fresh. The heads contain large amounts of: vitamins (C, A, K, B complex), mineral salts (720 mg per 100 g, of which 234 mg potassium, 37 mg calcium, 24 mg phosphorus, 11 mg magnesium, the rest being iron and zinc) (Burzo et al., 2005), as well as significant amounts of sugar, polyphenols and cellulose. It is a low-calorie vegetable, being recommended in all diets. Eating this plant reduces the risk of heart disease, cancer and cataracts. Are very rich in vegetable fiber, which can significantly reduce cholesterol and prevent constipation, can induce a feeling of satiety much faster and thus help to lose weight or keep weight within optimal limits. *Lactuca sativa* is a remineralizing, purifying, emollient vegetable (Pârvu, 2006). The leaves can be eaten fresh, in the form of a salad, in early spring and autumn.

Urtica dioica, considered in ancient times the queen of plants, we must not miss our diet. It is consumed only in spring, from March to May, when they are young. It ensures a constant supply of biologically active substances, which restore our tone in the spring. Nettle is rich in chlorophyll, mineral salts (calcium, magnesium, potassium, iron, silicon), protein, pantothenic acid, folic acid, vitamins (B1, B2, C, K, beta-carotene). Nettle vitaminizes and remineralizes the body, eliminates anemia, balances the body's defense system with detoxifying effect, has a beneficial effect in bronchitis and asthma, fights cough, promotes the elimination of uric acid by the kidneys, is a hair tonic and fights dandruff. It is found in spontaneous flora all over the globe except in very cold or excessively hot areas. It is an herbaceous, dioecious, perennial plant that grows up to 150 cm in height. In the soil it forms a thin, cylindrical, whitish, long and branched rhizome. The stems are straight, with 4 edges the leaves are opposite, toothed at the edges. Both the stem and the leaves are provided with stinging hairs. At the base, the leaves are cordate. It blooms in June (Drăghici, 2009)

MATERIALS AND METHODS

The experiments were carried out in the "Engineering Processing" and "Food Biotechnology" laboratories, from the Faculty of Biotechnologies, USAMV Bucharest.

Raw milk. As coagulation substrate was used non-pasteurized raw cow milk delivered in the automatic equipment from UASMV Bucharest campus, originated from the didactical farm Belciugatele.

Plants sources of vegetal proteases. All the plants tested in this study (*Taraxacum officinale*, *Rumex acetosa*, *Lactuca sativa* and *Urtica dioica*) were harvested during spring season from natural habitats in Bucharest-Ilfov geographical. For the test the plants were conditioned as aqueous extracts, as follows: sort the leaves, flowers, roots and stems; crushing and grinding of the selected plant material takes place; the preparation of the extract consists in the addition of distilled water over the ground vegetable material; stir the mixture; followed by filtration of the mixture and centrifugation for 15 minutes at 40°C, then separation of the supernatant to obtain the extract; the last step is to store the extract in refrigerated conditions at 4°C.

Animal rennet used in experiments. Rennet is an enzyme used to coagulate milk in the process of forming cheeses. Commercially purchased rennet, called IDEAL, is a source of chymosin, obtained by fermentation (Figure 1) and was employed as control. The use instructions refer to the use of 2 ml of solution for 10 l of warm milk (35°C) and shaking the solution before use. The milk-clotting time mentioned in the instructions is 45 minutes. The net mass of the package is 100 ml, and the coagulation power of the curd is 180 IMCU*/ml, (*international milk coagulation units). Indications: Heat the milk to 35ºC and add 2 ml of rennet to 10 L of milk**.**

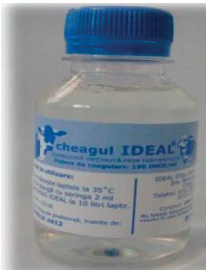

Figure 1. Commercial Rennet IDEAL

Methods used in experiments. In order to perform the experiments to highlight the ability of coagulation with plant extracts, different steps were performed, as follows. In an initial step the physical and chemical analysis of the raw cow's milk, was analyzed by the use of an EKOMILK analyser which provides information regarding the fat percentage, dry matter, density, added water, protein content; also, by classical tools, were determined the milk pH (pH-meter) and milk acidity (titration)). Further, the vegetal extracts from *Taraxacum officinale, Rumex acetosa, Lactuca sativa, Urtica dioica*, were prepared; the cleaned plants were prepared as aqueous extracts and these vegetal extracts were added in milk (20 ml aqueous extract in 100 ml milk); samples were incubated for coagulation at 35°C; the resulting curd was analyzed for the water activity, dry matter and organoleptic properties.

Physico-chemical analysis. In order to perform the experiments, the following physico-chemical analyzes were determined: determination of pH, water activity, dry matter, determination of acidity, milk-clotting activity and determinations using the Ekomilk device.

Determination of pH. was performed with the aid of a pH meter of the INOLAB 720 WTW series type with automatic temperature compensator.

Water activity was measured by the use of a Novasina system.

Dry matter. The principle of the method is based on removing water from the product by heating at a temperature of 100-110°C and calculating the dry mass in the sample according to the below formula

$$\% \text{ dry matter} = [(m_2 - m_0)/(m_1 - m_0)] * 100$$

m_1-mass of the ampoule and the product to be analyzed, in g; m_2-mass of the ampoule and the residue after drying, in g; m_0-mass of the ampoule, in g**.**

Determination of acidity. The acidity was determined by titration with an alkaline NaOH solution until the milk sample is neutralized in the presence of phenolphthalein as an indicator by the aid of an automatic titrator Schott Titronic basic type

Milk Clotting Activity. One of the important properties of coagulating enzymes is their coagulation capacity quantified by Milk Clotting Activity (MCA). MCA is expressed by the amount of milk, taken in volumes, at 35°C, for 40 minutes (2400 seconds). MCA is calculated with the formula:

MCA = (2400*V)/(T*E) in which MCA = Milk Clotting Activity; E = volume of coagulated milk (in liters); T = coagulation time (in seconds).

The coagulation time represents the necessary period from the moment of the introduction of the coagulating enzyme in the milk until the appearance of the first curd flakes.

The coagulated milk sample is left until the curd is hardened and its appearance is appreciated as hard, firm curd, soft curd, dusty curd.

Soxhlet units are defined as the volume of fresh milk that can be coagulated by a unit volume of curd in 40 minutes (2400s) at 35°C (Costin G. M., 2003).

Yield calculation. The calculation relation is as follows:

R (%) = 100 $*$ CB / CL, where: R - yield (%); CB - the amount of cheese / curd obtained (kg) and CL - the amount of milk used (l). (Palicica et al., 2007)

Organoleptic analysis. In order to perform the sensory analysis of curd samples, certain quality indicators are monitored, such as: appearance, appearance in section, color, consistency, smell and aroma of the samples (Banu C., 2007), and the scoring system is performed using the method by comparison with a unit scoring scale (Table 1), from 1 to 5.

The notations used for the curd samples thus obtained were: PMI - sample of 100 ml of milk + 0.02 µl of commercially purchased animal rennet; P1 - test of 100 ml milk + 20 ml aqueous extract of *Rumex acetosa*; P2 - sample of 100 ml milk + 20 ml aqueous extract of *Taraxacum officinale*; P3- 100 ml milk + 20 ml extract of *Urtica dioica*; P4 - 100 ml milk + 20 ml *Lactuca sativa* extract.

Table 1. Scoring scale for organoleptic analysis

Quality appreciation stage	Number of points	General description of appreciation stage
Excelent	5	Excelent quality
Very good	4	Quality fully according with the specific of the product
Good	3	Good quality, proper
Satisfying	2	The product has slight defects that can be accepted
Unsatisfying	1	The product has obvious, multiple and systematic defects
Adulterated	0	The product has severe defects and can no longer be consumed

RESULTS AND DISCUSSIONS

The study of the effect of using plant coagulants that have as a source plants from spontaneous and cultivated flora, respectively *Taraxacum officinale, Rumex acetosa, Urtica dioica* and *Lactuca sativa*, in the process of obtaining cheeses, on the sensory and physico-chemical qualities of the finished product was performed by using as raw material unpasteurised cow's milk according to the working methods described above.

Results of physico-chemical determinations of cow's milk used as raw material

Obtaining cheese can be described as the process of removing water, lactose and some mineral salts from milk in order to produce a concentrate of fat and protein (Ciocîrlie, 2002).

Transforming milk into cheese is a more complex process that involves concentrating protein along with a variable fraction of fat and minerals, eliminating a significant amount of water and lactose (Costin, 2003).
The Table 2 shows the results of the physico-chemical analyzes obtained for the cow's milk sample.

Table 2. Results of physico-chemical analysis of cow's milk used as raw material

Sample	Analyzed parameters						
	pH	Acidity (°T)	Fat (%)	Non-fat dry mater (%)	Density (g/cm³)	Water added (%)	Protein (%)
Raw cow milk	6.6	19	3.53	7.97	1.027	0	3.01
Reference values*	6.4-6.7	15-21	3.5±0.1	8-8.5	1.027-1.033	0	3-3.2

*State Standard STAS 143-84 on the quality of raw cow's milk

As can be seen in the table above, the analyzed cow's milk had a pH of 6.6 and an acidity of 19 degrees Thorner, elements that reflect its quality and freshness, according to the reference values in STAS 143-84. Following the determinations performed, a fat percentage of 3.53% and a dry matter value of 7.97% were recorded. Also, cow's milk had a density of 1,027g/cm^3 and a total protein content of 3.01%, values that fall within the reference ranges of STAS 143-84 for fresh milk.

Results of physico-chemical determinations

The curd samples obtained by adding vegetable rennet, from different plant sources, were analyzed physico-chemically, and the results obtained are presented as followed.
The curd samples obtained from the addition of plant rennet were analyzed physico-chemically in order to determine the resulted volume of whey, the amount of curd obtained, the determination of the pH of the whey, the water activity index of the curd samples, the dry matter content of the curd samples and the calculation of the yield, and the results obtained are presented in Table 3.
As shown in Table 3 the control sample of milk with rennet Ideal which is comparable to sample 2 of milk with *Taraxacum officinale* extract, both resulting in the same value of 83 ml. The other vegetal extracts registered relatively higher whey volume (an increase of about 18.5% in volume) which is a satisfactorily result.

Table 3. Curd samples - Results of physico-chemical determinations

Sample code number	Whey volume (ml)	pH whey	aw curd	Dry matter curd, %	Yield, %
PMI	83	4.73	0.892	57.74	11.52
P1	99	4.56	0.888	34.51	14.76
P2	83	4.16	0.892	23.38	25.54
P3	98,5	5.05	0.894	66.67	6.59
P4	98	5.27	0.889	46.01	6.34

In terms of water activity, the data obtained for all samples are comparable and vary between 0.88 and 0.89, which is an index value that support an increase in the shelf life of the finished product.
The pH of the analyzed whey had close values, between 4.16 and 5.27, which indicates a slightly acidic environment that favored the coagulation of cow's milk, while the initial milk pH was around 6.6 (Table 3).
The dry matter determined for the curd samples obtained reveals both the degree of separation of the curd from the whey following the filtration / separation operation, and the ability of the three-dimensional structure of the curd to retain water. This varies in a fairly large range for the analyzed samples, respectively 23.38-66.67.
Regarding the efficiency of the coagulation process (yield, %), which reflects the amount of curd obtained from the volume of milk tested, respectively 100 ml of milk, it is observed in Table 3 that the lowest value was presented by the P4 sample with *Lactuca sativa* (6.34%), closely followed by the P3 sample with *Urtica doica* (6.59%). Almost double yield was registered in the case of sample P1 with *Rumex acetosa* (14.76%) while for the sample P2 with *Taraxacum oficinale* the volume of separated whey was significantly higher 25.54%). Actually, *Taracaxum officinale* has been reported before as a potential milk coagulant (Mahajan & Chaudhari, 2014). Also, the possibility of using *Rumex* juice in the amount of 9 ± 0.5% as a coagulant of milk proteins has been confirmed by Grek et al. 2017.
The MCA (Milk Clotting Activity) was determined under small scale laboratory conditions, in 20 ml glasses tubes (Figure 2).
The results obtained from the determination of the milk clotting activity of cow's milk curd

with the addition of different vegetable coagulants are presented in the Table 4.

Table 4. Milk Clotting Activity of different plant extract proteases

Sample code number	Milk Clotting Activity (%)
PMI – Commercially rennet	0.0089
P1 – *Rumex acetosa*	0.0038
P2 – *Taraxacum officinale*	0.0017
P3 – *Urtica dioica*	0.0048
P4 - *Lactuca sativa*	0.0029

Table 4 shows the results obtained from the coagulation power test, according to the method proposed by Soxhlet. Thus, it is observed that the highest coagulation power is found in sample 3 with *Urtica dioica*, followed by sample 1 with *Rumex acetosa*, sample 4 with *Lactuca sativa* and sample 2 with *Taraxacum officinale*. However, the MCA's recorded in the plant extract samples have lower values than the commercially purchased rennet reference sample.

Different authors have used many different methods and units, so this makes it difficult to compare the coagulation capacity of different coagulants of plant origin. An important role is also played by a prior selection of the type of milk on which the coagulation capacity of these coagulants from plant sources is tested, in order to obtain specific or desired varieties of cheese. Despite the successes achieved for certain types of preparations, the production of cheeses obtained from coagulation with clot of vegetable origin on an industrial scale is quite limited and marginal.

Figure 2. Milk Clotting Activity Determination under small scale laboratory conditions

Results of sensorial analysis

The sensory analysis of the milk samples coagulated with vegetal rennet from different vegetal sources consisted in the use of the method, described in the previous chapter, respectively the awarding of points to the quality indicators according to the sensory analysis. The results are described in Table 5.

Table 5. Senzory Analyses - Average scores

Sample code number	Number of points awarded to quality indicators					Average score
	Appearance	Texture	Firmness	Smell	Color	
PMI	4	4	3	4.5	5	4.1
P1	4	4	3	4	4.5	3.9
P2	4	4	2	4	5	3.8
P3	1	1	0.5	4	4.5	2.2
P4	2	2	1.5	4	4.5	2.8

The highest score was obtained by the PMI sample with Ideal rennet, followed at a very short interval by sample 1 with *Rumex acetosa* and sample 2 with *Taraxacum officinale*.

Lower scores were obtained in sample 4 with *Lactuca sativa* and sample 3 with *Urtica dioica*. The appearance of the curd is shown in Figure 3.

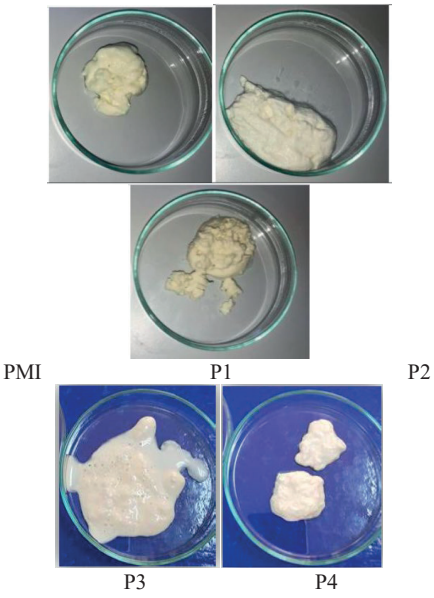

Figure 3. Curd obtained with different types of vegetal rennet (PMI - Commercially rennet; P1 - *Rumex acetosa;* P2 - *Taraxacum officinale;* P3 - *Urtica dioica* P4 - *Lactuca sativa*)

CONCLUSIONS

Following the study carried out and based on data from the literature, a series of conclusions can be issued, which are presented below.

Coagulation of milk with coagulating enzymes of vegetable origin is a feasible solution in the cheese-making process.

Several plant sources have been used to study the coagulation capacity of milk, including *Taraxacum officinale, Rumex acetosa, Urtica dioica, Lactuca sativa*. These plants are found in the spontaneous and cultivated flora of Romania, so they were harvested and used in the form of aqueous extracts. When studying a potential replacement for animal clot, it is particularly important to perform a test of milk coagulation activity.

From the experimental results presented in this article, a series of conclusions can be drawn, which are briefly presented below.

Following the physico-chemical determinations carried out on the raw cow's milk used as raw material for the preparation of the curd, a very good quality and freshness was found, and the values recorded were within the limits provided by the standards in force.

Following the determination of the dry matter for the curd samples obtained, it was observed that the milk sample to which the *Taraxacum officinale* extract was added had the lowest dry matter content and therefore the lowest energy value.

In the milk sample in which *Rumex acetosa* extract was added, a curd formed very well separated from the whey, obtaining the largest volume of whey among the analyzed samples.

The addition of a source of fresh and ground plant rennet, respectively of *Rumex acetosa*, in cow's milk leads to obtaining a low value of the water activity index, which implies an increase in the shelf life of the finished product. In this respect, the results obtained underlined the fact that the sample with the addition of *Rumex acetosa* reached the lowest value of the aw index. At the opposite pole was the test milk sample clotted with commercial animal rennet, which recorded the highest value of the water activity index.

Regarding the coagulation activity, the best results were obtained with the extract of *Urtica dioica*, the value being, but at half compared to the animal rennet used.

The results obtained after the organoleptic analysis of the curd samples obtained with plant rennet from different plant sources suggest that the sample with the addition of *Rumex acetosa* was preferred, being the sample that obtained the highest score, respectively 4.6 on a 5-point scale.

Data obtained from physico-chemical analyzes of the raw material and the finished product and organoleptic analyzes of curd samples with the addition of different plant coagulants led to interesting results in terms of quality and preference of finished products. From the point of view of preservability, there was a decrease in the values of the aw index in the case of the addition of coagulants compared to the control sample.

The studied plants, respectively *Rumex acetosa*, *Taraxacum officinale*, *Urtica dioica* and *Lactuca sativa* proved that they have the potential to coagulate cow's milk and can be a source of milk-clotting enzymes in cheese-making.

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STUDY CONCERNING THE POTENTIAL OF DRIED SEA BUCKTHORN AND LINGONBERRIES TO DEVELOP VALUE-ADDED PORK PRODUCTS

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Abstract

The paper presents a study on the possibility to enrich in bioactive compounds some baked meat products by using in their manufacturing recipe dried and ground sea buckthorn and lingonberries. Both sea buckthorn and lingonberries are valuable fruits due to their high content in bioactive compounds. Pork is also a food appreciated for its content in proteins with high biological value. The aim of the study was to obtain nutritionally balanced products, which benefit at the same time from meat proteins and antioxidant compounds from fruits. In order to preserve as much as possible, the nutrients coming from ingredients, the fruits were dehydrated at a moderate temperature of 45-50°C, and the baking of the designed products was conducted according to a technological diagram in the temperature range 55-70°C. Thus, there were prepared 10 baked products with addition of dried and ground fruits, using pork from different anatomical part such as tenderloin, loin, ham, shoulder and belly. After preparation, the samples were assessed in terms of total antioxidants capacity, total phenolic content and microbiological properties. The experimental results recorded for designed baked products compared to fresh meat samples, without any addition, showed a significant increase in the total antioxidant capacity (up to 4 times) and in the total phenolic content (up to 2 times). The microbiological analysis consisting of assessing the presence and number of coliforms in the fresh meat and in the baked samples with incorporation of dry fruits, both unpackaged and packaged in vacuum and stored at 2-4°C. The microbiological analysis was performed immediately after baked products obtaining, respectively every 7 days for unpackaged products and every 10 days for the packaged samples. Our results reveal the high potential of dried sea buckthorn and lingonberries to be included as valuable ingredients to design value-added pork products.

Key words*: microbiological properties, sea buckthorn and lingonberries, total antioxidant capacity, total phenolic content, value-added pork products.*

INTRODUCTION

Meat and meat products are known as valuable sources of nutrients due their content in proteins with high biological value, vitamins, minerals, and different other micronutrients which the human body needs. Meat, especially pork, beef and lamb, has been part of human nutrition since ancient times and continues to be a dominant food in the diet of the modern consumer (Savu et. al., 2002; McAfee et al., 2010). In order to be eaten, the meat must be subjected to heat treatment. Also, cooking process makes the meat safe for consumption and for this reason it is often processed at high temperatures for a long time. But this practice has the great drawback that it causes a considerable loss of nutrients in the finished product, such as, water soluble vitamins, fats due to the fusion process, simultaneously with the initiation of browning reactions (Xiong, 2017). Also, the high temperature affects meat proteins being proved that at temperatures up to 100°C these are denatured (Bertram et al., 2006; Suleman et al., 2020). To solve these problems and preserve as much of the nutrients

as possible, modern technologies use the technics consisting of application of *Low-Temperature for a Long-Time* in meat processing. This method has many advantages primarily because the doneness can be controlled facilitating the establishment of the thermal balance between the product subjected to baking and the heating medium with effect on improving of meat tenderness. However, this method has the disadvantage that the aroma of the products is lower compared to that formed in meat prepared at high temperatures (Dominguez-Hernandez, 2018). The assortment of meat products is very varied, which are processed by applying different degrees of degradation of muscle structure along with the use of different food ingredients in order to improve their sensory characteristics and their preservation (Jiménez-Colmenero, 2001; Vitaglione & Fogliano, 2004; Nicorescu et al., 2018). There are many suppositions that processed meat consumption may cause different diseases in human body due to the ingredients used in the manufacturing formula and the technological parameters applied during processing (Jiménez-Colmenero, 2001; Vitaglione & Fogliano, 2004). The main factor considered responsible for unwanted changes in meat during processing is oxidation and associated effects (Kanner, 2007; Negre-Salvayre, 2008). The last studies in antioxidant topic have allowed meat researchers to identify new methods of minimizing the damages caused during meat processing by addressing technologies based on low temperatures and using of natural antioxidants instead of the synthetic ones which could have unwanted implication on the human health (Engel, 2015; Predescu, 2016; Kamala Kumari, 2019). A new trend in meat products preservation is the replacement of nitrites and a portion of salt with herbs and berries due their antioxidants and antimicrobial compounds (Haugaard, 2014). Also, meat scientist showed a real interest in the association of meat products with fruits and vegetables to improve their sensorial properties and to increase their biologically active properties by enriching them in vitamins, antioxidants, minerals, etc. (Bazhenova et al., 2020). Take into consideration the presented data, the aim of our study was to assess the potential of two much appreciated fruits, lingonberries and sea buckthorn to improve the biologically active properties of some pork products.

Bitueva & Ayusheeva (2011) used pre-crushed dehydrated pulp of lingonberry into minced meat products in order to replace 13-15% of bread. Lingonberries are recognized and highly valued for their antioxidant properties and the content in biologically active compounds, such as: minerals, vitamin C, carotene, and organic acids (Bitueva & Ayusheeva, 2011).

Sea buckthorn (*Hippophae rhamnoides* L.) is considered one of the most valuable fruits in terms of composition in bioactive compounds due to the high content in polyphenols, of which quercetine and flavonols are present in various forms in large amounts, vitamins, carotenoids and minerals (Rösch et al., 2003; Guliyev et al., 2014). Sea buckthorn is widely used as therapeutic treatment in various diseases of the human body among the best known positive effects on health is the reduction of cholesterol levels in the blood, increase of immunity, preventing thrombosis (Khan et al., 2010; Ma et al., 2019; Shkolnikova et al., 2019). Although the beneficial effects on health are well known, there are few studies of the use of sea buckthorn in the processing of meat products.

The goal of this paper was to presents the effect of using dehydrated lingonberries and sea buckthorn on the antioxidant and microbial properties of cooked pork products.

MATERIALS AND METHODS

Fruit mixtures preparation

After thawing, fruits of lingonberries and sea buckthorn were dehydrated at a moderate temperature of 45-50°C, in order to preserve their nutrients and then were ground with a laboratory mill to a granulation close to that of semolina flour and mixed 3:1w/w.

Baked pork products preparation

10 samples of pork from different anatomical part such as tenderloin, loin, ham, shoulder and belly were prepared by addition of mixture of dried and ground fruits as a filling, but also on the surface in order to form a crust meant to improve the appearance of the products. Along with the dehydrated fruits in the basic manufacturing formula of all baked pork

products, salt and pepper were used. In addition to these ingredients, cinnamon was added to 5 of the samples and chili was added to the other 5 samples (Table 1). After that, the meat samples were backed in a smoking cell with a closed smoke flap according to a technological diagram in the temperature range 55-70°C until it reached 58°C in the technological center of the product. After cooling some of the meat samples were packed in vacuum and together with the unpacked ones were kept at 2-4°C until the analyzes were performed.

The total polyphenolic content was determined by Folin-Ciocalteu method (Folin & Ciocalteu, 1927; Singleton et al., 1999). The method consists in measures the reductive capacity of polyphenols from samples compared to hexavalent molybdenum in polyphosphomolybdate from Folin-Ciocalteu reagent. In order to perform the analysis, 2 g of each sample (fresh meat, baked products and dehydrated fruits were mixed with 20 mL of 70% methanol solution. After two hours, 0.5 mL from each prepared solution was mixed with 2.5 mL of Folin-Ciocalteu reagent 1:10 v/v aqueous solution and 2 mL of a 7.5% sodium carbonate solution. After 30 minutes of incubation in the dark the absorbance of the mixture was read at 750nm wave lengths using a UV–VIS spectrophotometer (SPECORD 205, Analytic Jena). The total polyphenols concentration was expressed as mg gallic acid equivalents per 100 g of sample.

The total antioxidant capacity of the samples was evaluated by CUPRAC method was used (Özyürek et al., 2011). The method consists in the reduction of the copper-neocuproine complex in the presence of ammonium acetate. The product of the reduction reaction is the yellow complex copper-neocuproine [Cu(Nc)2]+, with has a maximum absorption at 450 nm wavelengths. TROLOX (6hydroxy-2,5,7,8-tetramethylchromate-2-carboxylic acid) as references substance was used. The analysis consisted in mixing of 1 mL of copper solution with 1 mL of alcoholic ligand solution, 1mL of acetate buffer and 1.1 mL of sample. After keeping in the dark for 30 minutes the absorbance of the blank at 450nm is determined. The results were expressed in mM TE/100 g sample.

All results are expressed as mean values ± standard deviation (SD) and were obtained in triplicate. The Microsoft Excel 2010 program was used for statistical data processing.

Bacteriological analysis consisting of assessing the presence and number of coliforms germs in the baked samples with incorporation of dry fruits, both unpackaged and packaged in vacuum and stored at 2-4°C was performed immediately after products processing, respectively after 7 days for unpackaged products and every 10 days for 3 weeks in the case of vacuum packed samples (thermoscientific.com/microbiology, 2013). The analysis was performed according to standardized procedures, ISO 4832:2006. Samples were collected under aseptic conditions. After insemination by incorporation and solidification of the culture medium - MacConkey agar, the Petri dishes were placed in a thermostat at 44°C for 24 hours. This culture medium was used to differentiate the members of the coliform group into lactose-positive and lactose-negative germs, respectively.

The coding of the samples is presented in Table 1.

Table 1. Sample coding

Sample name	Sample code
Dehydrated Sea buckthorn	DSb
Dehydrated lingonberries	DL
Dehydrated fruits (lingonberries and Sea buckthorn) mixture	DFM
Baked pork ham with stuffing and crust of dehydrated fruit and chili	P1
Baked pork ham with stuffing and crust of dehydrated fruit and cinnamon	P2
Baked pork shoulder with stuffing and crust of dehydrated fruit and chili	S1
Baked pork shoulder with stuffing and crust of dehydrated fruit and cinnamon	S2
Baked pork tenderloin with stuffing and crust of dehydrated fruit and chili	M1
Baked pork tenderloin with stuffing and crust of dehydrated fruit and cinnamon	M2
Baked pork loin with stuffing and crust of dehydrated fruit and chili	C1
Baked pork loin with stuffing and crust of dehydrated fruit and cinnamon	C2
Baked pork belly with stuffing and crust of dehydrated fruit and chili	PI 1
Baked pork belly with stuffing and crust of dehydrated fruit and cinnamon	PI 2

RESULTS AND DISCUSSIONS

Total antioxidant capacity (TAC) of the raw materials used in manufacturing formula of the baked pork products (DL, DSb, DFM, pork: ham, shoulder, loin, tenderloin, belly) is presented in Table 2 and the influence of the addition of the dehydrated fruit mixture on the antioxidant capacity of the baked pork products is shown in the Figure 1.

Table 2. The total antioxidant capacity (TAC) of the raw materials used in manufacturing formula of the baked pork products

Sample	TAC (mM TE/g)
DSb	29.23±0.021
DL	29.45±0.020
DFM	29.40±0.019
ham	5.03±0.035
belly	4.52±0.032
shoulder	6.01±0.034
loin	6.12 ±0.035
tenderloin	5.85±0.030

The highest antioxidant capacity was registered for dry fruits and their mixture being in range of 29.45-29.23 mM TE/g, as is shown in table 2. The results are according with reported literature data (Bazhenova et al., 2020; Dróżdż et al., 2018). The lingonberries are one of the most appreciated fruits due to their richness in antioxidant compounds, such as: vitamins, organic acids or polyphenolic compounds (Dróżdż et al., 2018). Among the samples of fresh pork the highest antioxidant activity was determined for loin (6.12 mM TE/g) similar to the shoulder (6.01 mM TE/g). The lower TAC value was registered for belly (4.52mM TE/g). The obtained values are correlated with those reported by Serpen et al. (Serpen et al., 2012). Meat and also meat products are considered an important source of bioactive compounds such as vitamins, minerals, proteins or fatty acids. Of these, vitamins (group B, E or C) are considered responsible for the antioxidant activity of meat and come mainly from animal feed (Pogorzelska-Nowicka et al., 2018). The data in Table 2 show that the total antioxidant activity of mixtures of dehydrated fruits is 6.5 times higher than that of belly, 5.85 times higher than that of ham, 5.02 times higher than that of tenderloin, 4.88 times higher than that of loin and shoulder. Figure 1 presents the antioxidant capacity of baked pork products with addition of dried and ground fruits.

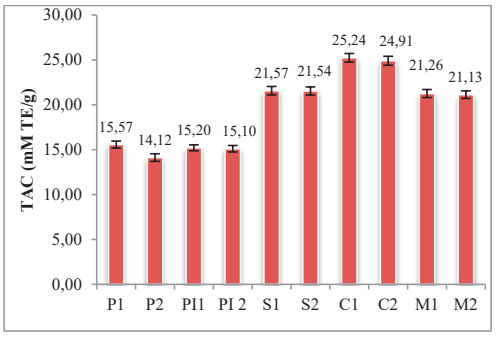

Figure 1. The antioxidant capacity of baked pork products with addition of dried and ground fruits mixture

The addition of dried and grounded fruits mixtures in pork products manufacturing formula has decisively influenced the total antioxidant activities of all baked samples. The TAC of final products is correlated with the TAC of raw meat. As seen in figure 1 and by reference to the data in Table 2 the increasing of total antioxidant capacity was 4.1 times (C1, C2), 3.6 times (S1, S2) and (M1, M2), 3 times (P1, P2), respectively 3.3 times (PI1, PI2) compared to fresh meat samples. The results are similar with that observed by Bazhenova et al., regarding the influence of lingonberry extract on the antioxidant capacity of meat paste (Bazhenova et al., 2020). Using of chili or cinnamon in receipts of pork samples did not influenced the total antioxidant capacity of finished products. The greatly increasing of TAC in baked pork products with the addition of dried fruit mixture is due to the abundance of antioxidant compounds in the fruit.

Total phenolic content (TPC) of the raw materials (DL, DSb, DFM, pork: ham, shoulder, loin, tenderloin, belly) used in manufacturing formula of the baked pork products is presented in Table 3 and the total polyphenol content of baked pork products with addition of the dehydrated fruit mixture is shown in the Figure 2.

The total phenolic content of dried lingonberries and Sea buckthorn (Table 3) are similar and correlated with the value determined for their mixture.

Table 3. The total phenolic content (TPC) of the raw materials used in manufacturing formula of the baked pork products

Sample	TPC (mg GAE/100 g)
DSb	91.49±0.021
DL	96.18±0.020
DFM	95.12±0.019
ham	15.64±0.035
belly	12.24±0.032
shoulder	12.05±0.034
loin	12.41±0.035
tenderloin	22.84±0.030

As results from Table 2, the highest content in phenols was registered by dehydrated lingonberries and is similar those reported in literature (Dróżdż et al., 2018). The phenol content of raw meat samples was substantial lower than the dried fruits mixture: 4.16 times lower tenderloin, 6.1 times lower in case of ham, and 7.7 times lower in average for loin, belly and shoulder. The highest phenolic content was determined for tenderloin (22.84 mg GAE/100 g) and the lower by shoulder (12.05 mg GAE/100 g).

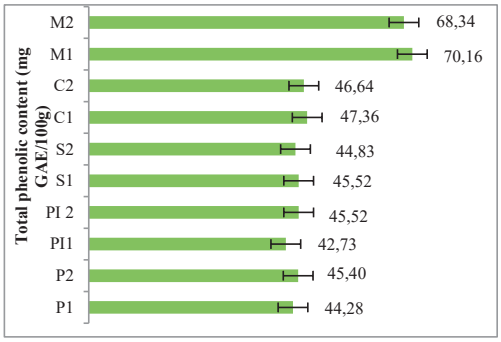

Figure 2. The total phenolic content of baked pork products with addition of dried and ground fruits

Both lingonberries and sea buckthorn are fruits recognized for their substantial content in polyphenols, which have a significant contribution to their antioxidant properties. Their use as a mixture in dehydrated and grounded form (DFM) has led to an increase in the polyphenol content of cooked baked meat products, as shown in the figure 2. Correlated with the phenolic content of fresh meat, addition of dried and ground fruit mixtures led to an increase in the TPC of baked pork products about 1.4 times in case of tenderloin (M1, M2) on average 2 times in loin (C1, C2), shoulder (S1, S2), ham (P1, P2) and belly (PI1, PI2). Similar to the total antioxidant activity, the content in polyphenols of finished baked products was not influenced by the use of cinnamon or chili in their manufacturing formula. Even there many studies which revealed the strong antioxidant character of these spices (Nagy et al., 2015; Wijewardhana et al., 2019), use in low concentrations (1%) in baked pork products did not affect their total phenolic content.

The results of bacteriological analysis of baked pork products with dehydrated and ground fruits showed the absence of coliform germs in all samples after 24 hours of thermostating at 44°C. Keeping the samples in the thermostat at the same temperature for another 24 hours led to the same results, indicating the absence of coliform germs in all processed pork products. Some photos of Petri dishes grown with the analyzed samples in order to isolate and identify the coliform germs are selectively presented in Figures 3-6.

Figure 3. Evaluation of the presence and number of coliform germs in baked pork ham with addition of dehydrated fruit mixture and chili (P1, P2)

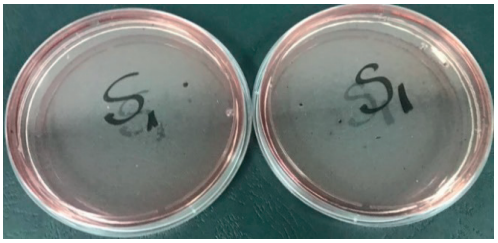

Figure 4. Evaluation of the presence and number of coliform germs in baked pork shoulder with addition of dehydrated fruit mixture and chili (S1, S2)

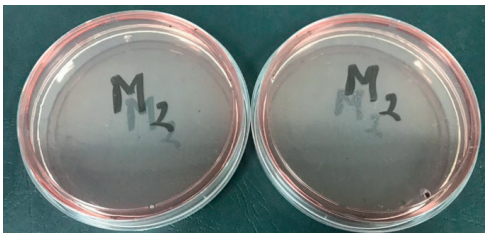

Figure 5. Evaluation of the presence and number of coliform germs in baked pork tenderloin with stuffing and crust of dehydrated fruit and cinnamon (M1, M2)

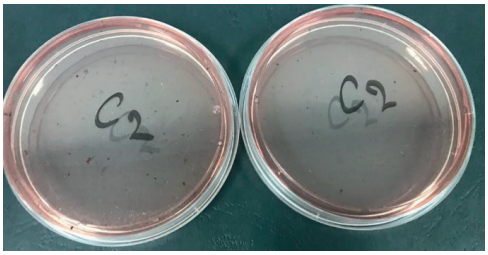

Figure 6. Evaluation of the presence and number of coliform germs in baked pork loin with stuffing and crust of dehydrated fruit and cinnamon (C1, C2)

In order to assess the microbiological stability of the baked pork product with addition of dried and ground fruits mixtures the development of coliform germs was verified after 7 days for unpackaged products and every 10 days for 3 weeks in the case of vacuum packed samples. The obtained results were the same with those registered immediately after processing and are useful for establishing the shelf life of products. Also it has to be mentioned that the using of cinnamon or chili in the manufacturing formula of the products did not influence the results of microbiological analyzes. The microbiological stability of baked pork product with the addition of dried fruits mixture could be attributed to the use of lingonberries and sea buckthorn in their manufacturing formula. The obtained results are according to those reported in the literature (Apostolidis et al., 2008; Wu et al., 2008; Wu et al., 2009; Caillet et al., 2012; Lacombe, 2012) which reveals that many foodborne pathogens including coliforms are inhibited by berries and help preserve food.

CONCLUSIONS

The addition of dehydrated and ground lingonberries and sea buckthorn mixture led to increase of the total antioxidant capacity of designed baked products compared to fresh meat samples up to 4 times and in the total phenolic content up to 2 times. The microbiological analysis conducted on both unpackaged and packaged in vacuum baked pork with dried fruits mixture and stored at 2-4°C demonstrated the absence of coliform germs after 7 days for unpackaged products and after 30 days for the packaged samples. The results of the study reveal the high potential of dried sea buckthorn and lingonberries to be included as valuable ingredients to design value-added pork products.

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STUDY ON THE CHEMICAL COMPOSITION AND NITROGEN FRACTION OF MILK FROM DIFFERENT ANIMAL SPECIES

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Abstract

The dairy industry occupies a very important place in the economy of our country. In order to obtain high quality dairy products, it is necessary to use a good quality raw material milk, which involves determining its chemical composition in particular. At present, on the shelves of our stores in the country there are products obtained from the processing of cow's milk, buffalo's milk, goat's milk and, last but not least, sheep's milk. For these reasons, in this paper we set out to perform a chemical analysis of milk from these species of interest. Following the determinations, the sheep's milk proved to be with the highest percentage of fat (7.70 ± 0.06%), SNF (11.40 ± 0.09%), TS (19.10 ± 0.04%), TP (4.98±0.04%) and in the casein content (3.65 ± 0.04%) being followed by the milk collected from the buffaloes. This study can support processors, especially those who process only cow's milk but also consumers who will be able to evaluate their products.

Key words*: milk, casein, quality, sheep.*

INTRODUCTION

Consumers have become more concerned about the consistency and protection of food items in recent years, and they have developed a strong interest in learning more about food authenticity and food fraud. In other words, customers want more detailed facts about their food, such as what they're purchasing, where it came from, and where and how it was made (McGrath et al., 2018).

World milk production derives from cows, buffaloes, goats, sheep, and camels, with buffalo milk being the second most consumed type after cow's milk (FAO, 2000).

In Romania, the most consumed types of milk are cow's milk, goat's milk, buffalo's milk and sheep's milk.

Cow milk is composed of different components including water, fats, proteins, ash, and lactose. The nitrogen-containing milk proteins can be classified into three main categories: caseins, whey (serum) proteins, and nonprotein nitrogen, which are also subdivided into several fractions (Urgu et al., 2019).

Milk is a biotic substance that animals have evolved to feed their newborns and provide essential nutrition for growth and development. Lactoferrin and lactoperoxidase are two milk proteins that have antimicrobial and immune-modulatory properties (Chen et al., 2019).

Goat's milk is an essential contribution to human nutrition, especially for people who are lactose-intolerant or sensitive to cow's milk. Goat's milk has been associated with low allergenic reactivity, antioxidant and anti-inflammatory effects, and prevention of atherosclerosis and cardiovascular diseases (Haenlein, 2004; O'Shea et al., 2004; Russell et al., 2011; Lad et al., 2017).

Buffalo milk is thought to contain almost all of the protective compounds present in other milks, such as proteins, peptides, fatty acids, vitamins, and other bioactive compounds. Total calcium, medium chain fatty acids, CLA, and retinol and tocopherol content are all higher in buffalo milk than in cow milk. Specific groups of gangliosides, for example, can only be found in buffalo milk (Berger et al., 2005).

As for sheep's milk, it is more used in obtaining cheeses, products that are in Romania are quite appreciated by consumers. Typical products have been developed according to local resources available. The production of cheese

with particular characteristics can be carried out only if genetic diversity in sheep rearing is retained. Milk composition, and especially proteins and fat, may vary according to genetic diversity of the animals and different feeding systems, giving peculiar features to the milk utilized to make typical milk products. Most sheep milk produced in the world is processed into cheese, yogurt and other dairy products. The specific composition of sheep milk makes it especially valuable nutritionally and for consumer health. The nutritional importance of sheep milk is due to its higher total solids and major nutrient contents than goat and cow milk (Ospanov and Toxanbayeva, 2020).

Although milk is a food appreciated by all consumers, its composition differs from species to species. We can also have differences in the case of milk from the same species, the influencing factors being given by the breeding system, diet, age of the animals, etc. (Kittivachra et al., 2007).

The largest group of milk proteins is caseins and whey proteins, which are present in varying ratios in various milk organisms. The casein to whey protein ratio in human milk is 40:60, in quine milk it is 50:50, and in cow, pig, goat, and buffalo milk it is 80:20 (Fox et al., 2000).

Proteins are the most essential components of the human diet, providing major chemical, biochemical, and functional properties. These proteins are considered high-quality proteins because of their high biological importance, high digestibility (97–98%), and fast absorption and utilization in the body. Casein, in particular, is an extraordinarily versatile food source because it provides a steady and gradual release of amino acids into the bloodstream (Schaafsma, 2000).

Present research set out to perform a study on the quality of milk composition from species of interest in our region, including cow, buffalo, goat, and sheep milk.

MATERIALS AND METHODS

Collecting milk samples

For each type of milk analyzed (cow's milk, buffalo, goat's and sheep's milk) ten samples were collected in sterile bottles, the milk coming from farms located in the NE region of Romania as follows: cow's milk was collected from a female located in Iași county, the buffalo milk was brought from a farm in Neamț county, the goat's milk came from a farm in Vaslui county and the sheep's milk from a farm in Suceava county.

The samples were brought in special bags provided with ice boxes and in the laboratory were stored in a refrigeration system at + 4ºC. Qualitative analyzes were performed on five samples within 24 hours in the Qualitative Milk Analysis laboratory at USAMV-Iași.

Physicochemical analysis

The pH value was determined with an electronic pH meter (WTW InaLab).

The AOAC method no. 925.23 (AOAC, 2005) was used to assess solids (TS) by dehydration in a Memmert UFE 700 forced air oven Water (W) content resulted from the difference, according to the relation: Water (%) = 100% - DM (%).

Fat of milk was determined by following Gerber method according to Dick et al., 2001.

Regarding the non-fat solid (SNF) content, this acetate was calculated by difference: SNF = TS (%) - Fat (%).

Crude ash content was assessed via incinerating at 550ºC, in a Super Therm C311 oven after prior combustion with a Bunsen funnel, until samples ceased to smoke, in accordance with AOAC 945.46 specification (AOAC, 2005).

The crude protein (CP), true protein (TP), casein, noncasein- nitrogen (NCN), whey proteins and non proteinnitrogen (NPN) contents were determined by using Kjeldahl method applied on a Velp Scientifica DK 6 digestion and UDK 7 distillation system according to standard protocol of IDF (1993). The total nitrogen content was multiplied by 6.38, which generated the crude protein content. The TP in the milk sample were determined by treating with 12% trichloroacetic acid. The nitrogen (%) was converted to NPN and NCN contents by using the conversion factor 3.60 and 6.25, respectively. Protein (nitrogen) fractions were calculated using the formulas described by Rafiq et al. (2016):

TP = CP–NPN,

Casein (N %) = Total protein (N%)–NCN (N %)

Whey protein = NCN–NPN.

Quantitative determination of amino acids was performed using the method described in the literature and using hight performance amino acid analyzer for the separation of amino acids, while tryptophan was determinated colorimetrically according to the method of (Opienska-Blauth et al., 1963; Ratu et al, 2017).

RESULTS AND DISCUSSIONS

The pH value is a very important qualitative parameter used especially in the milk processing industry. As can be seen in Table 1 with regard to the analysis of this parameter, no very large differences were observed between the milk samples analyzed.

Regarding the chemical composition of the analyzed milk samples, it can be seen that we have differences from one species to another. Therefore, for cow's milk the dry matter content was 12.45 ± 0.04% lower by 6.65% than that of sheep's milk and 4.84% lower than that of buffalo milk. For goat's milk the average value of fat content was 12.38 ± 0.18% (Table 1).

Regarding the fat content, the milk that had the highest value was the one from sheep, the average value being 7.70 ± 0.06%. Buffalo milk registered a fat content of 6.97 ± 0.04% and cow's milk of 3.86 ± 0.02% being also the lowest percentage in terms of this quality parameter. It was also considered necessary to calculate the non-fat dry matter, an index for which the highest average value was for goat's milk (11.40 ± 0.09%) followed by buffalo milk with an average value of 10.32 ± 0.04% of the milk. cow's milk (8.59 ± 0.05%) and goat's milk where the mean was 8.13 ± 0.18%.

When we talk about the protein level in milk, we must keep in mind that this is one of the most important parameters. According to the results obtained by the new parameters CP, TP, Casein, WP, NCN and NPN highlighted different values for the milk from each analyzed species.

For example, the highest values were obtained for sheep's milk where the average value for TP was 4.98 ± 0.04% followed by buffalo milk with an average of 3.98 ± 0.04%, after that from cow where the mean was at a level of 3.24 ± 0.02% and goat's milk where the mean value for TP was only 3.02 ± 0.04% (Table 2).

Table 1. The chemical composition of different milk species

Species	pH	W (%)	TS (%)	Fat (%)	SNF (%)	Ash (%)
Caw	6.51±0.04	87.55±0.04	12.45±0.04	3.86±0.02	8.59±0.05	0.69±0.004
Buffalo	6.62±0.01	82.70±0.02	17.29±0.02	6.97±0.04	10.32±0.04	0.84±0.02
Goat	6.48±0.01	87.62±0.18	12.38±0.18	4.25±0.02	8.13±0.18	0.81±0.003
Sheep	6.48±0.01	80.90±0.04	19.10±0.04	7.70±0.06	11.40±0.09	0.85±0.01

W, water content; TS, total solids; SNF, solid non-fat; SD, standard deviation.
All values are ±SD which represent data average of five sample

Table 2. The fractions protein of different milk species

Species	CP (%)	TP (%)	Casein (%)	WP (%)	NCN (%)	NPN (%)
Caw	3.57±0.02	3.24±0.02	2.48±0.01	0.44±0.02	0.76±0.02	0.32±0.004
Buffalo	5.20±0.05	3.98±0.04	3.07±0.04	0.51±0.01	0.90±0.01	0.39±0.01
Goat	3.42±0.04	3.02±0.04	2.10±0.04	0.51±0.01	0.92±0.01	0.40±0.004
Sheep	5.63±0.03	4.98±0.04	3.65±0.04	0.67±0.004	1.32±0.01	0.65±0.01

CP, crude protein; TP, true protein; WP, whey proteins; NCN, non-casein nitrogen; NPN, non- protein nitrogen; SD, standard deviation. All values are mean±SD, representing data average of five samples.

Another very important parameter regarding the milk processing part, especially when we talk about cheese processing is represented by the casein content of milk, the main protein in it, being also the protein that remains in the cheese. Therefore, the milk that recorded the highest value in terms of casein content was sheep's milk where the average value was 3.65 ± 0.04% followed by buffalo milk where the average was 3.07 ± 0.04% and that of a cow for which the average casein content was 2.48 ± 0.01%.

Analyzes were also performed to determine the content of the main essential and non-essential amino acids. Therefore, for cow's milk, the highest level of essential amino acids was found in the case of leucine, namely 324.02 ± 0.32 mg /100 g followed by lysine where the

content was 261.38±0.25 mg/100 g. Among the non-essential amino acid content, the highest level was found in the case of glutamic acid (717.2 ± 0.20 mg/100 g) followed by proline where the average value was 302.23 ± 0.19 mg/100 g with variation limits between 302 mg/100 g and 303 mg/100 g.

In the case of non-essential amino acids determined for cow's milk, the lowest level was found in the case of Arginine (190.92 ± 0.33 mg/100 g) (Table 3).

For milk from buffaloes in terms of content of essential amino acids - leucine recorded a higher content compared to the content from cow's milk, namely 398.00 ± 0.32 mg/100 g.

Differences were also noted in the lysine content, where the mean value for buffalo milk was 310.20 ± 0.73 mg/100 g (Table 4).

Table 3. The main amino acid content of proteins in COW milk

Specification	n	$\overline{X} \pm s_{\overline{X}}$	V%	Min.	Max.
Essential amino acids (mg/100g milk)					
Valine	5	190.92±0.33	0.38	190	192
Isoleucine		189.60±0.24	0.29	189	190
Leucine		324.02±0.32	0.22	323	235
Lysine		261.38±0.25	0.21	260.9	262
Threonine		153.34±0.19	0.28	153	154
Phenylalanine		171.34±0.19	0.25	171	172
Non-essential amino acids (mg/100g milk)					
Arginine	5	122.07±0.32	0.58	121	123
Asparagic acid		218.23±0.19	0.20	218	219
Glutamic acid		717.2±0.20	0.06	716.9	718
Proline		302.23±0.19	0.14	302	303
Serine		187.01±0.32	0.38	186	188
Threonine		185.05±0.32	0.38	184	186

Table 4. The main amino acid content of proteins in BUFFALO milk

Specification	n	$\overline{X} \pm s_{\overline{X}}$	V%	Min.	Max.
Essential amino acids (mg/100g milk)					
Valine	5	240.20±0.37	0.35	239	241
Isoleucine		210.80±0.37	0.40	210	212
Leucine		398.00±0.32	0.18	397	399
Lysine		310.20±0.73	0..53	308	312
Threonine		195.60±0.51	0.58	194	197
Phenylalanine		278.60±0.51	0.58	194	197
Non-essential amino acids (mg/100g milk)					
Arginine	5	129.40±0.51	0.88	128	131
Asparagic acid		362.80±0.58	0.36	361	364
Glutamic acid		561.20±0.86	0.34	559	564
Proline		370.00±0.71	0.43	368	372
Serine		269.80±0.86	0.71	267	272
Threonine		199.00±0.71	0.79	197	201

Regarding the analysis of the results obtained for non-essential amino acids from buffalo milk, it can be seen that in the case of Glutamic acid the average level obtained by us was 561.20 ± 0.86 mg/100 g and that of proline recorded an average value of 370.00 ± 0.71 mg/100 g. The lowest content was also found in the case of Arginine, where the mean value was 129.40 ± 0.51 mg/100 g slightly higher compared to the level of cow's milk (Table 4).

For goat's milk in the case of essential amino acids we had average values of 308.8 ± 0.37 mg /100 g for Leucine, 234.60 ± 0.51 mg/100 g for Lysine and only 137.80 ± 0.58 mg/100 g for Phenylalanine. In the case of non-essential amino acids, the mean level was 107.00 ± 0.71 mg/100 g for Threonine and 595.60 ± 0.51 mg/100 g for Glutamic acid (Table 5).

Table 5. The main amino acid content of proteins in GOAT milk

Specification	n	$\overline{X} \pm s_{\overline{X}}$	V%	Min.	Max.
Essential amino acids (mg/100g milk)					
Valine	5	192.60±0.51	0.59	191	194
Isoleucine		173.00±0.71	0.91	171	175
Leucine		308.8±0.37	0.27	308	310
Lysine		234.60±0.51	0.49	233	236
Threonine		144.60±0.51	0.79	143	146
Phenylalanine		137.80±0.58	0.95	136	139
Non-essential amino acids (mg/100g milk)					
Arginine	5	111.01±0.71	1.42	109	113
Asparagic acid		249.8±0.37	0.33	249	251
Glutamic acid		595.60±0.51	0.19	594	597
Proline		273.00±0.71	0.58	271	275
Serine		155.20±0.58	0.84	154	157
Threonine		107.00±0.71	1.48	105	109

The last type of milk analyzed was the one from sheep, milk for which the protein level was the highest, which is also noticeable in the case of the level of essential and non-essential amino acids. Therefore, the average value obtained for valine was 371.00 ± 0.32 mg/100 g, much higher than the average value of milk from other species. A strong difference is also noted in the case of glutamic acid, for which the mean value was 1166.00 ± 0.71 mg/100 g (Table 6).

Table 6. The main amino acid content of proteins in SHEEP's milk

Specification	n	$\overline{X} \pm s_{\overline{X}}$	V%	Minima	Maxima
Essential amino acids (mg/100g milk)					
Valine	5	371.00±0.32	0.19	370	372
Isoleucine		278.8±0.37	0.30	278	280
Leucine		519.40±0.51	0.22	518	521
Lysine		572.20±0.58	0.23	571	574
Threonine		233.40±0.51	0.49	232	235
Phenylalanine		269.80±0.58	0.48	268	271
Non-essential amino acids (mg/100g milk)					
Arginine	5	207.4±0.51	0.55	206	209
Asparagic acid		272.6±0.51	0.42	271	274
Glutamic acid		1166.00±0.71	0.14	1164	1168
Proline		537.00±0.71	0.29	535	539
Serine		321.40±0.51	0.35	320	323
Threonine		193.00±0.71	0.82	191	195

CONCLUSIONS

Following the analyzes performed, regarding the chemical composition of milk samples from different species, we can conclude that fat was the most inconsistent component while the ash content showed minimal variations between milk samples. Therefore, it can be seen that sheep's milk has the highest levels in terms of fat, SNF, TS and ash content, followed by buffalo milk.

Regarding the protein fractions such as CP, TP, casein, whey proteins, NCN and NPN content, the results obtained showed that there are differences between milk from different species.

As a final conclusion, taking into account that the chemical composition of milk is a very important aspect in terms of its processing, it is appropriate that those working in this field be informed about the raw material used.

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THE EFFECT OF THE STORAGE TO THE MICROSTRUCTURE OF ANGKAK SAUSAGES OF LAYING CHICKEN MEAT WITH THE SCANNING ELECTRON MICROSCOPE (SEM) METHOD

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Abstract

The purpose of this study was to evaluate the microstructure of the sausage meat of laying hens without giving Angkak (0%), with 0.5% Angkak, 1% Angkak and 1.5% Angkak and storage at 0 days, 10 days, 20 days and 30 days at 5^0C with the method Scanning Electro Microscope (SEM). The method used is descriptive observation using a Scanning electron microscope. The results showed that the higher the addition of Angkak concentrations in sausages the greater the cavity on the surface of the sausage structure. At 0 day storage, the surface of the structure was still compact and there was little bacterial contamination. After 10 days of storage, the surface texture began to loosen and the cavities began to spread. Storage for 20 days the surface texture of the sausage is more cavity and begins to grow bacteria. 30 days of storage, there was a visible growth of fungus on the surface of the meat sausage. The conclusion is that the addition of Angkak affects the formation of starch granules and texture on the sausage surface. At 20 days of storage there was no effect of preservation on the addition of Angkak, it was shown the growth of bacteria and fungi. Furthermore, the 30 day shelf life creates a new ecosystem that allows mold to appear on the surface of the sausages.

Key words: *Angkak sausage, microstructure, scanning electron microscope, storage.*

INTRODUCTION

Laying hens chicken meat has low quality because the cutting is done at old age and does not produce so that the meat tenderness is lower and is less liked by the community. To increase the preference for rejected layer chicken meat, it is is necessary to innovate and diversify the rejected layer chicken meat, one of which is by making sausages (Purnamasari, 2012).

Sausage or sausage comes from the Latin salsus which means to be salted is meat that is prepared through salting. Sausage is a food made from mashed, ground, seasoned meat and then wrapped in a casing that is symmetrical and has a distinctive taste (Ernawati, 2015). Sausage is a source of protein (Elvira, 2009). The characteristics of a good sausage are chewy texture, do not contain preservatives, are free from harmful chemicals and do not contain harmful synthetic dyes (Palandeng et al., 2016). The composition of sausage processing consists of animal tissue, water, ingredients for curing, spices, fillers and binders. The basic principles of making sausages include the stages of grinding and mixing the meat with spices, filling in the shell, smoking, drying and storing. The ingredients for curing (cured meat) in the manufacture of sausages are salt (NaCl), sugar, and Angkak.

The purpose of curing is to get a stable color, aroma, texture, good taste and to extend the shelf life of the product (Soeparno, 2011). Nitrite salt functions as a curing agent but can be a precursor to carcinogens because it reacts with amines from protein components to form nitrosamines (Zahran and Kassem, 2011). One of the ingredients that can replace the function of nitrite as a preservative, flavor giver, texture improvement as well as natural dyes and colors that are not harmful is Angkak.

Angkak is fermented rice using the fungus Monascus purpureus so that its appearance is red. Angkak has been used widely in Asia as a natural food coloring in fish, Chinese cheese, red wine, and sausages (Blanc, Lorel and Goma, 1997). Apart from being a natural dye,

Angkak is also used as a flavoring, preservative, texture improvement and medicine because it contains nutritious bioactive ingredients. *Monascus purpureus* fungus produces pigments that are not toxic and do not interfere with the immune system (Fardiaz and Zakaria, 1996). The red color of angkak is very potential as a substitute for synthetic red which is currently very widely used in various food products. As a natural dye, Angkak has fairly stable properties, can mix with other color pigments and is non-toxic. Monascus mushrooms which produce Angkak by converting the substrate of starch into several metabolites, such as alcohol, antibiotic agents, antihypertensives, enzymes, fatty acids, aromatic compounds, ketones, organic acids, pigments and vitamins (Yongsmith, Tabloka, Yongmantichal and Bavavoda, 1993). In addition to the curling material, Angkak can also be used as a binder (Rumondor et al., 2019).

The purpose of this study was to evaluate the structure of the sausage meat of laying hens without giving Angkak (0%), 0.5% Angkak, 1% Angkak and 1.5% Angkak and storage at 0 days, 10 days, 20 days and 30 days at 5 0C with the method Scanning Electro Microscope (SEM). The method used is descriptive observation using a Scanning electron microscope.

MATERIALS AND METHODS

This research is an experimental laboratory conducted in the Microbiology Laboratory of Gajah Mada University. Microstructure testing of chicken meat Angkak sausage was carried out to determine the microstructure of sausages without giving Angkak, giving Angkak 0.5 %, 1 % and 1.5% and Angkak sausages stored at 0 days, 10 days, 20 days and 30 days.

Angkak Sausage Microstructure Research Procedure Using SEM Method

The method used is microscope observation using a Scanning electron microscope. The sausage sample to be observed is fixed first. Substance or 3% gluterldehyde substance which is diluted at 0.1 M phosphate buffer for 2 - 4 hours. Then washed with 0.1 M phosphate buffer with a pH of 7.3, carried out 3 times for 10 minutes. After fixation 2% osmium was added in 0.1 M phosphate buffer for 2 - 4 hours at room temperature. Then do the dehydration with 100% ethanol for 2 times for 15 minutes. Then it was dried and coated with gold, after which it was observed on the Scanning Electron Microscope. SEM equipment preparation: turn on the switch beside the tool (SEM) and leave it for 30 minutes to warm up the tool, set the specimen on the specimen holder. Then press the EVAC / AIR button to enter air into the specimen chamber. LED light (which is blinking and yellow). To indicate that air has entered the specimen camber, the AIR LED light will light up constantly and not blink again. Insert the sample slowly by adjusting the sample support to the main unit. Then adjust the position of the sample surface you want to see by turning the XY button, its position can be seen on the display screen. Furthermore, an enlargement is carried out on each sample observation.

RESULTS AND DISCUSSIONS

Angkak Sausage Microstructure

Analysis of differences in the treatment of Angkak on sausages using a Scanning Electron Microscope at a magnification of 100x. Scanning Electron Microscope results (Figure 1) show a difference in the structure of sausages without the addition of Angkak (Figure 1a) and the addition of Angkak (Figures 1a, b, c and d).

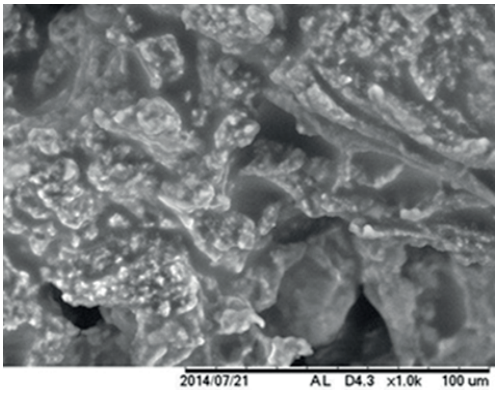

Figure 1a. Sausage Without Giving Angkak (0%)

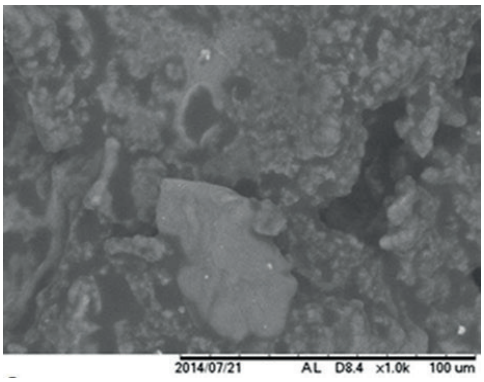

Figure 1b. Sausage with the addition of Angkak 0.5%

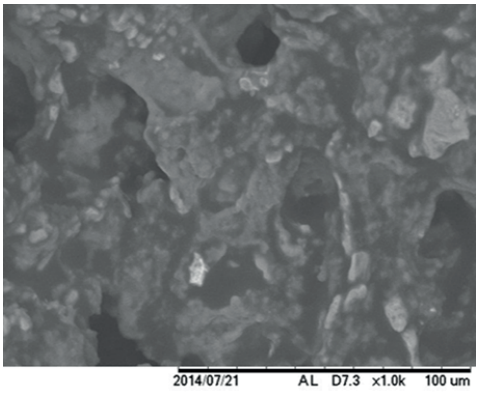

Figure 1c. Sausage with the addition of Angkak 1%

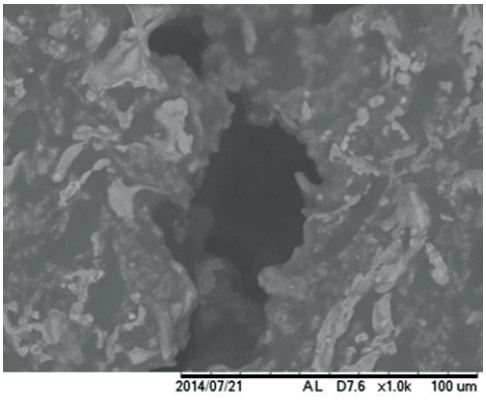

Figure 1d. Sausage with the addition of Angkak 1.5%

The presence of starch granules from tapioca which are irregularly rounded and the surface of a slightly rough texture, seen in sausages without the addition of Angkak (sausages with the addition of 0.5% Angkak), visible starch granules from Angkak rice in the form of small spheres and starch from tapioca flour. Sausage with the addition of 1.0% and 1.5% Angkak showed the presence of starch granules in the form of small spheres and irregular spheres originating from Angkak rice flour and tapioca flour. Qualitative SEM analysis results inform that the addition of Angkak affects the formation of starch granules and texture on the sausage surface.

The difference in surface texture and presence of pore cavities both qualitatively (the size of the cavity) and quantitatively (the number of cavities) formed in Figure 1 is due to differences in the concentration of Angkak given to each treatment. The higher the increase in the concentration of Angkak in sausages the more and the greater the cavity on the surface of the sausage structure. This shows a decrease in water content in the cells due to the osmosis and dehydration processes as a result of the release of water through the evaporation of water substance (H2). The cooking temperature also affects the structure of the sausages, the process of increasing the temperature results in the sausage cavity being wider and easier to absorb water (Ayu and Yuwono, 2014). Carbon dioxide (CO_2) and water (H2) easily evaporate, this is due to the gas produced through the fermentation process of this Angkak will try to make a way out that shows the presence of air cavities on the surface of the sausage. Gas production in the fermentation process of Angkak is preceded by the release of energy from carbohydrates in the form of glycogen in meat and starch from rice in the form of one part of the phosphate. The amylase enzyme from Angkak converts starch or starch into a simple monosaccharide. This concurs with Pattanagu et al. (2007) that Angkak produces α-amylase, β-amylase enzymes which can improve the texture of meat sausages. The release of this energy changes ATP to ADP + Pi, then there is the release of water (HOH) which reacts with carbohydrates to form vinegar (antibiotics) and its aromatic compounds as well as carbon dioxide (CO_2) and water (H_2).

Angkak Sausage Microstructure during Storage

Angkak comes from fermented rice using the fungus *Monascus purpureus* which is a natural coloring agent and also as a preservative that

can suppress the moisture content of sausages, inhibit the growth of decomposing bacteria and contain bioactive ingredients. The fungus *Monascus purpureus* produces Angkak by converting a starch substrate which is alcohol, antibiotic and aromatic compounds (Yongsmith, 1993). The results of observations through the Scanning Electron Microscope in Figure 2, the sausage shelf life of 0 days, 10 days, 20 days and 30 days at a temperature of 5 0C with a magnification of 100x, which is indicated by a circle sign, namely bacterial growth.

At 0 day storage, the surface of the structure was still compact and there was little bacterial contamination. After 10 days of storage, the surface texture began to loosen and the cavities began to spread. Storage for 20 days the surface texture of the sausage is more cavity and begins to grow bacteria. Storage of 30 days has seen a growth of fungus on the surface of the meat sausage.

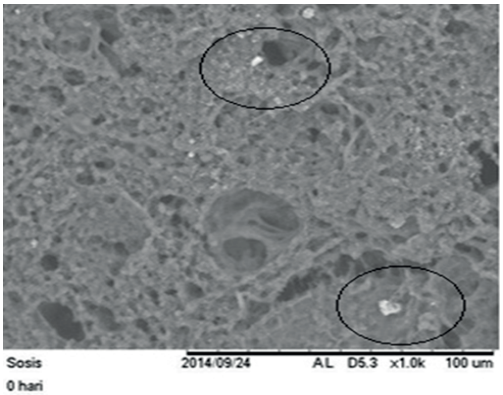

Figure 2A. Sausages shelf life of 0 days

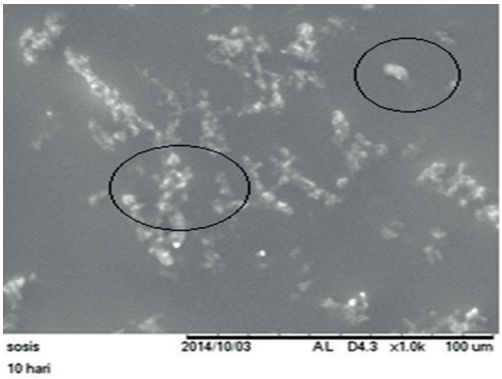

Figure 2B. Sausages shelf life of 10 days

The results of the analysis showed that at 0 days of storage at first there were no visible physical, biological and chemical changes. 10 days of storage, there is an effect of giving Angkak, while in 20 days of storage there is no effect of preservation on the addition of Angkak, it is shown the growth of bacteria and fungi. Furthermore, the shelf life of more than 20 days to 30 days creates a new ecosystem that allows the appearance of mold on the surface of the sausage. Angkak compounds are able to control water content, dehydration and cell osmotic pressure so that it can suppress the decomposing microbial population in sausages.

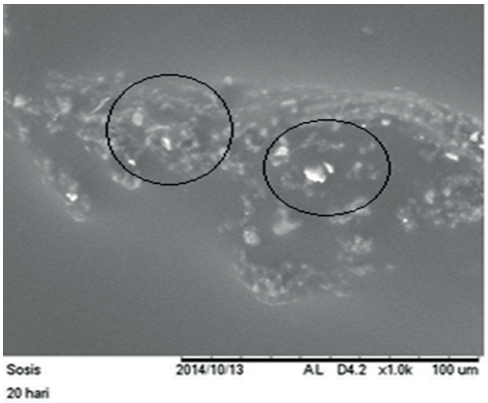

Figure 2C. Sausages shelf life of 20 days

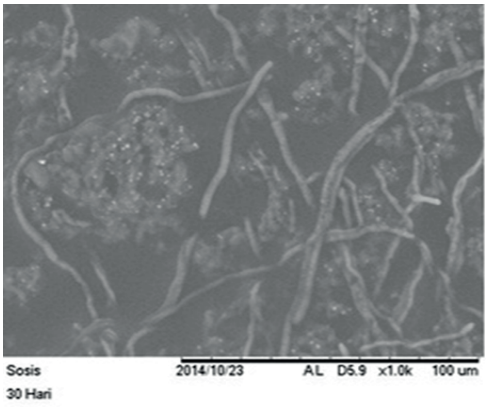

Figure 2D. Sausages shelf life of 30 days

The change in sausage in Figure 2 occurs due to the re-fermentation process by fungi. The end result of fermentation is in the form of gas and water which is released resulting in structural changes in the sausages in the form

of physical changes in the presence of a space or cavity which is the way out of the gas.

The carbon dioxide produced from Angkak evaporates when the space or cavity is still small and the cavity space extends or increases. The enlarged space or cavity for the production of carbon dioxide gas evaporates and decreases along with the decreasing power of carbon dioxide production. As an antibiotic agent that kills thermophilic bacteria, the compounds that play a role are Ankalactone compounds from Angkak which are more acidic (Mostafa and Abbady, 2014). According to Barbara (2001) bacteria that can damage sausages are *Staphylococcus aureus* which grows at a pH of 5.0 - 6.5 which is not too acidic and a material with a high protein content.

The lovastatin compound in Angkak together with water will react to become acid, carbon dioxide and water. This carbon dioxide will evaporate, thus inhibiting the growth of the bacteria *Pseudomonas* sp, *Bacillus cereus* and *Bacillus stearothermophyllus*. The results of the reaction of the lovastatin compound and water have the ability to bind and release water. The production of carbon dioxide gas also comes from the garlic seasoning as an anti-bacterial which can bind water and change the osmotic pressure in cells.

Inhibition of the decay process due to reduced water content which inhibits the growth of decomposing bacteria from the sausages. Evaporation of light gaseous carbon dioxide and oxygen will come out of the sausage and leave a pore in the form of a cavity as a way out of gas in the sausage. This is supported by Adams & Moss (2002) that the growth of microorganism contaminants on the surface of sausages can result in changes in color, taste and smell.

CONCLUSIONS

The addition of Angkak affects the formation of starch granules and texture on the sausage surface. At 20 days of storage there was no effect of preservation on the addition of Angkak, it was shown the growth of bacteria and fungi. Furthermore, the 30 day shelf life creates a new ecosystem that allows mould to appear on the surface of the sausages.

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STUDY ON THE USE OF MILK AND DAIRY PRODUCTS IN THE DIET OF CHILDREN IN SCHOOLS

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Abstract

School milk program are common in many countries around the world for a good reason. Nutritionists and social policy experts believe that encouraging healthy eating should be the main goal of this general and universal program. The benefits of school milk are many, so milk and dairy products are considered the main sources of nutrients needed for the harmonious development of children. The paper provides information on the benefits of consuming by dairy products on the student`s body, the presentation of the categories of dairy products that are included in the program and how this program has influenced the consumption of milk among the population.

Key words*: children, food choose, milk, school program.*

INTRODUCTION

We live in a world that is developing at a dizzying tempo. Thus, there are countries with different stages of economic development and progress. The SARS CoV-2 pandemic more intensifies the vulnerabilities and deficiencies of the world's food systems. Most activities and processes affect food production, distribution and consumption, which are hampered by border restrictions, quarantines and other containment measures. And all this hinders the progress of the fight against hunger. Although we are still in the midst of a global pandemic wave, we can estimate a doubling of the population vulnerable to famine, as a result of the economic and health crisis triggered by SARS CoV-2, reconfigure the needs and necessities of the population, transportation restrictions and increased spending on food chain.

According to a study conducted by the ONU in 2019, hunger affects over 690 million people, and the COVID-19 crisis could increase the figure by another 130 million people worldwide, is more than 10% of the planet's population. The ONU report highlights worse figures from year to year, this situation worsening due to wars, global warming, economic and health crises. The nutritional status of children's diets around the world has suffered as a result of trade actions that have increased dependence on food imports (Brownell et al., 2006).

Most of these people live in low- and middle-income countries, and especially in rural areas. They depend, to a large extent, on agriculture and animal husbandry. High food costs and low affordability mean that billions of people cannot eat healthy or nutrient-rich food. This hunger includes chronic food shortages and malnutrition, in the form of deficiencies in nutrients and micronutrients.

This problem is also encountered in developed countries. People who do not eat enough food, often become hungry, and in the long run this can lead to malnutrition. It is important to note that sometimes it is not hunger that leads to this condition, but a series of diseases and conditions that prevent digestion and absorption of nutrients (celiac disease, digestive problems, etc.). Most often, nutrient deficiencies are found in children, generally due to inadequate eating behaviour (Kusz & Kilar, 2020). The most common deficiency in the world is iron and calcium deficiency which leads to anaemia.

All of this has led many heads of state, government and organizations around the world to step in to end world hunger and improve food security in all parts of the world (FAO, 2014). School feeding has been

identified as a means of addressing the reduction of the proportion of people suffering from hunger worldwide and ensuring universal enrolment in primary education (Bundy et al., 2009; Buhl et al., 2010).

MATERIALS AND METHODS

This paper is based on a review of the narrative literature on the importance of feeding children in schools, studies on milk administration programs in schools implemented in different country. The study focuses on the strongest evidence available where possible, with the latest publications consulted. The effectiveness and effect of the presence of milk and dairy products in school milk programs was also investigated.

RESULTS AND DISCUSSIONS

The evolution of mankind has been marked by the succession over the centuries of several stages of socio-political order and civilizations, each characterized by its administrative, economic, religious and cultural peculiarities, with which they have evolved and changed over time - as it is known - food possibilities and habits (Graur et al., 2006).

The WHO Global School Health Initiative was launched in 1995, with a mandate to use schools as a means of strengthening health promotion and education activities at the local, national, regional and global levels - thus improving the health of students, families and families. to all members of the community (WHO, 2006). The term "health-promoting schools" has been developed to recognize educational institutions that "constantly strengthen their capacity as a healthy environment for life, learning and work" (WHO, 2006; Bulletin of the IDF N° 505/ 2020)

Schools and educational institutions have been identified as one of the fundamental settings for health promotion and establishing healthy eating and lifestyle patterns (Scriven & Stiddard, 2003). Schools can provide an important opportunity for prevention (Carter et al., 2002), as they provide the most effective way of reaching large numbers of people, including youth, school staff, families and community members (WHO, 1998).

Approaches to school food and nutrition policy

The World Health Organization (WHO), in 2020, declared the epidemic caused by the new coronavirus (COVID-19) as a global pandemic. This pandemic has caused an unprecedented global health crisis, with serious socioeconomic consequences and intense human suffering, which mainly affects people with chronic diseases. In addition, medical personnel, who were key during the COVID-19 crisis, were exposed to high health risks (Regulation (UE) 2021/522).

According to the new EU policies, schools may be important, protected settings for learning healthy dietary preferences and habits. Specific actions to promote healthy diets in school settings include free or subsidized fruits and vegetables, food- or nutrient-based standards for the foods and meals available in schools, changes to the presentation of food choices at points of offer and nutrition education and skills to increase nutrition literacy and capacity. Appropriate action should be taken to ensure the availability of healthier foods and to limit the availability of HFSS products. (HFSS products - High Fat, Sugar and salt foods)

Diet patterns have a significant influence on health and well-being. A healthy diet during childhood and adolescence reduces the risk of immediate health problems related to nutrition, which are a major concern for school children, namely obesity, tooth decay and lack of physical activity.

In addition, young people who have developed healthy eating habits at the beginning of life are more likely to maintain them and thus have a reduced risk of chronic diseases such as cardiovascular disease, cancer, type II diabetes and osteoporosis in adulthood.

Childhood and adolescence are critical periods for health and development, because the physiological need for nutrients increases and the consumption of a diet of high nutritional quality is particularly important. Eating habits, lifestyle and behaviour patterns are established during this period, which can persist throughout adulthood.

It is therefore the responsibility of each country, school or authority to decide which of the proposed suggestions presented in this guide are the most appropriate and applicable to the context-specific circumstances.

Milk's Role in School Meal Programs. School is the environment in which the child spends up to a third of the day, and the assimilation of the daily caloric needs should be achieved through a snack taken during school hours (Arpinte et al., 2009). During school, social factors greatly influence their dietary profile. The eating habits, food choices and meal quality of young people reflect the weak influence of the family and the increasing pressure of colleagues / social.

Changes in eating habits can be associated with the need to express their freedom from parental control and identity falsification.

Independence can be expressed through increased consumption of meals (fast meals) outside the home or school.

Psychological, social and environmental factors influence food preferences, which increase with age, and are subject to social and psychological changes. From a social point of view, young people experience peer pressure in many areas, including nutrition, and group behavior becomes the norm. Eating habits are strongly affected by cultural pressures. Thus, many teenagers and children feel pressured to have an "ideal" body shape. The desire to have a socially adequate body and the stigma of obesity can have a significant effect on body image and self-esteem among young people.

Other influences on food attitudes and choices include: religion, culture, metabolic problems, the family food model, access to information, food marketing and advertising, and life in social media.

Consumption of nutrient-rich foods, such as milk, which are readily available in school meals, is also associated with improved teacher and behaviour. Participation in school breakfast or lunch programs is associated with improved grades, standardized test scores, and school attendance. In addition, by adding nutrient-rich foods, especially fruits, vegetables and dairy products, which are missing from students' diets, their academic performance has improved.

The nutrition and health benefits of milk. Dairy products, fruits, nutrient-rich vegetables, and protein-rich foods (of plant or animal origin) are the most expensive food groups in the world.

Energy requirements result from basal metabolic rate, growth rate and physical activity. The intensity of physical activity varies with age, being lower in children between 2-5 years than in children between 6-10 years (Torun, 2011; Graur et al., 2005; Gibney et al., 2005). Calculation of energy requirements (ER) is calculated according to the formula:

ER (kcal/day) = 1000 + 100 x age (years)

Table 1. Energy requirements of children and adolescents

Age (Years)	Daily energy requirement kcal	
	kcal/d[a]	kcal/kg/d
1-3	1000-1200	102
4-6	1300-1400	90
7-10	1800-2000	70
11-13	2000-2200	60
14-18	2200-2400	50

[a]gender and activity level
Source (Torun, 2011; Graur et al., 2005; Lutter et al., 2003; Gibney et al., 2005)

Milk is a nutrient-rich food that provides significant value to school meals and feeds students. Very palatable, milk - along with other dairy foods - plays an important role in children's diets. By encouraging milk consumption in schools, the nutrient gaps that exist in students' diets can be eliminated.

Dairy and dairy products have been an important part of the human diet for about 8,000 years, and are part of the official nutritional recommendations of many countries around the world. They provide a package of key nutrients that are difficult to obtain in diets with limited or no dairy products, such as vegan or restrictive dairy diets. Indeed, dairy products are rich in calcium, protein, potassium and phosphorus. They contribute about 52-65% of the reference dietary intake (DRI) of calcium and 20-28% of the protein requirement, depending on the age of the consumer (Smit et al., 1999; Feskanich et al., 2003; Skinner et al., 2011, Dragomir et al., 2012). The contribution of dairy products to the provision of recommended calcium intakes has largely led to dietary recommendations for dairy consumption in most guidelines. Up to two-thirds of the calcium intake of the population in Western countries is supplied by dairy products (Gueguen et al., 2000; Gueguen L., 2011),

while dairy foods account for only 9-12% of total energy consumption (Bonjour JP., 2011).
Milk offers health attributes that are different from herbal foods and other foods of animal origin, which can be very difficult to replace in a healthy diet for most people. The milk's unique nutrient package works together to provide multiple health benefits, including optimal growth and development in children and a reduced risk of chronic diseases such as type 2 diabetes and heart disease.
Milk and dairy products provide a suitable amount of nutrients for building bones, especially calcium, vitamin D, protein, phosphorus, magnesium, potassium, vitamin B12 and zinc. However, beyond bone health, milk, with its unique package of nutrients, is also the main food source for three of the four public health nutrients in children's diets.
Children and adolescents have a higher need for calcium, protein and minerals, so milk is one of the first food options. Milk is an almost complete food; it contains about 125 mg of calcium per 100 ml of milk, vitamin D, plus an optimal ratio of calcium - phosphorus, so it is one of the 5 food groups recommended by nutritionists to be consumed in every day. To meet the daily requirement of calcium, dairy products, green vegetables and mineral waters are important; they are easily accessible sources of calcium. Dietary Calcium Intake for children and young people, by age group are shown in Table 2 (Ciucu C., 2015; Bouziani et al., 2018; American Academy Of Pediatrics, 1999).

Table 2. Dietary Calcium Intake for children and young people, by age group

Age category	Calcium intake mg/day
1-3 years	500 mg
4-8 years	800 mg
9-18 years	1300 mg

Dairy products are good dietary sources of calcium due to their high calcium and nutrient content, high absorption rate, availability and relatively low cost, which makes regular consumption of dairy products possible. They provide more calcium, protein, magnesium, potassium, zinc and phosphorus per calorie than any other typical food found in the adult diet (Heaney, 2009; Caroli et al., 2011)
Many dietary recommendations include consuming 3 servings of dairy products per day (e.g., 1 glass of milk, 1 serving of cheese, 1 yogurt) - an amount that provides most of the calcium DRI to the general population. For example, 250 mg of calcium can be obtained from a 200 ml glass of milk, a portion of 125 g of yogurt or 35 g of hard cheese (Rozenberg et al., 2016; USD, 2013).
It stands out that milk is the only food needed in the school lunch program. The required serving size is 250 ml of milk. Some studies showed that there was a dose dependent effect, while we were unable to conduct the dose - response analysis, more work should be done to elucidate the dosage and effects of milk consumption on human health.
European Commission's Health Promotion and Disease Prevention Knowledge Gateway, offer a reference point for public health policy makers in creating a balanced meal (Food-Based Dietary Guidelines in Europe).
In each country, there is a personalized list of subsidized dairy products in the states, all respecting the tradition, culture and local availability. In addition to milk, we can also find yogurts and cheeses.
Due to the high incidence of people with intolerances and metabolic diseases, the list of products included in school meals has diversified so we also find vegetable milk (based on soy, rice, oats or almonds), and drinks based on calcium fortified plants have become part of nutritional recommendations as alternatives to milk in several countries, such as the United States, Sweden, Australia and Brazil. However, there is a resistance in their consumption, because their nutritional properties depend on the raw material used, processing, fortification with vitamins and minerals and the addition of other ingredients, such as sugar and oil (Mäkinen et al., 2016). The importance of raising consumer awareness of such products is underlined, as there are now cases of severe nutritional deficiencies in children reported as a result of inappropriate consumption of herbal beverages (Le Louer et al. 2014; Mäkinen et al., 2016; Ellis et al., 2015).
For pre-schoolers or students who suffer from intolerance to gluten and/or lactose and/or any other ingredient or compound and/or any

product distributed, they will benefit from products appropriate to their situation, within the daily value provided. Lactose-free or low-lactose milk (i.e., <0.01% and <0.1% lactose by weight), suitable for consumption by lactose-intolerant individuals, is obtained by adding beta-galactosidase to milk before heating, thus leading to the release of glucose and galactose.

National Approaches to Milk in Schools.

In many peoples' minds, school milk is synonymous with milk being subsidized, or even given free. However, there are three categories of milk distribution: free, subsidized and full-cost. Each country adopts one method or more, so that children benefit from a correct and adequate diet.

Looking back in time, at 110 years or older, school milk programs in countries such as United Kingdom, the United States and the Australia, where school milk interventions were created as a social safety net for children, providing Milk for school consumption takes place in many countries around the world.

Milk and dairy products play an important role in healthy eating patterns, and dairy milk continues to be an important component of school meals globally (Chen, 1989; Bulletin of the IDF, 2020).

In Regulation (EU) no. 1308/2013 states that "in order to encourage healthy eating habits among children […]." The European Commission allocates annually the budget for the EU School Meal Programs both for the distribution of milk and dairy products, and for the distribution of fruits and vegetables to schoolchildren. National funds can also be used to supplement the EU budget (EU school scheme, 2020).

Since 1977, in European Union has been available School Milk Scheme, through which grants for the sale of reduced- rate milk products in schools. At the moment, two schemes are active in the European Union two similar nutritional schemes in schools specifically targeting children:

- the *School Milk Scheme*, through which grant s have been available since 1977 for the sale of reduced- rate milk products in schools;
- the *School Fruit Scheme*, which has co-financed the distribution of fruit and vegetables in schools since the 2009/10 school year.

The two schemes mentioned were designed to help stabilize the market and promote healthy eating, thus contributing in the short term to increasing and maintaining the consumption of dairy and dairy products by young people and in the long term to a proper education on eating habits (Regulation (EC) No 1234/2007, No 13/2009; No 657/2008).

School milk programme around the world contribute to good health and nutrition for children in schools.

The benefits of school milk programs for school children:

- normal growth and development of the body;
- improving the general health of children;
- increasing school performance;
- acquiring quality nutritional habits;
- prevent and reduce early school absenteeism;
- productive and healthy future adults.

The concern for children's nutrition has been a permanent concern of society and the authorities. In the following are presented the programs developed on the world map in different moments of humanity.

The first initiative was in United Kingdom, *School Milk in Britain* start on the passage of the Education (Provision of Meals) Act of 1906, and the Education (Administrative Provisions) Act of 1907, establishing medical inspection in State schools, marked the beginning of the construction of the welfare state (Atkins, 2007). The program continues, and the list of products in school meals is very diverse.

In Switzerland, start program *Break-time Milk Day* in 1940. During World War II, children and adolescents in cities received between 600 and 700 ml of milk per day and up to one liter in rural areas. This measure was recommended by the Swiss Federal Commission for War Nutrition (EKKE) to meet the calorie requirement and prevent rickets caused by vitamin D deficiency.

Since 1997, there has been an EU recommendation for member countries to implement such programs, and according to studies, the impact on public health is beneficial. In fact, European society promotes a healthy lifestyle both in schools and among adults (Yilmaz, 2017, Bailey et al., 2020).

In *Romania* since 2002 there is *Bread and milk Programme*, with application OUG No. 96/2002 on the provision of dairy and bakery products for students in grades I-VIII in state and private education, as well as for preschool children in state and private kindergartens with a normal schedule of 4 hours. The "*Laptele si cornul/Milk and croissant*" program allows: access to education and prevention of early school leaving; social aspects; healthy eating and education for healthy eating; the economic impact of the program on society.

In *New Zealand*, until 1940, milk was available to over 80% of school children. For several years during World War II, students also received an apple a day. The students received 284 ml of milk/day (Dunstall, 1992; Tennant et al., 1994; New Zealand history online, 2017). The program was discontinued in 1967 and resumed in 2013 (Cornall, 2020). Findings from the Milk for Schools evaluation provide a useful and timely contribution to the research regarding the ability of an in school initiative to increase the proportion of children meeting recommended dietary guidelines (Marsh et al., 2018).

Australian Milk for School Children Program (1950-1970). In Sydney, as early as 1924 were operating School milk scheme funded by private benefactors. The State Grants (Milk for School Children) Act was passed by federal parliament in 1950 and by the end of 1951 most states were inflicting this benefit on children up to the age of 13. In 1973, School milk scheme is abolish. Last year, in 2020, the dairy industry wants the national school milk program to be revived to improve the health of school-age children (Rymill L., 2020).

USA since 1940, the *Special Milk Program* began as an officially subsidized program at 15 elementary schools in low-income areas of Chicago. The children were charged 1 cent per 250 ml of milk. Those who could not pay received free milk, at the cost of private donations. Since 1955 the program has become free for all students. Since 1977 the Special Milk Program becomes permanent and subsidized by the state. The students received min. 250 ml of milk/day. A gap in research remains regarding children's preferences for extrinsic properties of fluid milk, especially as it relates to labelling and graphics. In American children it understands how to create and market milk products that are appealing to children without compromising health outcomes through excessive calorie or fat intake is necessary to increase lifelong milk consumption (Bailey et al., 2020; Sipple et al., 2020).

South Africa and more country start de *School Nutrition Program* in 1994. National School Nutrition Program (NSNP) is the government program that provides one nutritious meal to all learners in Primary and Secondary Schools (Devereux et al., 2018; Laurie et al., 2017).

In *Russia* since 2005, are implemented the *School Milk Programs* in some regions. Most regions in Russia supply milk to grades 1-4, but some also supply upper-class children. School milk is always provided free of charge, financed from regional or municipal budgets. The school milk model with UHT treatment, guarantees food safety and allows efficient distribution without the need for refrigeration (tetrapak.com).

China School Milk Programme started since 2000 in some school. The participation of government, schools and licensed supplier and the support of the society, this programme achieve remarkable progress (WHO, 2017).

The *School Milk Programme* in *Japan* has a long history and tradition. During the period after the war, child malnourishment was a major issue across Japan. A more comprehensive solution was needed to solve this challenge as it became a serious social and education problem. In 1946, the First Educational Delegation recommended a systematic health education and a school lunch programme in Japanese schools, and in 1951, after attaining full independence, external aid to supply milk and foodstuffs for the school lunches was proposed to be stopped due to budget reasons. Since 1959, he supply locally produced liquid milk in schools is resumed.

India's Mid-Day Meal (MDM) Scheme is the world's largest school meal programme designed to improve the nutritional status of school children since 1995. It is a centrally sponsored scheme implemented in association with the State Government. Incorporating 200-250 ml milk daily into the menu of MDM at national level would be having long-lasting effects on society. It will improve the nutrition

and health of millions of vulnerable children and also stimulate the rural economy by boosting the demand of milk (The Indian Express, 2020; World Food Programme, 2017). While school milk programme still predominantly rely on government support, a number of examples of programme without a direct financial contribution from government can be cited. Children, and the food they eat, are influenced by an environment much wider than that of the school; however, school-based programme provide an excellent opportunity to promote milk consumption among children and in so doing establish a life-time's habit.

The development and implementation of school meal programs benefits both children and small local producers who manage to distribute their local production (Dragomir et al., 2017).

CONCLUSIONS

The purpose of the present work was to present different applications of meal scholar scheme around the world and importance for health children. Despite the current situation, governments and local authorities want to support healthy eating programs in schools. Ideally, governments should integrate nutrition into their approaches to agriculture; take action to eliminate the factors that increase the costs of food production, storage, transport, distribution and marketing - including by reducing inefficiencies, food loss and waste; support small-scale local producers to grow and sell more nutritious food and ensure market access; to prioritize the nutrition of children, as the category with the highest degree of need; to encourage behavior change through education and communication; to introduce nutrition into social protection systems and investment strategies at national level.

Experience shows that the fact that milk is more nutritious than competing beverages is not enough for it to maintain, let alone expand, its role in children's diets. School milk programme; therefore, represent an important vehicle for the promotion of milk. Such programme are currently seeing a resurgence of interest and are enjoying a renaissance as more imaginative and appealing ways to presenting milk to children are sought.

Schools and educational institutions provide a key environmental framework in which to facilitate actions that promote healthy choices as the norm. By focusing on establishing a holistic school approach to health and targeting the wider community, a concrete food and nutrition policy in schools can not only bring short-term improvements in the daily lives of young people, but also establish attitudes healthy, preventing the onset of obesity and chronic diseases in later life.

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***Emergency Ordinance no. 96 of August 16, 2002 on the provision of dairy and bakery products for students in state and private primary and secondary education, as well as for preschool children in state and private kindergartens with a normal 4-hour program

***Recital 2 of Regulation (EC) No 657/2008: 'In the light of the fight against obesity, and in order to provide children with healthy dairy products […]'. Recital 4 of Regulation (EC) No 13/2009: 'The clear health benefits of a School Fruit Scheme […]'.

***Recital 43 of Council Regulation (EC) No 1234/2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation) (OJ L 299, 16.11.2007, p. 1).

***Regulation (EU) 2021/522 of 24 March 2021 establishing a Union action program in the field of health ("the EU Health Program") for the period 2021-2027 and repealing Regulation (EU) No 282/2014

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STUDY ON SOME FOOD PRODUCT CONTAMINATION RATE WITH BACTERIA FROM *LISTERIA* GENUS

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Abstract

Included in the large spread high risk group, the Listeria bacteria genus can be diffused throw food products, causing the Listeriosis disease. Listeriosis is a bacterial infection which in serious forms and inadequately treated can reach a 70% mortality. In this study the bacteria were isolated from several categories of samples (poultry and bird organs, raw pork meat, raw beef meat, processed pork meat), which were collected from slaughterhouse houses and department stores. From analysed samples 40% of isolated strains were found in raw pork meat, 25% in raw beef meat, also 25 % in poultry and bird organs and 10% in processed pork meat. The isolation and identification technique was done with an official method, following these steps: pre- enrichment in unselective liquid mediums, enrichment in selective liquid mediums, isolation and identification, identity confirmations.

Key words*: bacteria, contamination, food product, identification, isolation*

INTRODUCTION

Listeria genus belongs to the family *Listeriaceae*, along with the *Brochothrix* genus. It includes 7 species: *Listeria denitrificans* (*Jonesia denitrificans*), *Listeria grayi* (*Listeria murrayi*), *Listeria innocua, Listeria ivanovii* (with two subspecies: *Listeria ivanovii* and *Listeria londoniensis*), *Listeria monocytogenes, Listeria seeligeri* and *Listeria welshimeri*.
Listeria monocytogenes is a polymorphic Gram-positive bacterium (short bacilli, cocobacilli, filamentous forms) (Figure 1).

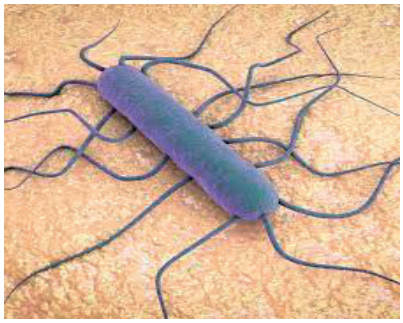

Figure 1. *Listeria monocytogenes*

It is non-capsulogenic, non-sporogenic. In cultures incubated at temperatures below 35°C it is mobile, with 4-5 flagella arranged peritrich, and at 37-46°C it becomes immobile or monoflagellate.
According to the classification made by the International Commission on Microbiological Specifications for Foods which grouped dangerous microorganisms depending on the severity of the risk, *Listeria monocytogenes* is included in the second group, with moderate risks, but with widespread potential (Apostu, 2004).
The natural infection is called listeriosis and affects both humans and many animal species. Man can become infected, either through the digestive tract through food or water (food poisoning), or through the air, genitals or wounds (Ivana, 2002).
Recently, there has been an increase in the percentage of contamination with Listeria of farm animals. In cattle and pigs, the skin and hair are important sources for *Listeria monocytogenes* spreading, with the possibility of its diffusion in carcasses (Tudor, 2002).
Paying attention to hygienic norms can reduce the number of contaminations, but these bacteria cannot be removed, which is why their determination can be a sanitary indicator of the overall hygiene conditions in slaughterhouses. (Dan, 2001).

In general, *Listeria* species are isolated from raw milk, cheese, fresh and frozen meat, chicken, seafood, fruits and vegetables.
Isolation of *Listeria monocytogenes* in food products is difficult and requires selective enrichment of samples before staining them on the surface of selective isolation media (Bărzoi, 2002).

MATERIALS AND METHODS

The study was conducted in 2016-2017 on several categories of meat samples, collected from slaughterhouses and sales units (Bucharest, Ilfov County): 10 samples of poultry and bird organs, 59 samples of raw pork meat, 21 samples raw beef meat, 10 samples processed pork. It is an attempt to highlight the existing interconnections between the quality of the raw meat, the attention paid to respect the hygienic norms on the technological flow of processing, the quality of the finished product and the consumers' safety.

Sample for analysis and initial suspension
For the preparation of the initial suspension, demi-Fraser broth (selective primary enrichment medium) or APT is used as the dilution liquid.
In general, to prepare the initial suspension, there is added 25 g of the test sample to 225 ml of primary enrichment medium, in order to maintain a 1/10 ratio between the test sample and the medium (mass/volume or volume/volume).
1. Primary enrichment
The initial suspension is incubated at 30°C for 25 hours.
2. Secondary enrichment
After incubation of the initial suspension (primary enrichment) for 25 h, 0.1 ml of the culture obtained is transferred to another tube containing 10 ml of Fraser broth (secondary enrichment medium).
The seeded medium is incubated at 37 ± 10°C for 24 ± 2 hours.
3. Stripping and identification
Using a bacteriological loop, the culture obtained at the primary enrichment (Demi-Fraser) will be dispersed on the surface of the first selective isolation medium (Listeria/ Agosti Ottaviani agar), so as to obtain isolated colonies.
In the same way is done with the second selective isolation medium (Palcam agar).
The procedure will be similar in the culture obtained at secondary enrichment (Fraser Broth), incubated for 24 ± 2 h at 37 ± 10°C, by dispersing the two selective media. At ALOA agar, if no signs of microbial development are observed after 24 ± 2 hours of incubation, or colony development is weak, incubation will be extended to 48 hours.
After 24 ± 2 h or 48 hours of incubation, the Petri dishes are examined in order to detect the presence of typical colonies: *Listeria* spp. or *Listeria monocytogenes*.
After incubation, the Petri dishes can be refrigerated at 5°C, before reading, for a maximum of 48 hours.
Is considered to be *Listeria monocytogenes* colonies if there are bluish-green colonies surrounded by an opaque halo (typical colonies). *Listeria ivanovii* colonies are also bluish-green surrounded by an opaque halo.
- *Listeria* spp. are considered likely bluish-green colonies surrounded or not by an opaque halo.
Note: Certain strains of *Listeria monocytogenes* exposed to stress conditions (especially acidity) may have a very weak, even absent, halo. There are also other organisms besides *Listeria* spp. which can produce blue colonies on ALOA agar.
The Petri dishes with Palcam Agar, after incubation at 37 ± 10°C, for 48 hours, are exposed to the air for 1 hour so that the environment regains its red-purple color. After 24 hours *Listeria* spp. forms small or very small olive or gray-green colonies, with a diameter of 1.5-2 mm, sometimes with a black center, but always with a black halo. After 48 hours, the colonies of *Listeria* spp. with a diameter of 1.5-2 mm are green, concave in the center and surrounded by a black halo.

Interpretation of morphological, and physiological properties and biochemical reactions
All species of *Listeria* spp., have the shape of small sticks, Gram-positive, are mobile and catalase positive (Table 1).

Table 1. Confirmation tests for *Listeria* spp.

Tests	***Listeria* spp.**	**Results**
mandatory	GRAM microscopic appearance	Thin and short sticks or coccobacilli form
	Catalase	+
optional	Voges-Proskauer test	+
	Motility at 25^0	+

L. monocytogenes differs from the rest of Listeria species by the characteristics specified in Table 2.

Table 2. Confirmation tests for *Listeria monocytogenes*

Tests	*Listeria monocytogenes*	Results
mandatory	GRAM microscopic appearance Beta-hemolysis L-rhamnose D-xylose	Thin and short sticks or coccobacilli form li + + -
optional	Catalase Motiliy CAMP test	+ + +
the microscopic appearance is optional in the case of the ALOA agar medium and in the case of the second medium (Palcam) if it allows the differentiation between pathogenic and non-pathogenic *Listeria* spp.		

Listeria monocytogenes has a characteristic and intense mobility and performs rolling movements that alternate with short periods of rest. On soft agar, the bacterium mobility is expressed by the culture development in an umbrella shape.

Catalase test: catalase is a hemoporphyrin enzyme that catalyzes the decomposition reaction of hydrogen peroxide. The reaction is evident when a culture loop is put in contact with a drop of hydrogen peroxide. The appearance of gas bubbles is interpreted as a positive reaction, being specific to *Listeria* species (Figure 2).

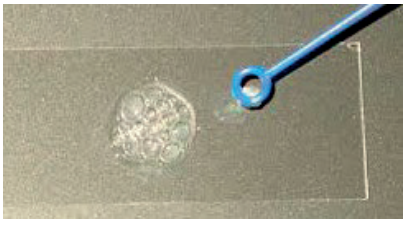

Figure 2. Catalase test

Carbohydrate fermentation: there is used peptone water with red-phenol. *Listeria monocytogenes* ferments glucose and rhamnose, with acid production, without gas production, and does not ferment xylose and mannitol.

Hemolysis test: this test can differentiate two closely related species, namely *Listeria monocytogenes* and *Listeria innocua*. The hemolytic species of *Listeria* species are: *Listeria monocytogenes, Listeria ivanovii* and *Listeria seeligeri.*

The method is as follows: the surface of the sheep's blood agar is stained with the bacterial culture which will be tested. There is an incubation time for 24 hours at 35-37°C. *Listeria monocytogenes* form small colonies with a small, clear halo around, specific to beta hemolysis. *Listeria ivanovii* has a strong hemolytic activity forming around the colonies clear, large areas, and *Listeria seeligeri* produces poor hemolysis.

CAMP test: It can clearly highlight the hemolytic characters and is achieved by seeding *Streptococcus aureus* and *Rhodococcus equi* in the streaks form, in one direction on the plate with blood agar, and the *Listeria* stems perpendicular to their trajectory without touching them. Hemolysis of *Listeria monocytogenes* strains is more exacerbated near the *Streptococcus aureus* stria, and for *Listeria ivanovii* it is intensified only near the *Rhodococcus equi* ridge (Figure 3) (Ivana, 2013).

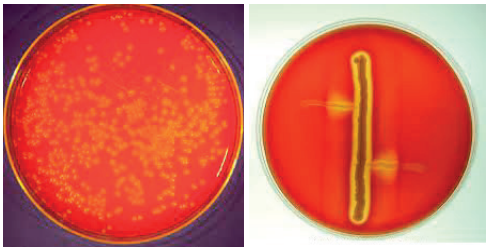

Figure 3. *Listeria* on blood agar, Camp Test

In Table 3, there are presented reactions at diferent tests completed in order to identify the *Listeria* species.

Table 3. Reactions to identify *Listeria* species

Species	Hemolysis	Acid production from:		CAMP test	
		Rhamnose	Xylose	*S. aureus*	*R. equi*
L. monocytogenes	+	+	-	+	-
L. innocua	-	V	-	-	-
L. ivanovii	+	-	+	-	+
L. seeligeri	(+)	-	+	(+)	-
L. welshimeri	-	V	+	-	-
L. grayi subsp. grayi	-	-	-	-	-
L. gravy subsp. murrayi	-	V	-	-	-
V: variable reaction; (+): weak reaction; +: over 90% with positive reactions; -: no reaction.					
NOTE: there are rare strains of *L. monocytogenes*, which do not give β-hemolysis, or CAMP test, under the conditions described in this procedure.					

RESULTS AND DISCUSSIONS

In the present paper, Listeria ssp. were isolated from several categories of samples collected from slaughterhouses and sales units, as previously presented. The results obtained are shown in Table 4 and Figure 4.

Table 4. Incidence of *Listeria* species in the analyzed samples

Sample type	Samples number	Positive results	%	Negative results	%
raw beef meat	21	1	4,76	20	96
poultry and bird organs	10	-	-	10	100
raw pork meat	59	2	3,38	57	97
processed pork	10	-	-	10	100
Total	100	3	3	97	97

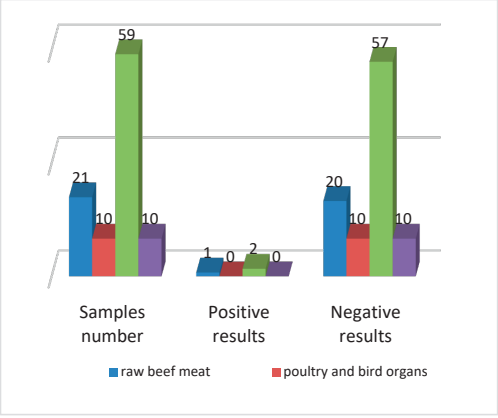

Figure 4. Incidence of *Listeria* species in the analyzed samples

The incidence of *Listeria* ssp. during this study period is shown in Table 5.

Table 5. *Listeria* species frequency results

Specie	Strains number	%
Listeria monocytogenes	1	50
Listeria ivanovii	1	50
Total	2	100

The incidence of *Listeria* species during 2016-2017 period, for the raw beef meat category is shown in Table 6 and Figure 5.

Table 6. Incidence of *Listeria* species in the period 2016-2017, for the raw beef meat category

Period	Samples number	*Listeria* ssp.	Number of isolated stems	%
2016	13	*Listeria monocyto genes*	1	7.69
2017	8	-	-	0
Total	21		1	4.76

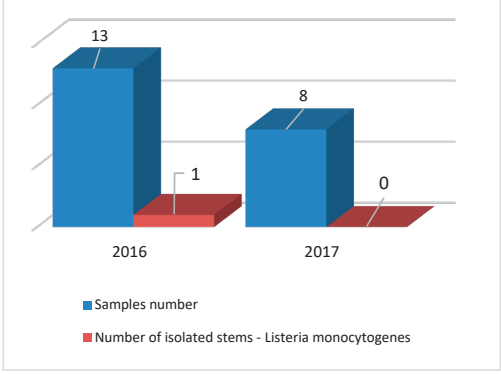

Figure 5. Incidence of *Listeria* species in the period 2016-2017, for the raw beef meat category

The incidence of listeries in 2016, for the category of beef raw material is 7.69%.
The incidence of *Listeria* species in the period 2016-2017, for raw pork meat category is shown in Table 7 and Figure 6.

Table 7. Incidence of *Listeria* species in the period 2016-2017, for raw pork meat category

Period	Samples number	*Listeria* ssp.	Number of isolated stems	%
2016	37	*Listeria monocytogenes*	1	2.7
2017	22	*Listeria ivanovii*	1	4.54
Total	59		2	3.38

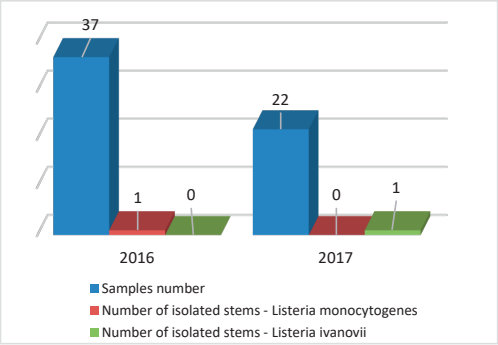

Figure 6. Incidence of *Listeria* species in the period 2016-2017, for the raw pork meat category

The incidence of listeries, for raw pork meat category was 2.7% in 2016 and 4.54% in 2017.

CONCLUSIONS

As a result of the research undertaken regarding the frequency of listeria species in meat samples, some conclusions may result.

There were analyzed 21 samples of beef raw material, 10 samples of poultry and organs, 59 samples of raw pork, 10 samples of processed pork, during 2016-2017 period. *Listeria* ssp was isolated in 3 samples (3%).

In raw beef meat category, in 2016, there was found the presence of 1 contaminated sample, with an incidence of 4.76% for *Listeria monocytogenes*.

In raw pork meat category in 2016, there was found the presence of 1 contaminated samples, with an incidence of 2.7% for *Listeria monocytogenes* and in 2017, there was found also the presence of 1 contaminated samples, with an incidence of 4.54% for *Listeria ivanovii*.

Listeria was not present in the poultry and bird organs or in the processed pork.

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FATTY ACID PROFILE IN EGGS AND EGGS PRODUCTS

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Abstract

Aim of this research was to determine the fatty acid profile of eggs from various poultry species, as well as the relationships between them, including fatty acids Ω-6 and Ω-3. The tests were carried out using a Perkin Elmer Clarus 500 chromatograph with a BP x 70 flame ionization detector and column on hen's eggs, quail, guinea fowl, and a pasteurized liquid mixture made from hen's eggs. The results found that quail eggs had the highest fatty acid content (101.485 g FAME / 100 g overall FAME), which was 0.90 percent higher than guinea fowl eggs, 1.48 percent higher than eggs, and 2.04 percent higher than the pasteurized chicken egg mixture. Quail eggs had an SFA/UFA ratio of 0.480, compared to 0.545 for pasteurized mix, 0.551 for chicken eggs, and 0.716 for guinea fowl eggs, and a PUFA/MUFA ratio 0.635 for pasteurized mix, 0.679 quail eggs, 0.730 hen eggs, and 0.744 for guinea fowl eggs. The researchers concluded that, in terms of fatty acid content and ratio, quail eggs are a safer alternative to chicken eggs in the human diet.

Key words*: eggs, fatty acid, guinea fowl, hens, quail, ratio Ω-6/Ω-3.*

INTRODUCTION

Lately, human nutrition is increasingly emphasizing the fat content of foods of animal origin and especially their composition in fatty acids (Gopinath et al., 2020).

Lipids from food have an energetic role (1 g of lipids produces about 9 kcal), structural (part of cell membranes), hormonal (steroid hormones), vitamin (fat-soluble vitamins) and organ insulators (mechanical, thermal and electrical) (Grandall et al., 2009; Hidalgo et al., 2008).

Saturated fatty acids can be synthesized by the human body, which is why they are not an essential part of the diet. This group of acids is responsible for the increase of low-density lipoproteins-LDL ("bad" cholesterol), because it reduces the synthesis of LDL receptors and their activity; Coconut oil, palm oil and butter have a strong hypercholesterolemic effect. To lower cholesterol, it is twice as effective to reduce the intake of saturated fatty acids (fats from dairy products and meat) than the additional intake of polyunsaturated fatty acids (Anton et al., 2003; Grobas et al., 2001; Jolivet et al., 2006).

Monounsaturated fatty acids can be produced by the human body; the most important is oleic acid, which is found in large amounts in olive, almond, avocado and peanut oil (Hu F.B et al., 2001; Xiao et al., 2020).

Polyunsaturated fatty acids are not produced by the human body, therefore an intake from exogenous sources is necessary, because they are indispensable for a proper diet; from the omega 6 group, the most representative is linoleic acid, contained in vegetable oils (sunflower, hemp, soybean, pumpkin and sesame) (Hur et al., 2003; Kovacs-Nolan et al., 2005; Imai et al., 2019).

Specialist studies have shown that some of the fatty acids, through the different physiological roles they play, influence the health of consumers (Katarzyna & Ignatowicz, 2019).

For example, conjugated linoleic acid is believed to have anticancer and antioxidant effects, but also to have a beneficial influence on the immune system (Schaefer, 2002; Seah et al., 2019).

The Ω-3 fatty acids (α-linolenic, eicosapentaenoic, docosapentaenoic and docosahexaenoic), together with linoleic acid contribute to fetal development and the prevention of premature births, lower blood cholesterol, improve visual acuity, but also intellectual development (Nistor et al., 2017) .

Diets with high levels of omega 9 monounsaturated fatty acids and with an optimal ratio between omega 6 and omega 3 help lower cholesterol, but are also recommended in the treatment of diabetes, arthritis, depression, etc (Radu-Rusu et al., 2014).
Nutritional imbalances due to low consumption of essential fatty acids (especially docosahexaenoic and eicosapentaenoic acid) can cause various diseases and therefore the recommendations of specialists aim to ingest them from natural sources, such as fish oil (Simopoulos, 1991).
Of all the products of animal origin, eggs are considered nutritionally complete foods, which is why they have been declared as the standard food of protein efficacy in children.
Worldwide, the highest consumption is recorded for chicken eggs, whose chemical composition depends, among other things, on the rearing system and the quality of the feed administered. However, in recent times, hen's eggs have been frequently challenged (as they contain harmful elements such as hormones, heavy metals, pathogens, etc. and as being involved in raising cholesterol), and eggs from other poultry species, such as quail, biblical, ostrich, etc.

MATERIALS AND METHODS

The research aimed to establish the profile of fatty acids and the relationships between them in eggs from several poultry species; to achieve the proposed purpose, chicken, quail and biblical eggs (30 pcs./lot) and pasteurized liquid mixture of chicken eggs (one packing unit, 1.5 kg) were studied.
The determinations were performed according to the methodology specific to scientific research.
The determination of the fatty acid content of the eggs was performed with the Perkin Elmer Clarus 500 chromatograph equipped with a flame ionization detector and a BP x 70 column, on previously dried samples at + 65°C (mass = 1 g). The principle of the method consists in transforming the fatty acids from the analyzed sample into methyl esters, separating them by chromatographic column and then identifying them by comparison with the standard chromatogram and quantifying the percentage of fatty acid esters (SR EN ISO 5508:2002). The calculation of the amount of fatty acid esters in the samples was made by relating the surface area of the sample to the standard and to the dilution used; expression was done in g AG/100 g lipids.
The ratio between the fatty acid groups was established by calculation, as follows:
SFA/UFA = saturated fatty acids (SFA)/totally unsaturated fatty acids (UFA);
PUFA/MUFA = polyunsaturated fatty acids (PUFA)/monounsaturated fatty acids (MUFA).
To establish the Ω-6/Ω-3 ratio, the total omega 6 fatty acids were calculated (c. Linoleic acid C18:2n6; linolenic acid C18:3n6; eicosadienic acid C20:2n6; eicosatrienoic acid C20:3n6; arachidonic acid C20:4n6; Decosatetraenoic acid C22:4n6) and, respectively, the total omega 3 fatty acids (Linolenic acid α C18:3n3; Octadecatetraenoic acid C18:4n3; Eicosatrienoic acid C20:3n3; Decosapentenoic acid C22:5n3; Decosahexaenoic acid C22:6n3).

RESULTS AND DISCUSSIONS

The sum of the fatty acids. From the sum of saturated, monounsaturated and polyunsaturated fatty acids, a total amount of fatty acids of 99.417 g FAME/100 g total FAME (methyl esters of fatty acids) resulted in the pasteurized mixture made from chicken eggs, of 99.985 g FAME/100 g total FAME for chicken eggs, 100.570 g FAME/100 g total FAME for biblical eggs and 101.485 g FAME/100 g total FAME for quail eggs (Table 1).

Table 1. Fatty acids content (g FAME/100 g total FAME)

Fatty acids		Chicken eggs	Pasteurized liquid mixture	Quail eggs	Biblical eggs
Saturated fat		***35.525***	***35.073***	***32.925***	***40.803***
Myristic acid	C14:0	0.290	0.333	0.675	0.552
Pentadecylic acid	C15:0	0.105	0.062	0.190	0.111
Palmitic acid	C16:0	24.520	25.532	21.250	26.220
Margaric acid	C17:0	0.190	0.134	0.160	0.240
Stearic acid	C18:0	10.420	9.012	10.650	13.680

Fatty acids	Chicken eggs	Pasteurized liquid mixture	Quail eggs	Biblical eggs	Fatty acids
Monounsaturated acids		***37.855***	***39.356***	***40.835***	***33.689***
Myristoleic acid	C14:1	0.075	0.062	0.555	0.102
Pentadecanoic acid	C15:1	0.100	0.054	0.405	0
Palmitoleic acid	C16:1	3.150	3.482	3.970	3.590
Heptadecanoic acid	C17:1	0.120	0.071	0.210	0.133
Cis oleic acid	C18:1n9	34.040	35.374	35.250	29.670
Erucic acid	C22:1n9	0.085	0.092	0.110	0
Neurronic acid	C24:1n9	0.285	0.221	0.335	0.194
Polyunsaturated acids		***26.605***	***24.988***	***27.725***	***26.078***
Conjugated linoleic acid	C18:2	0	0	1.000	0.190
Cis linoleic acid (Ω-6)	C18:2n6	19.580	18.674	16.730	17.590
Linolenic acid y (Ω-6)	C18:3n6	0.145	0.111	0.120	0.133
Linolenic acid α (Ω-3)	C18:3n3	0.420	0.434	1.295	1.050
Octadecatetraenoic acid (Ω3)	C18:4n3	0	0	1.700	0.501
Eicosadienic acid (Ω-6)	C20:2n6	0.165	0.142	0.070	1.000
Eicosatrienoic acid (Ω-6)	C20:3n6	0.275	0.163	0.215	0.234
Eicosatrienoic acid (Ω-3)	C20:3n3	0.395	0.384	0.395	0.355
Arachidonic acid (Ω-6)	C20:4n6	3.500	2.901	2.790	2.900
Decosatetraenoic acid (Ω-6)	C22:4n6	0.835	0.964	0.850	0.210
Decosapentenoic acid (Ω-3)	C22:5n3	0.185	0.142	0.400	0.145
Decosahexaenoic acid (Ω-3)	C22:6n3	1.105	1.073	2.160	1.770
Total fatty acids		**99.985**	**99.417**	**101.485**	**100.570**

Fatty acid profile. For saturated fatty acids, the lowest content was measured in quail egg yolk, of only 32.925 g FAME/100 g total FAME (methyl esters of fatty acids), and the highest in biblical egg yolk, of 40.803 g FAME/100 g total FAME. In the case of chicken eggs, the saturated fatty acids recorded levels of 35.525 g FAME/100 g total FAME in the case of those in shell and 35.073 g FAME/100 g total FAME in the case of those processed into pasteurized mixture.

As expected, C16:0 palmitic acid predominated among the saturated fatty acids (quail = 21.250 g FAME/100 g; chicken = 24.52 g FAME/100 g; pasteurized chicken mixture = 25.532 g FAME/100 g; biblical = 26.220 g FAME/100 g) and stearic acid C18:0 (with values between 9.012 g FAME/100 g total FAME as it was in the pasteurized liquid mixture and 13.680 g FAME/100 g total FAME in biblical eggs).

In the case of monounsaturated fatty acids, the highest content was found in quail eggs (40.835 g FAME/100 g total FAME), followed by a decrease in the pasteurized mixture of chicken eggs (39.356 g FAME/100 g total FAME), eggs chicken (37.855 g FAME/100 g total FAME) and biblical ones with the lowest level (33.689 g FAME/100 g total FAME).

The highest amounts detected were for cis oleic acid C18:1n9 (29.670-35.374 g FAME/100 g total FAME) and palmitoleic acid C16:1 (3.150-3.970 g FAME/100 g total FAME). It should be noted that quail eggs found the highest amounts for each of the monounsaturated fatty acids tested, with the exception of the cis oleic acid in which they were surpassed only by the pasteurized mixture of chicken eggs.

Another finding was that no monounsaturated pentadecanoic and erucic acids were identified in biblical eggs.

Polyunsaturated fatty acids also recorded higher levels in quail eggs (27.725 g FAME/100 g total FAME), followed at a distance by chicken eggs (26.605 g FAME/100 g total FAME), by biblical eggs (26.078 g FAME/100 g total FAME) and pasteurized mixture of chicken eggs (26.078 g FAME/100 g total FAME).

Quantitatively detached cis linoleic acid C18:2n6, with values between 16.730 g FAME/100 g total FAME as it was for quail eggs and 19.580 g FAME/100 g total FAME for chicken eggs, but also arachidonic acid C20:4n6 with values between 2.790 g FAME/100 g FAME for quail eggs and 3.500 g FAME/100 g total FAME for chicken eggs.

C18:2 conjugated linoleic acid credited with anticancer effects was found only in quail eggs

(1.000 g FAME/100 g) and biblical eggs (0.190 g FAME/100 g)

The ratio of fatty acids. The closest ratio between saturated fatty acids (SFA) and total unsaturated fatty acids (UFA) was for quail eggs (0.480), and the widest for biblical eggs (0.716); in the case of chicken eggs, intermediate values were found, both for those in shell (0.551) and for those transformed into pasteurized mixture (0.545) (Table 2).

The ratio between polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) was 0.703 for hen's eggs, 0.635 for pasteurized mixture made from hen's eggs, 0.679 for quail eggs and 0.774 for eggs, respectively of biblical.

Omega 3 and omega 6 acid content. Omega 3 fatty acids were found in an amount of 2.033 g in pasteurized molasses, 2.105 g in chicken eggs, 3.821 in biblical eggs and 5.950 in quail eggs; for omega 6 fatty acids were detected amounts of 24.500 g for chicken eggs, 22.955 g for pasteurized mixture, 22.067 g for biblical eggs and, respectively, only 20.775 g for quail eggs Table 3).

Table 2. Ratio between fatty acid groups

The system growth	Fatty acids				Fatty acid ratio	
	SFA Saturated fatty acids	**MUFA** Monounsaturated fatty acids	**PUFA** Polyunsaturated fatty acids	**UFA** Totally unsaturated acids	SFA/ UFA	PUFA/ MUFA
Chicken eggs	35.525	37.855	26.605	64.460	0.551	0.703
Pasteurized liquid mixture	35.073	39.356	24.988	64.344	0.545	0.635
Quail eggs	32.925	40.835	27.725	68.560	0.480	0.679
Biblical eggs	42.803	33.689	26.078	59.767	0.716	0.774

Table 3. Omega 3 and omega 6 fatty acid content

Specification	Ω3	Ω6	Ω6 / Ω3
Chicken eggs	2.105	24.500	11.639
Pasteurized liquid mixture	2.033	22.955	11.291
Quail eggs	5.950	20.775	3.492
Biblical eggs	3.821	22.067	5.775

Regarding the ratio between omega 6 and omega 3 acids, the values obtained were very high in chicken eggs (11.639) and pasteurized mixture (11.291) and much lower in biblical eggs (5.775) and especially in quail eggs (3.492).

CONCLUSIONS

The analysis of the data on the total fatty acid content showed that the quail eggs had the highest level (101.485 g FAME/100 g total FAME), 0.90% higher than the biblical eggs, with 1.48% than for hen's eggs and 2.04% compared to the pasteurized mixture of hen's eggs.

The highest amount of saturated fatty acids (40.803 g FAME/100 g total FAME) was found in biblical eggs, 12.95% higher than in chicken eggs, by 14.04% compared to pasteurized melange and 19.30% than in quail eggs.

As for mono and polyunsaturated fatty acids, the highest level (68.560 g FAME/100 g total FAME) was recorded in quail eggs, followed at a long distance by chicken eggs (less by 5.98%), by the milling pasteurized chicken eggs (6.15% less) and biblical eggs (12.83%).

Consistent with the above data, the ratio between saturated fatty acids (SFA) and total unsaturated fatty acids (UFA) was only 0.480 in quail eggs, compared to 0.545-0.716 found in the other categories of eggs analyzed.

Regarding the ratio between polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), the values resulting from the calculations performed were 0.635 for the pasteurized mixture, 0.679 for quail eggs, 0.703 for chicken eggs and 0.774 for eggs, respectively of biblical.

The best ratio between omega 6 and omega 3 fatty acids was recorded in quail eggs, of only 3.492, followed quite closely by biblical eggs by 5.775; In the case of chicken eggs, the ratio between the two fatty acids was much higher,

standing at 11.291 (processed eggs) and 11.639 (natural eggs), respectively.

In conclusion, it can be stated that the fatty acid profile of quail eggs is a good one, as well as the ratios between the fatty acid groups, being able to successfully replace chicken eggs in the human diet; the eggs of other poultry species considered as food alternatives (biblical ones) contain large amounts of saturated fatty acids, to the detriment of monounsaturated and polyunsaturated ones.

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RESULTS OF OIL PRESS STUDIES FOR THE PRODUCTION OF ECOLOGICALLY CLEAN BUTTER

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Abstract

Introduced an oil press for the production of environmentally friendly butter. A research methodology for substantiating optimal parameters is presented. Based on the theory of probability and mathematical statistics, the regression equation describing the complexity of the production of environmentally friendly butter was determined. When solving it, the optimal design and kinematic parameters of the oil press. Its optimal parameters were established, which were: the value of the angular velocity of the screw 6.3 s^{-1}, the change in the pitch of the screw winding in the zones of 17.7 mm and the utilization factor of the throughput of the loading zone of the screw 0.6. At the same time, the technological labor intensity of the oil press per cycle was 18.385 man-hours. Research in production conditions has confirmed the correctness of finding the optimal values for the parameters of the oil press. The resulting technological labor intensity per cycle was 18.4 man-hours. with a productivity of 3.85 kg/h, which confirms the convergence of research results in laboratory and production conditions. The mass fraction of moisture in the original oil grain was 24-27.8%, and butter - less than 20%, but more than 16%, which meets all the requirements for peasant unsalted sweet cream butter.

Key words: *auger, butter, butter grain, casing, environmentally friendly, oil press.*

INTRODUCTION

The main task of the country's agriculture and dairy farming in particular, there is a further increase in the production of dairy products necessary for the population, on the basis of reducing their cost and creating generally available solutions for the mechanization of processing dairy products with minimized labor costs (Yashin et al., 2018).

The most important parameter that determines the labor intensity of devices for the production of butter and energy consumption for compaction is the speed of rolling. Back pressure in screw machines with an open chamber is created as a result of friction of the compressed mass against the channel walls. In presses with a closed chamber, the material fed into the seal channel is compressed between the stamp and the stop.

The processed material is pushed out of the chamber by the subsequent stroke of the stamp with the retracted stop. It is obvious that the energy intensity of the process is much lower here than when pressing in an open chamber, however, presses with spiral working bodies received the greatest distribution due to their cheapness, durability, simplicity and low energy consumption, in contrast to roller ones.

According to the operating mode, oil presses are also divided into three groups, differing in the number of working bodies.

A single-screw press in comparison with a multi-screw press has a number of advantages: higher productivity, reduced load on bearings, transmissions and drives, greater reliability in operation, low cost, proven technology, simple design.

In a single-screw press, the entire screw channel is filled with material: the conditions for forming are more favorable than in a two-screw analogue.

By the location of the working body, oil presses are also divided into three groups, which differ in the position and direction of the working bodies in space, the unconditional advantage of installations with an inclined arrangement of the working body is that, unlike horizontal presses, there is no partial mixing of the processed material -la with the removed liquid, which indicates the expediency of using inclined presses due to the absence of partial mixing of the squeezed material with the removed liquid (oil grain and a layer of butter with the removed buttermilk).

In the direction of winding the auger working body, oil presses come with both left and right winding, this, of course, an important criterion plays a major role when choosing a working body during the assembly of the machine, since the direction of winding will depend on the direction supply and processing of material (Yashin, 2021).

By the type of the screw surface of the working body, oil presses are also divided into three groups, depending on the geometric parameters of the screw winding.

Existing, serially produced devices for the manufacture and processing of butter are focused on high throughput and their use when processing small batches of dairy products in conditions of farms with a small production program is not advisable. Therefore, promising devices are those with a relatively low power consumption, with minimized labor intensity, highly economical, easy to operate and maintain, capable of performing several technological operations, and most importantly, having a low cost (Yashin, 2020).

The production of butter is carried out mainly according to two technologies: conversion of high-fat cream and churning (discontinuous and continuous), the use of which is determined by the volume of production and grade of oil (Yashin et al., 2019; Melken, 1991).

Based on the foregoing, it can be concluded that the work devoted to the development and aimed at finding and determining the optimal parameters of the oil press, which ensures a decrease in the labor intensity of the manufacture of butter, while observing quality indicators, are relevant and practically significant for agricultural production.

MATERIALS AND METHODS

The oil press (Figure 1) consists of three main parts [PF patent, 2013, 2017, 2018). The first part is recording and includes a laptop ASUS X540S 2 and an electronic multimeter MAS-345 5.

The second part is a control one and has an IEK 7 automatic switch and a Vesper E2-8300 6 speed converter. The third part is an executive part and contains an AIR71A2SU 3 electric motor, an SG80N 4 worm gearbox parts.

The oil press works as follows (Figure 2). The oil grain to be processed into butter is loaded into the hopper 5, while the shutter 6 is in the closed position. Then the electric motor 2 is started, transmitting the torque through the gearbox 3 and the clutch 4 to the screw shaft 8.

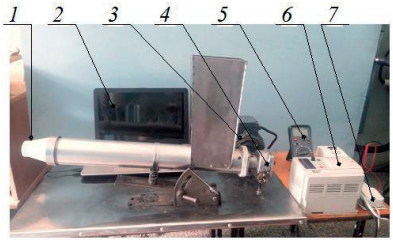

Figure 1. General view of the laboratory installation of the oil press: 1 - model of the oil press; 2 - ASUS X540S laptop; 3 - AIR71A2SU electric motor; 4 - worm gearbox SG80H; 5 - electronic multimeter MAS-345; 6 - frequency converter Vesper E2-8300; 7 - automatic circuit breaker IEK

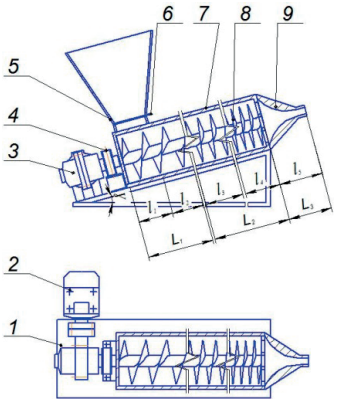

Figure 2. Structural diagram of the oil press: 1 - frame; 2 - electric motor; 3 - reducer; 4 - clutch; 5 - loading hopper; 6 - damper; 7 - casing; 8 - auger; 9 - forming nozzle; γ - auger ascent angle; l_1 - loading area; l_2 - primary mixing zone; l_3 - primary compression zone; l_4 - secondary compression zone; l_5 - forming zone; L_1 - section for loading and separating excess moisture; L_2 - compression section; L_3 - forming section

When the nominal angular velocity is reached, the shutter 6 opens and the oil grain from the loading hopper 5, enters the loading zone of the oil press auger and moves to the next zone with the separation of excess moisture and its removal from the hole in the casing 7, where mixing and texturing of the oil grain occurs.

Then the processed product, passing through the compression zones, undergoes compaction and plasticization with mixing. Towards the end of the secondary compression zone, the processed product acquires a continuous homogeneous structure, which is facilitated by the ongoing processes of intensified compression and the stepwise execution of the screw. From the secondary compression zone, the compressed mass leaves the screw and enters the molding zone in the form of a swirling flow, which is exposed to the forming nozzle 9 with a profile.

When using a polymer sleeve for food products on a forming nozzle, it is possible to pack and pack butter, which will reduce the labor intensity of making butter from the resulting oil grain when churning, since this oil press allows you to perform several technological operations - processing of oil grains and packaging.

The three-factor experiment was based on D - the optimal plan, carried out in order to obtain regression equations for the process of making butter and determine the optimal values of the parameters of the oil press.

When carrying out a three-factor experiment, the evaluation criterion was the labor intensity of processing oil grain, and the moisture content and consistency were used as limitations.

To implement a three-factor experiment, in order to give the factor x2 (change of the screw pitch in sections) an intermediate (zero) value of the level of variation, a set of replaceable screws with the first pitch t = 0.06 m was made (Figure 3).

Figure 3. A set of augers with a variable pitch in the sections: 1 - a screw with a pitch change of 20 mm, at t0 = 0.06 m; 2 - auger with a step change of 15 mm, at t0 = 0.06 m; 3 - auger with a step change of 10 mm, at t0 = 0.06 m

Mathematical processing of the results was carried out using the computer programs Statistica 6.0, MathCAD 2001RUS, Microsoft Excel on a PC. In this case, the statistical processing of the results of the three-factor experiment was carried out first by the Multiple Regression module of the Statistica 6.0 program when attempting to describe it by a linear model and by the Nonlinear Estimation module of the Statistica 6.0 program when describing by higher-order models. When determining the adequacy of the model (by multiple correlation coefficient and F-test), we used the data of statistical processing and the Microsoft Excel program. When determining the optimal values of the factors, the program MathCAD 2001 RUS was used, for which its listing was developed for solving the problem of finding the extremum of a function (an adequate model of the regression equation).

RESULTS AND DISCUSSION

The matrix and the results of the three-factor experiment are presented in Table 1. The results are defined as the average of triplicate.

Table 1. Matrix and results of a three-factor experiment

Experience number	ω	Changing the pitch of the auger in sections	Throughput ratio	Labor intensity, man-h.
	x_1	x_2	x_3	T
1	1	1	1	40.74
2	1	1	-1	46.34
3	1	-1	1	43.83
4	1	-1	-1	48.58
5	-1	1	1	46.51
6	-1	1	-1	41.72
7	-1	-1	-1	59.53
8	-1	-1	-1	53.92
9	1	0	0	26.77
10	-1	0	0	32.22
11	0	1	0	20.63
12	0	-1	0	28.27
12	0	0	1	33.95
14	0	0	-1	30.1

The volume of oil grain to be processed for each experiment was the same and amounted to the volume of the loading hopper equal to 3.85 liters. The processing time was defined as the time from the opening of the feed hopper flap to the end of the outflow of butter from the hole of the forming nozzle.

During the experiments, butter grains were produced from cream of constant fat content of 38%, temperature - 10°C, is corresponding to the requirements of GOST R 53435-2009 “Raw cream. Technical conditions”.

Before each experiment, the oil press was disassembled and assembled with washing of the parts in contact with oil grain and butter. For each experiment, according to the research matrix (Table 1), the adjustment was made to the specified parameters.

Analyzing the obtained values of Table 2, it can be noted that, with the exclusion of factors and their interactions x_2, $x_2 \cdot x_3$ from the considered dependence, the values of the regression coefficients for the factors and their interactions did not change in comparison with the results of table 4.3, in contrast to the Student's criteria for them. However, the tabular values of the Student's test at a confidence level of P = 0.95, t (6) = 2.447, which is lower than the obtained values for all factors and their interactions, which means they are statistically significant.

Table 2. Levels of significance of factors (parameters) on the labor intensity of making butter for the second order dependence (refined)

Factors	Coefficient regressions		Standard error	Student's criterion	Error severity level
	a		*Std. Err.*	*t (6)*	*p-level*
	a0	19.16188	0.725047	26.4284	0.000000
x_1	a1	-2.76400	0.359724	-7.6837	0.000254
x_2	a2	-3.81900	0.359724	-10.6165	0.000041
$x_1 \cdot x_2$	a12	2.48625	0.402184	6.1819	0.000824
$x_1 \cdot x_3$	a13	-2.59375	0.402184	-6.4492	0.000658
x_1^2	a11	10.33312	0.725047	14.2517	0.000007
x_2^2	a22	5.28812	0.725047	7.2935	0.000339
x_3^2	a33	12.86313	0.725047	17.7411	0.000002

According to the data obtained in Table 2, the mathematical dependence of the labor intensity of making butter of the parameters of an oil press finally in coded form will take the form:

$$\text{Tts} = 19.16188 - 2.76400\ x1 - 3.81900\ x2 + 2.48625\ x1\ x2 - 2.59375\ x1\ x3 + 10.33312x_1^2 + 5.28812x_2^2 + 12.86313x_3^2 \quad (1)$$

Moreover, the multiple correlation coefficient R = 0.995, and the F-test = 0.893. Consequently, the obtained mathematical relationship (1) adequately describes the results of the experiments.

To determine the optimal parameters of the oil press for the laboriousness of making butter, the data obtained were processed in the MathCAD program to find the extremum of the dependence (1). Why the obtained mathematical dependence (1) was differentiated by the variables x_1, x_2, x_3 and got a system of equations:

$$\begin{cases} -2,764 + 2,486 \cdot x_2 - 2,594 \cdot x_2 + 20,666 \cdot x_1 = 0 \\ -3,819 + 2,486 \cdot x_1 + 10,576 \cdot x_2 = 0 \\ -2,594 \cdot x_1 + 25,726 \cdot x_3 = 0 \end{cases} \quad (2)$$

By solving the system of equations (2), the optimal values of the parameters of the oil press in coded form were determined:

$$\begin{cases} x_1 = 0,094 \\ x_2 = 0,339 \\ x_3 = 0,0095 \end{cases} \quad (3)$$

With optimal values of the parameters of the oil press, the labor intensity of making butter (1) would be:

$$T_u^{\min} = 19,162 - 2,764 \cdot (x_1 = 0,094) - 3,819 \cdot (x_3 = 0,339) + 10,333 \cdot (x_1 = 0,094)^2 + 2,486 \cdot (x_1 = 0,094) \cdot (x_2 = 0,339) - 2,594 \cdot (x_1 = 0,094) \cdot (x_3 = 0,0095) + + 5,288 \cdot (x_2 = 0,339)^2 + 12,863 \cdot (x_3 = 0,0095)^2 = 18.385, \text{ people - hours} \quad (4)$$

The optimal values of the factors in the decoded form were: $\omega = 6{,}3$ s-1, $\Delta t = 17{,}7$ mm and $k_{загр} = 0{,}6$. In this case, the technological labor intensity per cycle of the oil press is $T_{mц} = 18{,}385$ people - h. The spread in the values of the labor intensity obtained from experimental studies in laboratory conditions at the optimal values of the parameters and according to the theoretical expression does not exceed 5%.

CONCLUSIONS

An experimental design model of an oil press has been developed and manufactured. Experimental studies in laboratory conditions made it possible to determine the optimal values of the angular velocity of the screw 6.3 s^{-1}, changing the pitch of the auger winding in the zones of 17.7 mm and the utilization factor of the throughput of the feeding zone of the auger 0.6. At the same time, the technological labor intensity of the oil press per cycle was 18.385 man-hours.

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STRUCTURE OF THE POPULATIONOF *ACANTHOCEPHALUS ANGUILLAE* IN *CARASSIUS GIBELIO* FROM TUNDJA RIVER, BULGARIA

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Abstract

During the ecological study of 19 specimens of Prussian carp (Carassius gibelio, Bloch, 1782) from Tundja River, by applying standard techniques for parasites, an infestation was found with the acanthocephalan species Acanthocephalus anguillae (Müller, 1780). Helminth parasites were recorded in 5 Prussian carp specimens (26.32%). The established helminth species is autogenic species, matured in fish. In the component community of Carassius gibelio from Tundja River A. anguillae is core species. This study is the first that presents the Prussian carp's endohelminth species biodiversity from Tundja River, Bulgaria. The established in this study parasite species is discussed and compared with previous researches of parasite communities of C. gibelio from Bulgaria.This is the first report of Acanthocephalus anguillae for the helminth communities of Prussian carp for river related to Aegian Basin in Bulgaria.

Key words*: Acanthocephalus anguillae, Carassius gibelio, helminths, Tundja River.*

INTRODUCTION

The Tundja River is the third-longest river in Bulgaria (349.5 km on Bulgarian territory) after the Danube and Iskar. The Tundja River springs from the central parts of Stara Planina north of Kalofer. After Kalofer passes from west to east between the mountain ranges of Stara Planina to the north and Sredna Gora to the south. After city of Sliven, the river flows to the southeast and below city of Yambol - to the south direction and leaves Bulgaria in the Edirne direction, where it merges with Maritsa River (Evros). The river is related to Aegean Basin and is included in the National monitoring program (Water Body Type BG3TU570R066) (Regulation 1/2011).

The Tundja River is impacted of domestic wastewater and, 'poor' to 'bad' nutrient quality prevails (Skoulikidis et al., 2009). Species composition of the ichthyofauna of Lower stream of Tundzha River is presented by 19 fish species belonging to 8 families (Kolev, 2014). Fish parasite communities and biodiversity from the Tundja River were studied not often (Kakacheva-Avramova, 1972; Kirin et al., 2013; Chunchukova & Kirin, 2020). This study is the first that presents the results of examinations of the Prussian carp's (*Carassius gibelio*, Bloch, 1782) endohelminth species biodiversity from Tundja River, Bulgaria.

MATERIALS AND METHODS

In the summer of 2019, fish and fish parasites are collected and examined from Tundja River (city of Yambol). The city of Yambol (42°29′N 26°30′E) is located in south-eastern Bulgaria and is situated on both banks of the Tundja River.

Almost the entire Lower Tundja River from the city of Yambol till before leaving Bulgarian boundaries is about to be pronouncing as Protected Zone (NATURA 2000 zones: Reka Tundzha 2 BG0000195).

A total of 19 specimens of Prussian carp from Tundja River are collected and examined in 2019. Fish are caught by angling. The scientific and common name of fish host is used according to the FishBase database (Froese & Pauly, 2021). The fish are examined immediately after their capture for gastrointestinal helmiths (an incomplete parasitological study), using standard techniques. The samples are counted and identified using keys of Bauer et al. (1981), Bauer (1987) and Bykhovskaya-Pavlovskaya (1985). Acanthocephalan specimens are examined as temporary slides in

ethanol-glycerin and identified (Petrochenko, 1956; Ergens & Lom, 1970; Bykhovskaya-Pavlovskaya, 1985).

The ecological terms prevalence (P%), mean intensity (MI) and mean abundance (MA) are used and calculated, based on Bush et al. (1997). The dominant structure of the component helminth communities was determined according to the criteria proposed by Kennedy (1993) based on the prevalence (P%) as: accidental (P% < 10), component (P% < 20) and core (P% >20) species.

RESULTS AND DISCUSSIONS

A total of 19 specimens Prussian carp (*Carassius gibelio* from Tundja River were collected and examined for parasites. Helminth parasites were recorded in 5 Prussian carp specimens (26.32%) from Tundja River. Only one parasite species was identified - the acanthocephalan species *Acanthocephalus anguillae* (Müller, 1780). The mean intensity of *A.anguillae* is 1.6 ± 0.8 and the mean abundance is 0.42 ± 0.82 (Table 1). The only established helminth species occurred as adult. *A. anguillae* is autogenic species matured in fish.

Carassius gibelio is classified as least concern species (LC=Least Concern; IUCN Red List Status). The Prussian carp is not included in the Red Data Book of the Republic of Bulgaria (Golemanski, 2011). *Carassius gibelio* is not typical for Bulgarian waters but is introduced (Stefanov, 2007). Prussian carp is a freshwater, benthopelagic, brackish, potamodromous fish species (Froese & Pauly, 2021). In Bulgaria, this species is widely distributed in most marshes, plain and sub mountain lakes, dams and rivers (Vassilev & Pehlivanov, 2005). Prussian carp is omnivorous and feeds on larvae of plankton, benthic invertebrates, plant material and detritus (Froese & Pauly, 2021). *Carassius gibelio* can tolerate low oxygen concentrations and pollution (Kottelat & Freyhof, 2007).

The life cycle of *A. anguillae* is accomplished with the precipitation of the intermediate crustacean host - *Asellus aquaticus* (Linnaeus, 1758) (Petrochenko, 1956; Kakacheva-Avramova, 1983; Bauer, 1987). *A. aquaticus* is a bioindicator for α-mesosaprobity (Johnson et al., 1993). Fish are definitive hosts for this acanthocephalan species, but there is also data from Bulgaria for paratenic host *Lutra lutra* (Dimitrova et al., 2008).

A. anguillae was reported as parasite of other cyprinids from Tundja River (Table 2).

Table 1. Ecological indices of the helminth parasite of *C. gibelio* from Tundja river
(N - number of examined fish specimens, n - number of infected hosts, p - number of parasites, P% - prevalence , MA - mean abundance, MI - mean intensity)

Helminth species	N	n	p	P%	MA ± SD	MI ± SD	Range
Acanthocephalus anguillae	19	5	8	26.32	0.42 ± 0.82	1.6 ± 0.8	1-3

Table 2. Fish species reported as hosts of *Acanthocephalus anguillae* from Tundja River in Bulgaria

Host	**References**
Alburnus alburnus	Kirin et al., 2013
Squalius cephalus	Chunchukova & Kirin, 2020
Chondrostoma vardarense	Chunchukova & Kirin, 2020

For the same cyprinid hosts from River Tundja was reported also the acanthocephalan species *Pomphorhynchus laevis* (Müller, 1776), but from earlier study (Kakacheva-Avramova, 1972).

The established in this study *Acanthocephalus anguillae* is an intestinal parasite of many freshwater fish in Bulgaria mainly from Cyprinidae family, and also there are records from families Salmonidae and Percidae (see Table 3). The records are from different rivers in Bulgaria, with the exception of the data for Srebarna Lake (Shukerova et al., 2010; Shukerova & Kirin, 2019), which is probably due to the connection of the Lake with the Danube River.

Table 3. Overview of fish species registered as hosts of *Acanthocephalus anguillae* in Bulgaria and their locality

Fish host	Locality	References
Cyprinidae Family		
Alburnus alburnus	Maritsa River	Kakacheva-Avramova (1965)
		Margaritov (1965)
	Tundja River	Chunchukova & Kirin (2020)
	Arda River	Kirin (2003)
Abramis brama	Danube River	Atanasov (2012)
		Chunchukova et al.(2016)
Barbus barbus	Danube River	Nachev & Sures (2009)
		Atanasov (2012)
		Chunchukova & Kirin (2018)
Barbus cyclolepis	Chepinska River	Margaritov (1965)
	Luda Yana River	Kirin (2002a)
	Arda River	Kirin (2003)
Blicca bjoerkna	Danube River	Margaritov (1966)
		Kakacheva-Avramova (1977)
Carassius gibelio	Danube River	Atanasov (2012)
Chondrostoma vardarense	Tundja River	Chunchukova & Kirin (2020)
Rutilus rutilus	Chepinska River, Bistrica River	Margaritov (1965)
	Srebarna Lake	Shukerova & Kirin (2019)
Squalius cephalus	Barzia River, Chuprenska River	Kakacheva-Avramova (1969)
	Stryama River	Kakacheva-Avramova (1973)
		Kirin et al. (2005)
	Palakaria River	Kakacheva-Avramova & Menkova (1978)
	Chepinska River	Margaritov (1965)
	Maritsa River	Kirin (2000a), Kirin (2000b)
	Chepelarska River	Kirin(2002b)
Leuciscus idus	Danube River	Margaritov (1959)
		Margaritov (1966)
		Kakacheva-Avramova (1977)
Squalius orpheus	Tundja River	Kirin et al. (2013)
Family Percidae		
Perca fluviatilis	Srebarna Lake	Shukerova et al. (2010)
Family Salmonidae		
Salmo trutta	Barzia River, Chuprenska River	Kakacheva-Avramova (1969)

The ichthyofauna of the Lower stream of River Tundja is presented by 19 fish species belonging to 8 families (Kolev, 2014). Fourteen of them were subject to ecogoparasitological investigation in previous studies (Kakacheva-Avramova, 1972; Kirin et al., 2013; Chunchukova & Kirin, 2020). For ten of the fish species was reported at least one acanthocephalan species in these studies. This is the first study of helminth fauna of *Carassius gibelio* from River Tundja. Generally the helminth fauna of Prussian carp was not very often studied in Bulgaria.

For Bulgaria were reported twenty three parasite species - *Paradilepis scolecina, Dactylogyrus anchoratus*, *D. extensus*, *D. formosus*, *D. intermedius, D. minutus, D. vastator, D. vistulae, D. wegeneri, Diplostomum helveticum, D. pseudospathaceum, D. rutili, Ancyrocephalus* sp., *Gyrodactylus medius, G. shulmani, G. sprostonae, Urocleidus similis, Paradiplozoon homoion, Posthodiplostomum cuticola*, *Raphidascaris acus* larvae, *Contracaecum microcephalum* larvae, *Acanthocephalus anguillae* and *Pomphorhynchus laevis* as helminth parasites of *Carassius gibelio* (Table. 4).

Table 4. Overview of parasite species of *Carassius gibelio* registered in Bulgaria

Authority / Helminth species	Margaritov (1959)	Margaritov (1964)	Margaritov (1966)	Kakacheva-Avramova (1977)	Grupcheva & Nedeva (1999)	Shukerova (2005)	Atanasov (2012)	This study
Paradilepis scolecina (Rudolphi, 1819)					•			
Dactylogyrus anchoratus (Dujardin, 1845)	•	•		•	•			
Dactylogyrus extensus Mueller & Van Cleave, 1932		•						
Dactylogyrus formosus Kulwiec, 1927		•						
Dactylogyrus intermedius Wegener, 1909					•			
Dactylogyrus minutus Kulwiec, 1927		•						
Dactylogyrus vastator Nybelin, 1924		•						
Dactylogyrus vistulae Prost, 1957					•			
Dactylogyrus wegeneri Kulwiec, 1927		•						
Diplostomum helveticum (Dubois, 1929)					•			
Diplostomum pseudospathaceum Niewiadomska, 1984							•	
Diplostomumrutili Razmashkin, 1969						•		
Ancyrocephalus sp. Creplin, 1839					•			
Gyrodactylus medius Kathariner, 1893		•						
Gyrodactylus shulmani Ling, 1962					•			
Gyrodactylus sprostonae Ling, 1962					•			
Urocleidus similis (Mueller, 1936)					•			
Paradiplozoon homoion (Bychowsky & Nagibina, 1959)					•			
Posthodiplostomum cuticola (Nordmann, 1832)						•		
Raphidascaris acus (Bloch, 1799), larvae						•		
Contracaecum microcephalum (Rudolphi,1809), larvae						•		
Acanthocephalusanguillae (Müller, 1780)							•	•
Pomphorhynchus laevis (Zoega in Muller, 1776)			•	•	•		•	

CONCLUSIONS

This is the first study of helminth fauna of *Carassius gibelio* from River Tundja. The determined helminth species *A. anguillae* is a core species for the helminth communities of Prussian carp's from the studied ecosystems. This is the first report of *Acanthocephalus anguillae* for the helminth communities of *C. gibelio* for river related to Aegian Basin in Bulgaria.

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EFFECTS OF FEEDING LEVEL ON GROWTH PERFORMANCE AND BODY COMPOSITION OF COMMON CARP (*CYPRINUS CARPIO*, LINNAEUS, 1758) IN RECIRCULATING AQUACULTURE SYSTEMS REARING

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Abstract

A feeding trial was conducted to determine the effects of different feeding levels on growth performance and body composition. During 31 days, the experiment was carried out in the "Dunărea de Jos" University facilities from Galați, Romania. 720 fish, with an initial weight of 10.92 ± 0.17 g, were randomly distributed in twelve RAS rearing unit, with the same stocking densities. Four experimental variants were created, in triplicate: V1 - 3% from body weight per day (% BW day^{-1}), V2 - 3.9% BW day^{-1}, V3 - 4.9% BW day^{-1} and V4 -6% BW day^{-1}. Fish were fed with 45% crude protein and 16% lipids at a feeding frequency of three meals per day. At the end of the experiment, significant differences ($p<0.05$) were recorded between the four experimental variants. The body composition showed significant differences ($p<0.05$) also. The highest protein content and the lowest lipid content being recorded in variant V4 with 6% BW day^{-1}.

Key words*: growth indicators, juveniles, pellets, proteins and lipids content.*

INTRODUCTION

Aquaculture is one of the fastest-growing sectors worldwide dedicated to food production (Food and Agriculture Organization, 2018). The highest demand for fish has led to the implementation of new and modern technologies to ensure high-quality products over the entire year. In this sense, the development of aquaculture in recirculating systems is beginning to gain ground (Timmons et al., 2018; EUMOFA, 2020), mainly to the higher production which can be obtained (Timmons et al., 2002).

Growing fish in RAS systems involves various factors which can influence the final production and profitability. The most important are aspects related to growth and feed efficiency. Therefore the optimization of feeding management is crucial. Since the cost of feeding represents an important proportion of aquaculture operational costs, it is very important to estimate the optimal ratio of feed suitable for the species, its developmental stage, and the conditions of rearing (Pillay and Katty, 2005; El-Sayed, 2013; Baki and Yücel, 2017). Therefore, in a RAS system, intensification of fish growing should take into consideration the use of a proper feeding level, in order to obtain higher productions and to keep a good quality of water. If fish are underfed, the competition for feed increase (Attia et al., 2011), the growth can be suppressed, and fish variability increases (Zhou et al., 2003). Also, fish welfare can be depreciated, making them more susceptible to diseases (Rowland et al., 2005; Lim et al., 2015). On the other hand, if fish are overfed, water quality can deteriorate, the production cost increases and fish growth is reduced (Cho et al., 2007; Kim et al., 2007).

Common carp (*Cyprinus carpio*) represents one of the world's most cultivated fish species, with over 8 million tonnes being produced in 2017 (Food and Agriculture Organization, 2019). Common carp is hardy and tolerant of a wide variety of conditions, disease-resistant, an aspect that makes it an ideal candidate for freshwater aquaculture (Mohapatra and Patra, 2014).

The effects of feeding level on fish growth and feed conversion efficiency have been studied for several fish species in the conditions of the RAS systems (Firas, 2017; Crețu et al., 2019;

Petrea et al., 2020), but there are several factors which influence the feeding level in a RAS system.

The optimum feeding rate is dependent on fish species, fish size, and rearing conditions. Therefore choosing good management practices is fundamental to the success of the production. In this context, this study aimed to investigate the effects of different feeding levels on the growth performance and body composition of common carp with an initial weight of 10.92 ± 0.17 in the condition of a RAS system.

MATERIALS AND METHODS

Experimental design

The experiment was carried out at the pilot system of the Faculty of Food Science and Engineering belonging to "Dunărea de Jos" University of Galați, in a pilot recirculating aquaculture system (RAS). The RAS system comprised twelve rearing units with a volume of 0,132 m^3 each, mechanical filter, biological filter, UV lamp for water sterilization and disinfection, pumps, and was described by Crețu M. (2013).

The common carp fry was obtained from the extra season natural reproduction in August 2018 (Figure 1).

Figure. 1 Common carp fry (photo original)

Before the trial, fish were acclimated in laboratory conditions in a tank of 500 L volume for one week. After fish acclimatization, 720 carp fingerlings with an initial weight of 10.92±0.17 g were randomly distributed in the RAS system to create the experimental variants.

The study design included four feeding rates, in triplicate: V1- 3% from body weight per day (% BW day^{-1}), V2- 3.9% BW day^{-1}, V3- 4.9% BW day^{-1} and V4-6% BW day^{-1}. The daily rations were supplied each day in three meals, at 09:00, 14:00, and 18:00 hours. The fish was offered a commercial diet with a content of 45% crude protein and 16% lipids (Table 1).

During the experimental period, the water quality parameters such as dissolved oxygen, temperature, and pH were recorded daily with the help of Hannah 98194, while the concentration of nitrogen compounds was measured twice per week with the help of the Spectroquant Nova 400 photometer compatible with Merck kits.

Table 1. Proximate composition of experimental diets

Ingredients	U.M.	Diet
Crude protein	%	45
Crude lipids	%	16
Crude cellulose	%	2
Ash	%	7
Calcium	%	1.3
Sodium	%	0.30
Phosphorus	%	1
Vitamin A	IU/kg	10 000
Vitamin E	mg/kg	200
Vitamin C	mg/kg	150
Fe	mg/kg	60
Cu	mg/kg	5
Zn	mg/kg	100
Mn	mg/kg	25
Ca	mg/kg	2.5
BHA (E320)	mg/kg	30
BHT (E321)	mg/kg	29
Ingredients: poultry meal, wheat, fish meal, concentrated sunflower, wheat feed, blood meal, fish oil, rapeseed cake, rapeseed oil, hemoglobin powder, sodium chloride, calcium carbonate.		

Fish growth performance

After 31 experimental days, fish were weighed, and the following technological efficiency indicators were calculated: weight gain, food conversion ratio, and specific growth rate using the following equations:

Weight Gain (WG, g)

WG = Final biomass (g) – Initial biomass (g),

Individual weight gain (IWG, g)

IWG = Final Weight (Wt) – Initial Weight (W0) (g/fish),

Survival rate (%) = (final number of fish / initial number of fish) ×100,

Feed Conversion Ratio (FCR, g/g)

FCR = Total feed (F)/Total weight gain (W),

Specific Growth Rate (SGR, % Body weight day^{-1})

SGR = [(LnWt–LnW0)/t] × 100,

Body composition analysis

At the end of the experiment, seven fishes from each replicate were sacrificed for the analysis of the proximate composition of the whole body. The ash, moisture, crude protein, and lipid contents of fish were estimated by AOAC (2000). The biochemical tests were performed with three replicates and calculated on a wet weight basis.

Data analysis

Data were analyzed by one-way (ANOVA) using SPSS, version 21 for Windows. Before ANOVA, the normality of the data used for analysis was checked by Kolmogorov-Smirnov test. If any differences between the experimental variants were registered, Duncan's test was used. All experimental values are expressed as mean ± SD. Significance was determined at $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

Water quality

Estimating the optimum ratio of feeding is important for determining nutrient requirements and production. In the present study, the analysis of the technical indicators was done in accordance with the monitoring of the main parameters of the water. The maintenance of good water quality is essential for the growth, survival, and production of fish species.

The water quality parameters except ammonium showed no significant differences ($p<0.05$) (Table 2). The ammonium concentrations significantly increased by increased ($p<0.05$) the feeding level. However, all the water parameters were in the optimal range for fish growing (Billard, 1995; Timmons et al., 2018).

The final weight, feed conversion ratio, specific growth rate, and protein efficiency ratio were significantly different (ANOVA, $p<0.05$) among the feeding levels. Data regarding the fish growth performance and feed conversion efficiencies of carp subjected to different feeding levels are presented in Table 3.

Initially, fish have similar weight, and no significant difference was recorded among the treatment ($p<0.05$). The weight homogeneity was verified and confirmed by Levene's test ($p>0.05$).

Table 2. The average values (± SD) of the main physicochemical parameters of water

Parameters	V1	V2	V3	V4
T°C	23.30±0.48^a	23.40±0.68^a	23.30±0.54^a	23.28±0.46^a
pH (pH units)	8.09±0.13^a	8.15±0.12^a	8.15±0.14^a	8.15±0.14^a
OD (mg L^{-1})	7.59±0.36^a	7.87±0.42^a	7.95±0.32^a	7.84±0.35^a
N-NO_2^- (mg L^{-1})	0.25 ±0.09^a	0.26±0.13^a	0.26±0.10^a	0.27±0.13^a
N-NO_3^- (mg L^{-1})	18.56±5.30^a	18.80±4.76^a	18.87±6.47^a	19.78±4.76^a
N-NH_4^+ (mg L^{-1})	0.21±0.11^a	0.32±0.09^b	0.38±0.16^b	0.45±0.18^c
P-PO_4^+ (mg L^{-1})	0.62±0.48^a	0.64±0.23^a	0.67±0.23^a	0.68±0.30^a

Note: data are presented as the mean of the triplicates; the data with the same letters were not statistically different.

Table 3. Growth performance indicators at the end of the experimental period

Growth parameters	Experimental variants			
	V1	V2	V3	V4
Initial biomass (g)	664.84±1.35^a	664.23±1.04^a	665.65±1.48^a	665±4.36^a
Final biomass (g)	1255.67±39.95^a	1102±2.65^b	1100±2.00^b	1093±2.00^b
Weight gain (g)	590.83±38.62^a	437.77±3.27^b	434.36±1.24^b	428±6.08^b
Survival (%)	98.44±1.50^a	94.64±4.73^a	97.22±1.92^a	97.22±1.92^a
Initial weight (g $fish^{-1}$)	11.08±0.02^a	11.07±0.02^a	11.09±0,02^a	11.08±0.07^a
Final weight (g $fish^{-1}$)	21.42±1.21^a	18.90±0.53^b	18.86±0.41^b	18.74±0.38^b
SGR (% day)	2.05±0.10^a	1.63±0.01^a	1.62±0.00^a	1.60±0.03^b
IWG (g)	10.34±1.19^a	7.83±0.53^b	7.77±0.38^b	7.66±0.34^b
FCR (g/g)	1.05±0.07^a	1.83±0.02^b	2.33±0.01^c	2.82±0.06^d

Note: data are presented as the mean of the triplicates; the data with the same letters were not statistically different.

After 31 experimental days the final mean weight was significantly higher ($p<0.05$) in V1 (21.42 ± 1.21g), while no significant differences ($p>0.05$) were recorded between the V2, V3, and V4 variants (18.90 ± 0.53 g in V2; 18.86 ± 0.40 g in V3, respectively 18.74 ± 0.37 g in V4).

Although in our experiment, the fish survival was high, ranging between 94.67 % and 98.33%, ANOVA analysis showed that the survival rate was not significantly ($p>0.05$) influenced by the feeding ratio. The highest survival rate is obtained at the lowest feeding ratio (V1).

Regarding the main technological indicators, it was observed that the increase in the feeding ratio did not always produce an increase in growth.

Significant differences ($p<0.05$) were recorded in the obtained values of FCR between the four experimental variants. The post hoc Duncan analysis divided the FCR values into four distinct groups belonging to each experimental variant. The best value of FCR was obtained at the lower feeding ratio (V1). A significant decline in feed conversion efficiency and protein efficiency ratio was observed at higher feeding rations which was a sign of loss of nutrients and wastage of food.

Increasing feeding wastage with the increasing of feeding ration was also reported by other authors in the case of common carp. Desai et al., 2009, studied four different rations (4%, 5%, 6%, and 7% BW day^{-1}) for common carp fry with the initial weight of 0.86 g and found that the optimum feed conversion efficiency was achieved at a lower feeding level (4% BW day^{-1}) at a temperature of 28°C and 32°C respectively. Also, Shimeno et al., 1997, observed that feeding common carp with slightly less than satiety levels, such as 90% and 80% of satiation, as compared to satiety feeding, achieved a somewhat higher feed efficiency ratio.

According to Van Ham et al., 2003, fish tend to optimize their digestion and retain nutrients more efficiently at lower feeding rates, and if they are fed above their appetite, food is wasted, and an artificially FCR will be registered (Khan et al., 2004). Also, Velázquez et al. (2006) say that reducing the daily amount of feed intake appears to compel fish to make the best use of the feed.

Comparing the obtained values of SGR between the experimental variants, the statistical analysis revealed significant differences ($p>0.05$) between the experimental variants. Better values of SGR were recorded in the V1, while no significant differences ($p>0.05$) were found between the V2, V3, and V4.

Effects of feeding levels on body composition and morphological indices are shown in Table 3. Fish body composition was significantly affected by feeding level ($p<0.05$). The findings of the present study showed that feeding level significantly affects water, protein, lipids, and ash content.

Body moisture content decreased significantly ($p<0.05$) with the increase of feeding levels. Regarding the protein content, higher values were obtained in the V4 variant, but there were no significant differences ($p>0.05$) between this variant and V1, V3, and V4.

Table 4. The proximate composition of common carp body composition reared at different stocking densities

Parameters	Experimental variants			
	V1	V2	V3	V4
Water (%)	72.68±0.17^{a}	72.98±0.16^{a}	70.43±0.17^{b}	70.43±0.13^{b}
Protein (%)	13.78±0.08^{a}	12.31±0.16^{b}	14.32±0.76^{a}	14.56±0.31^{a}
Lipid (%)	11.58±0.42^{a}	12.37±0.14^{b}	12.29±0.32^{b}	12.85±0.63^{b}
Ash (%)	1.47±0.03^{a}	1.67±0.02^{b}	1.72±0.07^{b}	1.78±0.05^{b}

Note: Data are presented as triplicate mean ± SD; the data with the same letters were not statistically different.

The lipids and ash content showed a significant increase ($p<0.05$) with the increasing of the feeding levels, the lowest values being registered in the V1 variant. The results obtained by us are comparable to those reported by other authors. Wang et al. (2019) reported for Nile tilapia an increase of lipid content in muscle and whole body with increasing feeding rates, fish fed 5% BW day^{-1} had higher values compared with that fed 3 % BW day^{-1}. Also,

similar results were obtained by Ahmad et al. (2012) for common carp fingerlings or by Crețu et al. (2019) for rainbow trout.
Increasing of protein and lipid content with the increasing of feeding level was also reported by Petrea et al., 2020 in the case of *Acipenser stellatus* fed at a feeding level of 1% BW day^{-1}, respectively 2% BW day^{-1}.
Generally, the increase of lipid content with the increasing of the level of feeding was observed when the fish are fed at higher rates than that needed for the maintenance requirement, the excess energy accumulates mainly in the form of lipid in the adipose tissues (Huang et al. 2015; Liu et al., 2018).

CONCLUSIONS

In aquaculture, establishing the appropriate feeding level is important to minimize the cost of production and to make the technological production process more profitable. The main conclusion of this study is that increase in feeding level does not increase significantly the growth performance of common carp. Based on the result of the present study, it can be concluded that the optimum feeding rate for *Cyprinus carpio* (with the individual weight ranging from 11 g to 21 g) was around 3% BW day^{-1} and a feeding ratio beyond this level lead to the obtaining of unsatisfactory technological indicators, which over time can lead to low economic efficiency.

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GROWTH TECHNOLOGIES FOR THE COMPLEX EXPLOITATION OF AQUATIC BASINS FROM THE TRADITIONAL FISH FARMS

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Abstract

The growth technologies for the complex exploitation of the aquatic basins from the traditional fish farms are regarding the location of some intensive growth modules such as battery of net pens (total surface/module 2000 m^2) or floating cages (total volume/module 576 m^3), within fish ponds. These growth modules allow obtaining additional quantities of fish on the same unit area and raising valuable species of different ages like catfish, carp, sturgeon, tilapia, etc, through the efficient exploitation of aquatic bioresources. For administered feed, FCR was in the range of 1.5-2.5 kg of feed/kg fish weight gain. Applying the growth technology into combined system it is possible to achieve a total production of 2,400 - 2,800 kg/ha (60-65% achieved in the host pond and 35-40% in the battery of net pens or floating cages). Bioeconomic studies demonstrates the profitability of the combined system and the developed technology, obtaining a profit rate of 1.26 for the pond-net pens system, respectively 1.56 for the floating cages-pond system.

Key words: *combined system, floating cages, net pens, polyculture, pond.*

INTRODUCTION

Romania has an important fishing potential represented by accumulation lakes (natural or artificial), dams, ponds, large rivers, high capacity irrigation canals. At this moment, their exploitation is carried out in extensive and semi-intensive systems, the productions being variable, depending on the natural productivity of the respective basins, most of the times, the production level being modest (FAO, 2013; MNSPA, 2014).

In the context of sustainable aquaculture, the challenges are set by the intensification of conventional aquaculture and the emerging integration within and between value chains (Little et al., 2016). Fish farmers need to better manage the growing demand for animal protein, adapting their service to needs and capacity (Marin et al., 2020).

The Fish Culture Research and Development Station Nucet (F.C.R.D.S. Nucet) proposes for traditional fish farming two combined fish growing system. Specifically, growth technologies for the complex exploitation of water basins in traditional fish farms have in mind the placement of intensive growth modules (such as floating cages, net pens) on the surface of fish ponds, which would allow obtaining additional quantities of fish on the same unit surface and growth of valuable species (catfish, carp, sturgeon, tilapia, etc.), in the conditions of efficient exploitation of aquatic bioresources (Billard & Marcel, 1985; Keramat, 2013).

The principle of the used method is based on the link between aquaculture production systems and the growth of fish species that occupy different niches in the food chain, in a single integrated system, so as to achieve the complex use of aquatic bioresources, nutrient recycling and reducing the effects on the growth environment (Bagental, 1978; Pope et al., 2010).

The proposed combined system aims to increase production capacity, diversify species, recycle nutrients in the production system, while testing the limits of the intensive growth system (Muir, 2000; Bucur et al., 2016).

MATERIALS AND METHODS

The technology of complex exploitation of the water basins from the traditional fish farms

imply the realization of two experimental models:
1. The first growth model in the combined system involved the placement of intensive growth modules (net pens battery) in a fish pond (Figure 1).

Figure 1. Battery of net pens located in the pond (*Original photo*)

Fish species for intensive farming are: carp (*Cyprinus carpio*) and catfish (*Silurus glanis*). This model is adapted to fish basins with a water depth of 1.5-2.0 m (Stickney, 2002).

a) *Main features*
The technology offers the possibility of intensive growth of carp and catfish in net pens located in ponds or reservoirs (where there is the possibility of maintaining a constant water level), with the administration of granulated feed and obtaining a high quality fish for restocking or market;
- the realization of the net pens does not imply high costs. The characteristics of the growth installation are the following:
  - net pens battery with an area of 2 000 m^2;
  - the surface of a net pen - 200 m^2;
  - height of the net pen - 2 m;
  - net pen length - 20 m;
  - net pen width -10 m;
- average water depth in net pens - 1.6 m (minimum 1.2 - maximum 2 m);
- the net pens battery is a closed enclosure with galvanized wire mesh with a mesh of 10 mm, fixed on pine pillars with a thickness of 15-20 cm, reinforced at the top with fir cabinets with a width of 10 cm and a thickness of 5 cm.
- the surface of the net pen battery represents maximum 10% of the total surface of the water basin in which they are located.

b) *Stocking formula*
For a net pen with an area of 200 m^2 and a minimum water depth of 1.2 m, the stocking formula (monoculture) is:
- carp C_1: 200 ex/net pen;
- catfish Sg_2: 200 ex/net pen.

c) *Average weight of fish for stocking*:
- carp C_1: 150 g/ex;
- catfish Sg_3: 700 g/ex.

d) *Fish feeding*
The carp feeding in the net pens battery was done with the Fish Feed Carp 32/2, 32/6, 32/8, 32/10 E Floating feed, produced by Furajny Hrany Ltd Lovech (Table 1).
Tthe catfish feeding in the net pen battery was done with the Aller 45-15 granulated feed, with the 7.2 mm granule size (Table 2).

Table 1. Chemical composition of feed Fish Feed Carp 32/2, 32/6, 32/8, 32/10 E Floating

Components	Values
Protein	32%
Lipid	10%
Raw fiber	2.9%
Calcium	1.4%
Phosphorus	1.8%
Moisture	10.0%
A vitamin (E672)	10000 IU.kg^{-1}
D_3 vitamin (E672)	2000 IU.kg^{-1}
E vitamin	200 mg.kg^{-1}
C vitamin	250 mg.kg^{-1}

Table 2. The structure on nutritional components of the Aller 45-15granulated feed

Components	Values
Protein	45%
Lipid	15%
Ash	7%
Cellulose	2.5%
Carbohydrates	29.5%
A vitamin (E672)	2500 IU.kg^{-1}
D_3 vitamin (E672)	500 IU.kg^{-1}
E vitamin	150 mg.kg^{-1}
C vitamin	100 mg.kg^{-1}

e) *The host pond stocking formula (1.0 ha module), in which the net pens battery is placed:*
- paddlefish P_2: 270 ex/ha;

- carp C_2: 450 ex/ha;
- grass carp Gc_2: 45 ex/ha;
- silver carp Sc_2: 45 ex/ha;
- pikeperch Pp_2: 90 ex/ha.

f) *Average weight of fish for stocking*:
- paddlefish P_2: 1400 - 1600 g/ex;
- carp C_1: 500 - 700 g/ex;
-grass carp Gc_2: 400 - 600 g/ex;
-silver carp Sc_2: 700 - 900 g/ex;
-pikeperch Pp_2: 400-600 g/ex.

g) *The growth cycle duration*: 150 days from the stocking time (end of April - beginning of May).

h) *Manure administration in the host pond*: 1000-2000 kg/ha (the amount being determined by the results of water analyzes).

2. The second growth model in the combined system involved the construction and placement of a floating cages module in a fish pond. Intensive rearing of the following fish species was carried out: paddlefish (*Polyodon spathula*), tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio*) (Figure 2).

Figure 2. The floating cages platform located in the pond (*Original photo*)

The growth model is adapted to fish basins with greater water depth.

a) *Main features*
Technology for intensive growth in floating cages located in a pond or reservoir (where there is a possibility to maintain a constant high water level), of paddlefish, tilapia and carp, offers the possibility of raising these species, with the administration of granulated fodder and can obtain high-quality fish intended for stocking or market.
The growth unit is composed of eight floating cages which have a square shape (Figure 2). The size of a single floating cage: 6 m x 6 m x 2 m, the cage surface - 36 sqm and the entire module surface is 288 sqm; useful volume - 72 m^3/floating cage; total volume: 576 m^3/module.
The floating cages are made of knotless fishing nets fastened on a frame fixed to the peripheral floats. On the frame, the fishnet is caught at the top at a 0.8 m height above the water (to prevent fish from escaping); in water, it is immersed cca 2 m.
Between the base of the cage and the bottom of the pond is at least 0.5 m, to allow the water flow to pass under the module, thus entraining some of the remaining organic waste.
The growing units surface (floating cages), does not represent more than 10% of the total surface of the water basin in which they are located.

b) *Stocking formula*
For a floating cage (72 m^3) with an 36 m^2 area and an average depth of 2.0 m, the stocking formula (monoculture) was the following:
- carp C_1: 500 ex/cage;
- paddlefish P_0: 1000 ex/cage;
- tilapia T_1: 1000 ex/cage.

c) *Average weight of fish for stocking*:
- carp C_1: 150 g/ex;
- paddlefish P_0: 20 g/ex;
- tilapia T_1: 70 g/ex.

d) *Fish feeding*
The carp and tilapia feeding from the floating cages was done with Fish Feed Carp 32/2, 32/6, 32/8, 32/10 E Floating feed, produced by Furajny Hrany Ltd Lovech (Table 1).
Feeding of the *Polyodon spathula* that was stocked in floating cages was done with floating feed Catco Pre Grower feed 15 EF, 2 mm grain size, produced by Coppens International GmbH (Table 3).

e) *The host pond stocking formula (1.0 ha module), in which the floating cages platform is placed*:
- paddlefish P_2: 270 ex/ha;

- carp C_2: 450 ex/ha;
- grass carp Gc_2: 45 ex/ha;
- silver carp Sc: 45 ex/ha;
- pikeperch Pp_2: 62 ex/ha;
- tench Te_4: 28 ex/ha.

Table 3. The structure on nutritional components of Catco Pre Grower 15 EF feed

Components	Values
Protein	50%
Lipid	15%
Fiber	0.9%
Ash	9%
Phosphorus	1.3%
Calcium	1.9%
Sodium	0.4%
A vitamin (E672)	10000 IU.kg^{-1}
D_3 vitamin (E672)	1382 IU.kg^{-1}
E vitamin	200 mg.kg^{-1}
C vitamin	150 mg.kg^{-1}

f) *Average weight of fish for stocking*:
- paddlefish P_2: 1400-1600 g/ex;
- carp C_1: 500-700 g/ex;
- grass carp Gc_2: 400-600 g/ex;
- silver carp Sc_2: 700-900 g/ex;
- pikeperch Pp_2: 400-600 g/ex;
- tench Te_4: 350-450g / ex.

g) *Growth cycle duration*: 150 days from the stocking time (end of April - beginning of May).

h) *Manure administration in the host basin*: 1000-2000 kg/ha (the quantity being determined by the results of water analyzes).

In both technological models, the ponds where the floating cages are located, will be exploited by stocking them with high economic value fish species like: carp, pikeperch, paddlefish, tench.
When choosing these fish species, it was first took into account the bioproductive characteristics that make them fit into the category of the valuable species, and secondly, their specificity to capitalize on various links in the food chain in the respective growth basins biocenoses.
The carp is a bentophagous species, consuming fodder, the sturgeon is mainly zooplanktonophagous, tench is detritophagous, and the pikeperch is a predator that will feed on wild fish species without economic value which, which compete for food with farmed fish. Uneaten fodder left by carp, as well as the resulting manure will stimulate the development of zooplankton, the trophic base for paddlefish (Costache et al., 2000; Costache et al., 2004).
In order to maintain the ecological balance and the sustainable exploitation of the ponds are introduced into the stocking formula the Asian species of cyprinids: silver carp and grass carp in a percentage of about 10%.
During the growing season, for the carp, grass carp and tench in the host pond, a combined feed with PB 22.22% was administered, consisting in the following ingredients: wheat, barley, corn, soybean and sunflower grists and fodder yeast (Oprea & Georgescu, 2000).
The feed was manually distributed in 3 rations/day, the whole growing period. The daily ration was a 0.8-5% of the total fish biomass. Every month, a control fishing was carried out to assess health condition and establish feed rations (Bogatu & Munteanu, 2008).
The environmental conditions were determined by physico-chemical and hydrobiological analyzes performed by taking monthly water samples from the growth basins.
The determination of the main hydrochemical parameters was done by known classical methods.
Temperature, dissolved oxygen and *pH* were determined using the WTW Multi 3320 multiparameter kit.
The organic substance - expressed by the chemical consumption of oxygen in potassium permanganate, was determined by the volumetric method (STAS 9887/74), and the principle of the method is to oxidize organic substances from water using potassium permanganate. The result of the analyzes can be expressed in two ways: mg $KMnO_4.l^{-1}$ and $mgO_2.l^{-1}$. Nitrite (NO_{2-}), nitrates (NO_3^-), and were determined by spectrophotometry (STAS 8900/1-71). The ammonium ion (NH_4^+) was determined from ammoniacal nitrogen; is determined spectrophotometrically (STAS 8683/83). The phosphorus ion ($P_2O_4^{3+}$) was also determined spectrophotometrically (STAS 10064/75). The determination of carbonates and bicarbonates was done volumetrically.

RESULTS AND DISCUSSIONS

Results obtained at F.C.R.D.S. Nucet, in the experiment of growing fish in a combined system, floating cages-pond and net pens-pond, after 150 days, are presented below.

1. Indicators of economic feasibility of the growth technology for the complex exploitation of the water basins from the traditional fish farms (net pens battery - pond system):

a) *Technological indicators at the end of the growth cycle obtained in the battery of net pens:*

- losses: 10-15%;
- total production: (C_{1-1+} + Sg_{3-3+}): 510 - 550 kg/net pen, of which:
  - carp (C_{1-1+}): 230-250 kg/net pen;
  - catfish (Sg_{3-3+}): 280-300 kg/net pen.
- individual mass at the end of the growth cycle (g/ex):
  - carp (C_{1+}): 1200-1600 g/ex;
  - catfish (Sg_{3+}): 1500-1700 g/ex.
- individual net growth (g/ex):
  - carp (C_{1+}): 1100-1400 g/ex;
  - catfish (Sg_{3+}): 800-1000 g/ex.
- Feed Conversion Ratio (FCR):
  - carp (C_{1+}): granulated feed with PB 32%, FCR = 1.6 kg of feed/kg fish weight gain;
  - catfish (Sg_{3+}): granulated feed with PB 45%.
- FCR = 1.9 kg of feed/kg fish wheight gain.

b) *Technological indicators at the end of the growth cycle, for the pond (1.0 ha module), in which the net pen battery is placed:*

- losses 5-20%;
- total production: 1500-1700 kg/ha, of which, by species:
  - paddlefish P_{2-2+}: 650-750 kg/ha;
  - carp C_{2-2+}: 650 - 700 kg/ha;
  - grass carp Gc_{2-2+}: 40-60 kg/ha;
  - silver carp Sc_{2-2+}: 80-120 kg/ha;
  - pikeperch Pp_{2-2+}: 60-80 kg/ha.
- average weight at the end of the growth cycle (g/ex*)*:
  - paddlefish P_{2+}: 2700-3000 g/ex;
  - carp C_{2+}: 1600-1900 g/ex;
  - grass carp Gc_{2+}: 1400-1500 g/ex;
  - silver carp Sc_{2+}: 2500-2700 g/ex;
  - pikeperch Pp_{2+}: 800-1000 g/ex.
- individual net growth (g/ex):
  - paddlefish P_{2+}: 1200-1500 g/ex;
  - carp C_{2+}: 1000-1300 g/ex;
  - grass carp Gc_{2+}: 900-1000 g/ex;
  - silver carp Sc_{2+}: 1700-1900 g/ex
  - pikeperch Pp_{2+}: 300-500 g/ex.
- Feed Conversion Ratio (FCR): 2.5 kg feed/kg fish wheight gain (only for consuming species (carp and grass carp), combined feed with PB 22.22%.

2. Indicators of economic feasibility of the growth technology for the complex exploitation of the water basins from the traditional fish farms (floating cages - pond system):

a) *Technological indicators obtained at the end of the growth cycle obtained in floating cages*:

- losses 10-20%;
- total production by species:
  - carp C_{1-1+}: 500-600 kg/cage;
  - paddlefish P_{0-0+}: 280-320 kg/cage;
  - tilapia T_{1-1+}: 280-300 kg/cage.
- average weight at the end of the growth cycle (g/ex):
  - carp C_{1+}: 1200-1400 g/ex;
  - paddlefish P_{0+}: 350-400 g/ex;
  - tilapia T_{1+}: 500-600 g/ex.
- individual net growth (g/ex):
  - carp C_{1+}: 1000 - 1200 g/ex;
  - paddlefish P_{0+}: 300-350 g/ex;
  - tilapia T_{1+}: 400-500 g/ex.
- Feed Conversion Ratio (FCR):
  - carp C_{1+}: 1.5 kg feed/kg fish weight gain, granulated feed with PB 32%;
  - paddlefish P_{0+}: 1.8 kg feed/kg fish wheight gain, granulated feed with PB 50%;
  - tilapia T_{1+}: 1.5 kg feed/kg fish wheight gain, granulated feed with PB 32%.

b) *Technological indicators obtained at the end of the growth cycle, for the pond (1.0 ha module), in which the floating cages platform is placed*:

- losses 9-20%;
- total production: 1600-1800 kg/ha, of which by species:
  - paddlefish P_{2-2+}: 680-750 kg/ha;
  - carp C_{2-2+}: 800-850 kg/ha;
  - grass carp Gc_{2-2+}: 40-60 kg/ha;
  - silver carp Sc_{2-2+}: 80-120 kg/ha;
  - pikeperch Pp_{2-2+}: 40-50 kg/ha;
  - tench Te_{4-4+}: 10-20 kg/ha.
- individual mass at the end of the growth cycle (g/ex):
  - paddlefish P_{2+}: 2800-3000 g/ex;
  - carp C_{2+}: 2000 - 2200 g/ex;
  - grass carp Gc_{2+}: 1300-1500 g/ex;

- silver carp Sc_{2+}: 2500-2800 g/ex;
- pikeperch Pp_{2+}: 800-1000 g/ex;
- tench Tc_{4+}: 600-700 g/ex.

- individual net growth (g/ex):
  - paddlefish (P_{2+}: 1300-1500 g/ex;
  - carp C_{2+}: 1400 - 1600 g/ex;
  - grass carp Gc_{2+}: 800-1000 g/ex
  - silver carp Sc_{2+}: 1700-2000 g/ex;
  - pikeperch Pp_{2+}: 300-500 g/ex;
  - tench Te_{4+}: 200 - 300 g/ex .
- Feed Conversion Ratio (FCR): 2.5 kg feed/kg fish wheight gain (only for consuming species (carp, grass carp and tench), combined feed with PB 22.22%.

The fish from the combined growth systems benefited from the following environmental conditions regarding the water temperature. The main physical parameter with major importance in fish farming, the water temperature, was between14 and 16°C during the fish stocking in two systems.

The evolution of the average monthly water temperature, during the growth cycle of the fish is shown in the figure below (Figure 3).

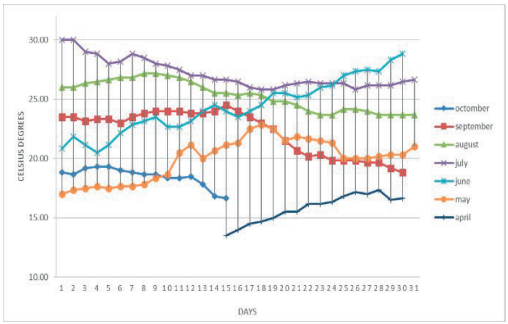

Figure 3. The evolution of the average monthly water temperature, during the growth season

Table 4. The main chemical parameters of the water, during the growing season

Parameter	U.M.	Recorded values	
		net pens battery - pond system	floating cages - pond system
pH	upH	6.8-7.4	6.9-7.5
Alkalinity	ml HCl. l^{-1}	2.6-3.5	2.8-3.8
Total hardness	(°D)	5.5-7.2	5.6-6.8
Dissolved Oxigen	mg $O_2.l^{-1}$	5.1-12.1	5.2-11.5
CCO-Mn	mg $KMnO_4.l^{-1}$	12.8-25.7	14.8-28.5
	mg $O_2.l^{-1}$	3.23-6.50	3.74-7.21
Nitrites (NO-2)	mg.l^{-1}	0.018-0.208	0.012-0.158
Nitrites (NO-3)	mg.l^{-1}	0.221-0.413	0.253-0.385
P from PO_4	mg.l^{-1}	0.028-0.210	0.025 - 0.285
Ammonium NH^+_4	mg.l^{-1}	0.141-0.250	0.163-0.350

The analysis of the water chemistry results shows that there are no notable differences between the values of the investigated hydrochemical parameters. The Table 4 shows the limits recorded (minimum and maximum) in the two combined growth systems.

Among the most important hydrochemical parameters, dissolved oxygen is a limited factor in the growth and survival of fish. The Figure 4 shows the level of dissolved oxygen in the water of the two growth systems, expressed in mg.l^{-1} O_2, during the entire growth cycle.

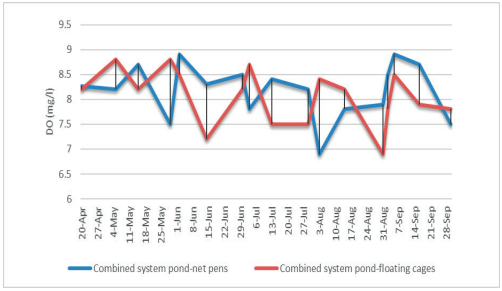

Figure 4. The level of dissolved oxygen (mg $O_2.l^{-1}$) recorded in the two growth systems

The values of the investigated hydrochemical parameters are within the normal range. The hydrochemical parameters were maintained within normal limits only by ensuring the optimal flow of water refreshness in the two combined fish farming systems (5-10 liters/sec/ha).

CONCLUSIONS

In the pond and net pens battery, the growth technology in combined system, makes possible a total production achievement of 2400-2700 kg/ha, of which 60-65% is realized in the pond, and 35-40% in net pens battery (10 cages/battery), without additional aeration, only with a permanent water intake (5-10 liters/sec/ha).

In the case of the combined net pens battery - pond system, the bioeconomy studies demonstrate the profitability of the developed system and the elaborate technology, by obtaining a 1.26 profit rate.

In the pond and floating cages platform, the growth technology in combined system makes possible a total production achievement of 2600-2800 kg/ha, from which 60-65% is

achieved in the pond, and 35 - 40% is obtained in floating cages, without additional aeration, only with a permanent intake of water (5-10 liters/sec/ha).

In the case of the combined floating cages - pond system, the bioeconomy studies demonstrate the profitability of the developed system and the elaborate technology, by obtaining a 1.56 profit rate.

The growth technology in intensive system like net pens and floating cages, located in ponds, is distinguished by the feasibility of applying in practice the developed technology with favorable arguments for the aquaculture sector being materialized by:

- investment recovery in a very short time;
- installation simplicity and low costs for building the investment;
- the possibility to achieve a production planning in time, depending on the needs of the market and consumption;
- high production per unit area;
- the quality of the obtained production by the fish species that are the object of the growth and the individual weight of the fish realized at the harvest;
- high biological material survival rate;
- the possibility of introducing mechanization and automation of the various stages of the technological process;
- reducing the growth period, simultaneously reducing the risk factor;
- superior capitalization of the administered fodder;
- allowing the introduction into the growth culture of some valuable fish species highly sought on the market, with sufficient materialized quantities by diversifying and increasing the production quality;
- offers the possibility to develop processing and marketing capacities, generating new jobs in rural areas;
- does not present technological risks, is non-aggressive, in a normal correlation with the environment and alines to the responsible aquaculture principles;
- determines the creation of an agri-food chain: production → processing → marketing and facilitates the association of economic agents in the sector;
- the investment costs for the design and realization of experimental growth models (floating cages/net pens) located in ponds represent only 10-15% of the capital invested in arranging one hectare of pond surface.

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ASPECTS REGARDING THE CONTROLLED REPRODUCTION OF PIKEPERCH (*SANDER LUCIOPERCA* LINNE, 1758) IN INDUSTRIAL AQUACULTURE SYSTEMS

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Abstract

Pike-perch is a very active predatory fish in its natural environment, but extremely sensitive in aquaculture units. Usually, the frequent manipulations may cause many losses in the fish farms, especially during artificial or controlled reproduction. This paper presents the obtained results at S.C.D.P. Nucet, in 2018-2020 period, regarding the pikeperch controlled reproduction in ponds units. The fish were divided into three experimental variants, in triplicate: V_1-controlled reproduction, without hormonal induction, V_2-controlled reproduction with hormonal induction (carp pituitary), V_3 -controlled reproduction, with hormonal induction (synthetic analogue hormone-Nerestin 5A). Each variant used 5 females and 5 males with an average body weight of 2 kg/fish. The best technological indicators were registered in V_3 variant, which was based on hormonal induction with Nerestin 5A hormone (88-92% eggs fertilization rate, 71-78% eggs hatching rate, about 2.7 million 7-8 days larvae). The smallest brood stock losses were recorded in V_1 variant (without hormonal induction).

Key words: *controlled reproduction, pikeperch, pituitary, ponds, synthetic hormone.*

INTRODUCTION

The pikeperch (*Sander lucioperca* L., 1758) is present in most of continental waters on the Romanian territory, being a valuable species from an alimentary and commercial point of view. This species is a promising candidate for fish diversification (Fontaine et al., 2009; Pourhosein Sarameh et al., 2012; Dalsgaard et al., 2013).

Pikeperch artificial reproduction is difficult to achieve due to the spawners sensitivity to manipulation (Kucharczyk et al., 2007), as a consequence, the mortality registration rates could raise up to 50%. Along the time, few studies have been focused on the pikeperch conttroled reproduction. Physiological processes in fish knowledges has facilitated the use of hormonal substances that stimulate maturation and reproduction (Rinchard et al., 2005).

MATERIALS AND METHODS

The researches were realised in the 2018-2020 period at the Fish Culture Research and Development Station Nucet. The experimental basins are located in the major riverbed of the Ilfov brook, downstream of the Ilfoveni accumulation dam. For the pikeperch conttroled reproduction, are needed: wintering ponds, prematuration ponds, maturation ponds, spawning ponds and the hatchery station.

Breeding mattresses, made of bundled willow roots ('mustaches') (*Salix babylonica* L.), caught on a nytal sieve and mounted on a wooden support with 1.0 x 1.0 m sides, were made for laying the eggs (Figure 1).

Figure 1. Pikeperch breeding mattress

The mattresses thus made are fixed directly on the bottom of the pond by fastening wooden poles or by fixing with weights, at 2-3 m from the shore with a 8-10 m distance between them.
Starting from the pikeperch breeding biology particularities in general (stages of ontogenetic development), the technology of reproduction includes:

- ✓ setting up groups of spawners;
- ✓ spawners selection and prematurization;
- ✓ conttroled reproduction ponds preparation and breeding mattress installation;
- ✓ spawners stimulation with hormonal substances;
- ✓ ponds stocking;
- ✓ monitoring the spawning process and the breeding mattresses control;
- ✓ collecting nests with embryonated eggs and introducing them into incubators;
- ✓ incubation, application of antifungal treatments and hatching process;
- ✓ larval growth until the age of 7-8 days old.

The spawners wintering took place in two ponds. In March, when the water temperature constantly reached 7-8°C values, the pikeperch spawners were transferred to prematurity ponds, separated by gender (Figure 2).

Figure 2. Pikeperch spawner taken out from wintering pond

For the experimental works of conttroled pikeperch reproduction, were selected 3-4 years old females, with an average weight of about 2 kg/ex, whose oocytes are in the 4th maturation stage. The selected males were the same age as the females, with an average weight of about 1.9 kg/ex. When the spawners where transfered from the wintering ponds to the prematurity ones, the oocyte polarization index was also determined, using oocytes extracted by probing (Zarski et al., 2011) (Figure 3).

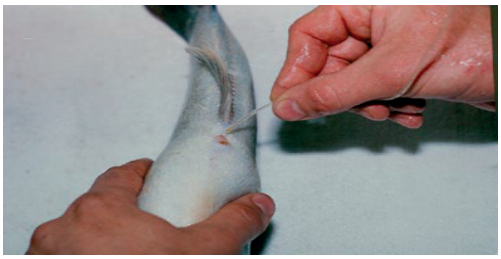

Figure 3. The determination of the maturation stage of oocytes

According to the scientific protocol, 3 experimental groups were formed, being selected 90 spawners (45 females and 45 males). The experiments were performed in nine breeding ponds with an average area of 1000 m^2/ponds. Before spawning, the three groups of females (15 ex/batch) were stocked separately in maturation basins. The males were all stocked in a maturation pond. The distribution in these ponds was made in the evening time, between 19^{00}-21^{00}, at a 11-16°C water temperature. The females from the 2 and 3 variants were injected the next day with the first dose of pituitary / synthetic hormones, and the second dose was administered 12-14 hours away. Simultaneously with the second dose, the spawners were introduced into the spawning ponds (5 ♀ + 5 ♂ / pond).
The experimental variants were the following:

- ▪ Variant 1 (V1) - without females hormonal stimulation; performed in triplicate (R1, R2, R3), in basins B1, B2 and B3;
- ▪ Variant 2 (V2) - with females hormonal stimulation with carp pituitary hormone, performed in triplicate (R1, R2, R3), in basins B4, B5 and B6;
- ▪ Variant 3 (V3) - with females hormonal stimulation with Nerestin 5A, performed in triplicate (R1, R2, R3), in the basins B7, B8 and B9.

For females stimulated with carp pituitary hormone, the total dose administered was 3.5 mg/ kg body weight, and for females stimulated with Nerestin 5A, the total dose administered was 0.15 ml/kg body weight. In this case males were not hormonally stimulated.
In each experimental variant, the gonado-somatic ratio (GSR) for females was calculated; its average being 10% of body weight.

RESULTS AND DISCUSSIONS

During the experiments, physico-chemical parameters of the water were periodic monitored. The obtained results interpretation was performed in accordance with the " Classification norm of surface water quality" provisions, correlated with the specialty literature data for aquaculture waters (OMMGA no. 161/2006) (Table 1).

Pikeperch families formed in 12-24 hours after their introduction into the reproduction ponds. According to data published by Zak & Demska-Zak (2005), the waiting time for laying eggs for a female can vary from 10 to 70 h after an HCG injection.

In 2018, reproduction began in basins B7, B8 and B9 (V3), followed by basins B1, B2 and B3 (V1) and basins B4, B5 and B6 (V2). The spawning season took place between 7-11 April.

In 2019, reproduction started in basins B7, B8 and B9 (V3), followed by basins B4, B5 and B6 (V2) and basins B1, B2 and B3 (V1). The spawning season took place between 11-15 April.

In 2020, spawning began in basins B7, B8 and B9 (V3), followed by basins B1, B2 and B3 (V1) and basins B4, B5 and B6 (V2). The spawning season took place between 13-17 April.

After laying the eggs on the breeding mattresses, the nests with fertilized eggs were collected and transported to the hatchery station, in water containers (Figure 4).

Figure 4. Pikeperch nest with embryonated eggs

The nests were introduced in "Nucet" type incubators, where a permanent water supply was ensured, with 8 liters/minute flow rate (Figure 5).

Figure 5. Pikeperch nest with embryonated eggs in "Nucet" type incubator

The data regarding the average values of these measurements, the average prolificacy and the type of hormone administered are presented in Table 2.

During the incubation, the eggs were bathed with a 37% formaldehyde solution (concentration 1.0-1.8 ml 37% formaldehyde/ 1 liter of water) to prevent the appearance and infestation with fungi. The first treatment was given 24 hours after the eggs were introduced to incubate. The exposure time was depending on the water temperature (10 min/10-12°C or 15 min/13-15°C). The process was repeated every 24 hours, until the embryo surrounds the entire yolk sac, the caudal reaches the eyes, the pigmentation is accentuated, the movements of the embryo become more intense and the heartbeat is observed.

In Table 3 are presented the results obtained for the conttroled reproduction of the pikeperch in the three experimental variants for each year of research.

During the incubation period, the water temperature was daily recorded, the average number of eggs laid in the nests and introduced to the incubator was evaluated, as well as the fertilization percentage.The incubation duration was 7-8 days at an average daily water temperature of 13.5°C. After hatching, the fasciculated willow roots ("mustaches") were removed from the "Nucet" type incubators.

Fish larvae were kept in incubators up to the age of 7-8 days, until the end of the vitelus reserves resorption period. The main indicators recorded in the experiments are presented in Tables 4-6.

Table 1. Water physical and chemical indicators for the 2018-2020 period (average values)

C.No.	Chemical parameter		M.U.	Parameter		
				Source	Experimental ponds	Optimal according to quality standards
				Average of the years 2018 - 2019 - 2020		
1	pH		pH unit	7.2	7.5	7-7.8
2	Alkalinity		mg /l	146	163	200-400
3	Calcium (Ca^{2+})		mg/l	38.2	46.8	90-120
4	Magnesiu (Mg^{2+})		mg/l	22.4	20.8	10-40
5	Ca^{2+}/ Mg^{2+}		mg/l	1.7	2.25	5
6	Organic Matter		mg $KMnO_4$/l	16	22.95	20-60
7	Oxygen		mg/l	10.4	8.8	5-12
8	Ammonia (NH^{+}_3)		mg/l	missing	missing	missing
9	Nitrates (NO^{-}_3)		mg/l	missing	0.21	2.5-4
10	Nitrogen (NO^{-}_2)		mg/l	0.002	0.004	0.03
11	Phosphates (PO^{3-}_4)		mg/l	missing	0.06	0.05-1.5
12	Clorides	Cl^{-}	mg/l	8.83	8.43	30
		Na Cl	mg/l	14.61	14.03	20
13	Ammonium (NH^{+}_4)		mg/l	missing	0.014	0.5-1
14	Total hardness		(^{0}D)	12.6	14.4	12

Table 2. Weight and number of the eggs according to the variant

Year	Variant 1(without hormonal stimulation)											
	V1R1				V1R2				V1R3			
	W (g/ex.)	Estimated amount of eggs Wi (g)	Eggs/ gram	Prolificity (milions)	W (g/ex.)	Estimated amount of eggs Wi (g)	Eggs/ gram	Prolificity (milions)	W (g/ex.)	Estimated amount of eggs Wi (g)	Eggs/ gram	Prolificiy (milions)
2018	2150	215	1260	1.3545	1890	189	1260	1.1907	2305	230.5	1260	1.452
2019	1840	184	1260	1.1592	2260	226	1260	1.4238	1990	199	1260	1.254
2020	1960	196	1260	1.2348	2140	214	1260	1.3482	2410	241	1260	1.518
	Variant 2 (stimulation with carp pituitary hormone)											
	V2R1				V2R2				V2R3			
2018	1990	199	1260	1.2537	2380	238	1260	1.4994	2220	222	1260	1.399
2019	2310	231	1260	1.4553	2120	212	1260	1.3356	2310	231	1260	1.455
2020	2080	208	1260	1.3104	2320	232	1260	1.4616	2150	215	1260	1.355
	Variant 3 (stimulation with synthetic hormone)											
	V3R1				V3R2				V3R3			
2018	2070	207	1260	1.3041	2410	241	1260	1.5183	2160	216	1260	1.361
2019	1890	189	1260	1.1907	2260	226	1260	1.4238	2310	231	1260	1.455
2020	2350	235	1260	1.4805	2120	212	1260	1.3356	2060	206	1260	1.298

Table 3. The obtained results during the spawning in the pikeperch conttroled reproduction

C.no.	Basin	Installed breeding mattresses	Families number	Variant	Number of collected nets	Maturated females	Maturation percent (%)
Year 2018							
1	B 1	5	5	V1 R1	3	3	60
2	B 2	5	5	V1 R2	4	4	40
3	B 3	5	5	V1 R3	3	3	60
4	B 4	5	5	V2 R1	4	4	80
5	B 5	5	5	V2 R2	4	4	60
6	B 6	5	5	V2 R3	4	4	60
7	B 7	5	5	V3 R1	4	4	80
8	B 8	5	5	V3 R2	5	5	100
9	B 9	5	5	V3 R3	5	5	100
Total V1		**15**	**15**	**V1**	**10**	**10**	**66,7**
Total V2		**15**	**15**	**V2**	**12**	**12**	**80**
Total V3		**15**	**15**	**V3**	**14**	**14**	**93.3**
Year 2019							
10	B 1	5	5	V1 R1	4	4	80
11	B 2	5	5	V1 R2	3	3	60
12	B 3	5	5	V1 R3	3	3	60
13	B 4	5	5	V2 R1	4	4	80
14	B 5	5	5	V2 R2	4	4	80
15	B 6	5	5	V2 R3	4	4	80
16	B 7	5	5	V3 R1	5	5	100
17	B 8	5	5	V3 R2	5	5	100
18	B 9	5	5	V3 R3	5	5	100
Total V1		**15**	**15**	**V1**	**10**	**10**	**66.7**
Total V2		**15**	**15**	**V2**	**12**	**12**	**80**
Total V3		**15**	**15**	**V3**	**15**	**15**	**100**
Year 2020							
19	B 1	5	5	V1 R1	4	4	80
20	B 2	5	5	V1 R2	4	4	80
21	B 3	5	5	V1 R3	3	3	60
22	B 4	5	5	V2 R1	4	4	80
23	B 5	5	5	V2 R2	3	3	60
24	B 6	5	5	V2 R3	4	4	80
25	B 7	5	5	V3 R1	5	5	100
26	B 8	5	5	V3 R2	5	5	100
27	B 9	5	5	V3 R3	4	4	80
Total V1		15	15	**V1**	**11**	**11**	**73.3**
Total V2		15	15	**V2**	**11**	**11**	**73.3**
Total V3		15	15	**V3**	**14**	**14**	**93.3**
Total							
Total V1		**45**	**45**	***V1***	**31**	**31**	**68.9**
Total V2		**45**	**45**	***V2***	**35**	**35**	**77.8**
Total V3		**45**	**45**	***V3***	**43**	**43**	**95.6**

Table 4. The main technological indicators in 2018

C.No.	Biotechnological Indicators	M.U.	V1	V2	V3
1	Pond	-	B 1-3	B 4-6	B 7-9
2	Surface	ha	0.3	0.3	0.3
3	Number of mattresses installed	-	15	15	15
4	Families number	-	15	15	15
5	Gender relation	♀/♂	1/1	1/1	1/1
6	Females characteristics	no.ex/g/ex	15/2115	15/2197	15/2213
7	Males characteristics	no.ex/g/ex	15/1880	15/2050	15/2017
8	Female hormonal stimulation	-	-	Pituitary hormone	Nerestin 5A
9	Dosage	mg/kg body weight	-	3.5	0.15
10	Reproduction period		07-11.04.2018	07-10.04.2018	07-11.04.2018
11	Reproductive water temperature	°C	11-15	11-15	11-15
12	Collected nets	no	10	12	14
13	Maturated females	ex/g/ex	10/20015	12/2197	14/2213
14	Maturation percentage	%	66.7	80.0	93.3
15	Average prolificacy	eggs/♀	266490	276780	278880
16	Eggs for incubation	mil	2.6649	3.3214	3.9043
17	Fertilization rate	%	88.1	89.4	92
18	Number of fertilized eggs	mil	2.348	2.969	3.592
19	Hatch percentage	%	74.3	74.8	77.8
20	Hatched larvae	mil	1.7444	2.2210	2.7946
21	Incubation survival	%	96.6	95.0	98.1
22	Larvae of 7 to 8 days viable	mil	1.6851	2.1100	2.7415
23	Larvae 7-8 days / ♀ matured	mil	0.1685	0.1758	0.1958
24	Larvae 7-8 days / kg ♀ matured	mil	0.0797	0.0800	0.0885
25	Fertilized eggs survival of larvae 7-8 days percentage	%	63.2	63.5	70.2

Table 5. The main technological indicators in 2019

C.No	Biotehnological Indicators	M.U.	V1	V2	V3
1	Pond	-	B 1-3	B 4-6	B 7-9
2	Surface	ha	0.3	0.3	0.3
3	Number of mattresses installed	-	15	15	15
4	Families number	-	15	15	15
5	Gender relation	♀/♂	1./1	1./1	1./1
6	Females characteristics	no.ex/g/ex	15/2030	15/2247	15/2153
7	Males characteristics	no.ex/g/ex	15/1970	15/2040	15/2007
8	Female hormonal stimulation	-	-	Pituitary hormone	Nerestin 5A
9	Dosage	mg/kg body weight	-	3.5	0.15
10	Reproduction period		11.-15.04.2019	11.-14.04.2019	11.-15.04.2019
11	Reproductive water temperature	°C	11.-15	11.-15	11.-15
12	Collected nets	no	11	12	15
13	Maturated females	ex/g/ex	11./2030	12/2247	15/2153
14	Maturation percentage	%	73.3	80	100

C.No	Biotehnological Indicators	M.U.	V1	V2	V3
15	Average prolificacy	eggs/♀	255780	283120	271280
16	Eggs for incubation	mil	2.8136	3.3974	4.0692
17	Fertilization rate	%	90.6	92.1	91.8
18	Number of fertilized eggs	mil	2.549	3.129	3.736
19	Hatch percentage	%	75.2	73.6	76.9
20	Hatched larvae	mil	1.9169	2.303	2.8726
21	Incubation survival	%	94.1	95.3	95.8
22	Larvae of 7 to 8 days viable	mil	1.8038	2.1947	2.752
23	Larvae 7 - 8 days / ♀ matured	mil	0.164	0.1829	0.1835
24	Larvae 7 - 8 days / kg ♀ matured	mil	0.081	0.0814	0.0852
25	Fertilized eggs survival of larvae 7 - 8 days percentage	%	64.1	64.6	67.6

Tabel 6. The main technological indicators in 2020

C.No.	Biotechnological Indicators	M.U.	V1	V2	V3
1	Pond	-	B 1-3	B 4-6	B 7-9
2	Surface	ha	0.3	0.3	0.3
3	Number of mattresses installed	-	15	15	15
4	Families number	-	15	15	15
5	Gender relation	♀/♂	1/1	1/1	1/1
6	Females characteristics	nr.ex/g/ex	15/2170	15/2183	15/2177
7	Males characteristics	nr.ex/g/ex	15/2003	15/1913	15/1917
8	Female hormonal stimulation	-	-	Pituitary hormone	Nerestin 5A
9	Dosage	mg/kg body weight	-	3.5	0.15
10	Reproduction period		13-17.04.2020	13-17.04.2020	13-16.04.2020
11	Reproductive water temperature	° C	11-16	11-16	11-16
12	Collected nets	no	11	11	14
13	Maturated females	ex/g/ex	11/2170	11/2183	14/2177
14	Maturation percentage	%	73.3	73.3	93.3
15	Average prolificacy	thousands eggs /♀	273420	275060	274300
16	Eggs for incubation	mil	3.0076	3.0257	3.8402
17	Fertilization rate	%	91.3	90.2	90.1
18	Number of fertilized eggs	mil	2.746	2.729	3.460
19	Hatch percentage	%	71.1	73.2	74.4
20	Hatched larvae	mil	1.9524	1.9977	2.5743
21	Incubation survival	%	95.2	94.4	95.3
22	Larvae of 7 to 8 days viable	mil	1.8587	1.8859	2.4533
23	Larvae 7 - 8 days / ♀ matured	mil	0.1690	0.1714	0.1752
24	Larvae 7 - 8 days / kg ♀ matured	mil	0.0779	0.0785	0.0805
25	Fertilized eggs survival of larvae 7 - 8 days percentage	%	61.8	62.3	63.9

Discusions

1. Spawners maturation percentage

- the best maturation percentage was obtained in 2019 in V3 (100%), and the lowest was obtained in 2018 in V1 (66.7%);
- in 2018 the highest maturation percentage was obtained in V3 (93.3%) and the lowest in V1 (66.7%), respectively 80% in V2;
- in 2019 the highest maturation percentage was obtained in V3 (100%) and the lowest in V1 (66.7%), respectively 80% in V2;
- in 2020 the highest maturation percentage was obtained in V3 (93.3%) and the lowest in V1, respectively V2 (73.3%).

Figures 6 and 7 show the variation of spawners maturity percentage on experimental variants and by years.

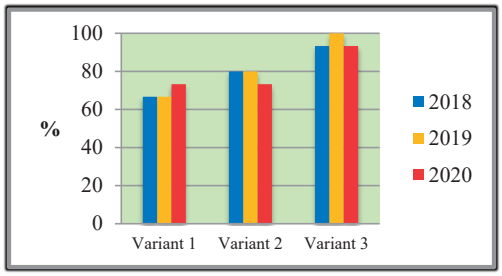

Figure 6. Variation of maturation percentage by experimental variants

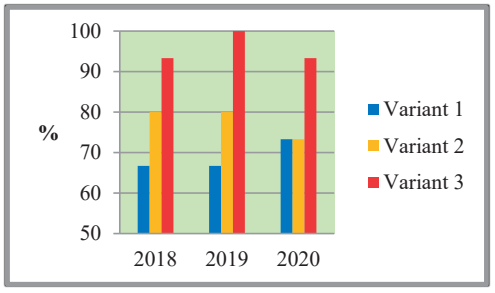

Figure 7. Variation of the maturation percentage per year

2. The total number of obtained eggs

- the highest eggsnumber was obtained in 2019 in V3 (4.0692 million), and the lowest number was obtained in 2018 in V1 (2.6649 million);
- in 2018, the highest eggs number was obtained in V3 (3.9043 million), and the lowest number was obtained in V1 (2.6464 million), respectively 3.3214 million in V2;
- in 2019, the highest eggs number of obtained in V3 (4.0692 million), and the lowest number was obtained in V1 (2.8136 million), respectively 3.3975 million in V2;
- in 2020, the highest eggs number was obtained in V3 (3.8402 million), and the lowest number was obtained in V1 (3.0076 million), respectively 3.0256 million in V2.
- Figure 8 shows the eggs number variation obtained per year and experimental variants.

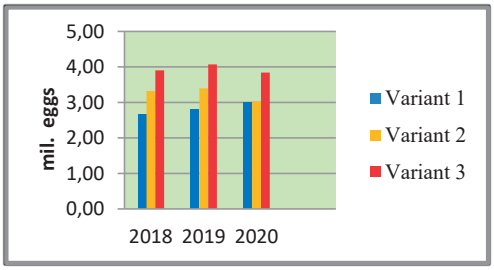

Figure 8. The eggs number variation obtained per year and experimental variants

3. Eggs fertilization percentage

- the best fertilization percentage was obtained in 2019 in V2 (92.1%), and the lowest percentage was obtained in 2018 in V1 (88.1%);
- in 2018 the best fertilization percentage was obtained in V3 (92.0%), and the lowest in V1 (88.1%), respectively 89.4% in V2;
- in 2019 the best fertilization percentage was obtained in V2 (92.1%), and the lowest in V1 (90.6%), respectively 91.8% in V3;
- in 2020 the best fertilization percentage was obtained in V1 (91.3%), and the lowest in V3 (90.1%), respectively 90.2% in V2.

Figure 9 shows the fertilization percentage variation by years and experimental variants.

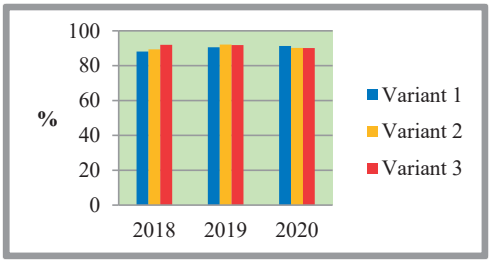

Figure 9. The fertilization percentage variation by years and experimental variants

4. Eggs hatching percentage

- the best hatching percentage was obtained in 2018 in V3 (77.8%), and the lowest percentage was obtained in 2020 in V1 (71.1%);
- in 2018, the best hatching percentage was obtained in V3 (77.8%), and the lowest in V1 (74.3%), respectively 74.8% in V2;
- in 2019, the best hatching percentage was obtained in V3 (76.9%), and the lowest in V2 (73.6%), respectively 75.2% in V1;
- in 2020, the best hatching percentage was obtained in V3 (74.4%), and the lowest in V1 (71.1%), respectively 73.2% in V2.

Figure 10 shows the hatching percentage variation by years and experimental variants.

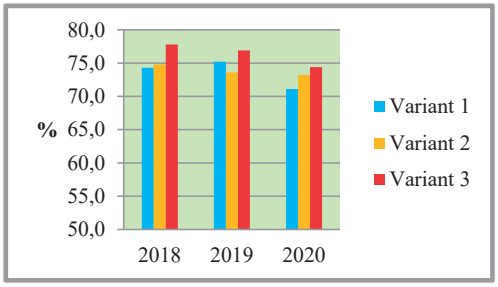

Figure 10. Hatching percentage variation by years and experimental variants

5. The total number of obtained 7 - 8 days larvae

- the highest number of 7-8 days old larvae of was obtained in 2019 in V3 (2.7519 mil.), and the lowest number of 7-8 days larvae was obtained in 2018 in V1 (1.6851 mil.);
- in 2018, the highest number of 7-8 days larvae was obtained in V3 (2.7415 mil.), and the smallest number of 7-8 days larvae was obtained in V1 (1.6851 mil.), respectively 2.1100 mil. in V2;
- in 2019, the highest number of 7-8 days larvae was obtained in V3 (2.7519 mil.), and the smallest number of 7-8 days larvae was obtained in V1 (1.8038 mil.), respectively 2.1948 mil. in V2;
- in 2020 the highest number of 7-8 days larvae was obtained in V3 (2.4533 mil.), and the smallest number of 7-8 days larvae was obtained in V1 (1.8587 mil.), respectively 1.8858 million in V2.

Figure 11 shows the 7-8 days larvae number variation per year and experimental variants.

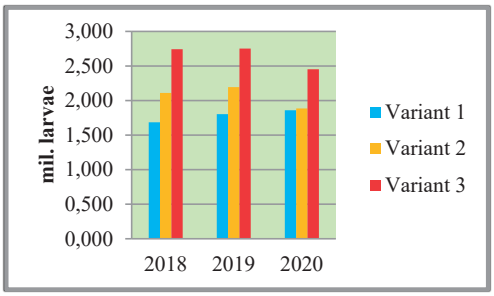

Figure 11. The number of days 7-8 larvae

6. Number of 7-8 days larvae/kg matured female

- the highest number of 7-8 days larvae/kg matured female was obtained in 2019 in V3 (0.0852 mil.), and the lowest number of 7-8 days larvae o/ kg of matured female was obtained in 2020 V1 (0.0779 million);
- in 2018, the highest number of 7-8 days larvae / kg of matured female was obtained in V3 (0.0885 million), and the lowest number was obtained in V1(0.0797 million), respectively 0.0800 million in V2;
- in 2019, the highest number of 7-8 days larvae / kg of matured female was obtained in V3 (0.0852 mil.), and the lowest number was obtained in V1 (0.0810 mil.), respectively 0.0814 million in V2;
- in 2020, the highest number of 7-8 days larvae / kg of matured female was obtained in V3 (0.0805 mil.), and the lowest number was obtained in V1 (0.0779 mil.), respectively 0.0785 million in V2.

Figure 12 shows the 7-8 days larvae/kg female matured number variation per year and experimental variants.

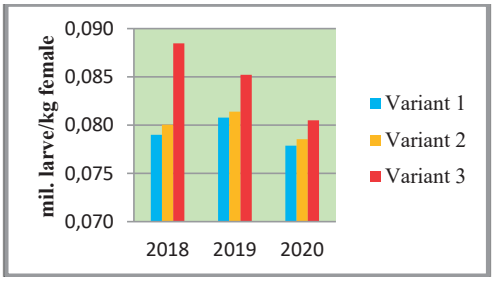

Figure 12. The number of 7-8 days larvae/kg female matur ed

7. Survival rate from fertilized egg stage to 7 - 8 day larval stage

- the best survival percentage was obtained in 2018 in V3 (70.2%), and the lowest percentage was obtained in 2020 in V1 (61.8%);
- in 2018, the best survival percentage was obtained in V3 (70.2%), and the lowest percentage was obtained in V1 (63.2%), respectively 63.5% in V2;
- in 2019, the best survival percentage was obtained in V3 (67.6%), and the lowest percentage was obtained in V1 (64.1%), respectively 64.6% in V2;
- in 2020, the best survival percentage was obtained in V3 (63.9%), and the lowest percentage was obtained in V1 (61.8%), respectively 62.3% in V2.

Figure 13 shows the survival rate variation from fertilized eggs to 7-8 days larvae per year and experimental variants.

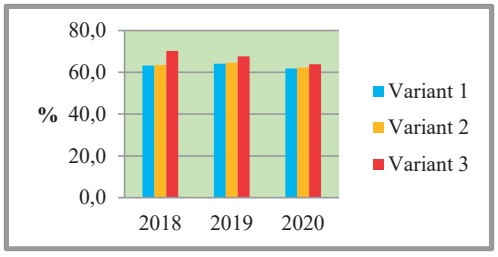

Figure13. The survival rate variation from fertilized eggs to 7-8 days larvae

CONCLUSIONS

The technology of conttroled pikeperch reproduction, practiced at Nucet Research Center, includes the following successive stages: in the spring beginning, when the water temperature reaches the value of 8-10°C, the spawners are fished from the winter ponds and are separated by sex. Next, they are introduced into the prematurity basins, where they are kept for about 3-4 weeks, depending on the water temperature. At a 10-12^{0}C water temperature, the advanced maturation stage females are hormonally stimulated (V1 and V2 variants). The unstimulated females from V1 variant and all the males are parked separately in maturation ponds. After the second dose of the hormone administered to the females, the spawners are introduced into natural-directed reproduction ponds, where the laying eggs mattresses have previously been introduced. The mattresses with fertilized eggs are collected and placed in "Nucet" type incubators in the breeding station. The advantage of this method is that the pikeperches are not manipulated when they are spawning their sexual products.

The best results were obtained in the synthetic hormone stimulation variant with (Nerestin 5A), in each of the three research years of (2018-2020). The females maturation percentage was 95.6%. The survival rate of larvae up to the age of 7-8 days was between 63.9% (2020) and 70.2% (2018). In this experiment, were also obtained good results in the variant without hormonal stimulation (V1), the survival rate of larvae up to the age of 7-8 days being between 61.8% (2020) and 64.1% (2018).

Due to the fact that the eggs were incubated in an optimal temperature range (11-15^{0}C), the hatching time was relatively short, resulting in homogeneous groups, which was later reflected in the results obtained in larval growth.

The choice of the hormone is made depending on the technological indicators obtained, but also on the price of the product. Nerestin 5A is a synthetic hormone, accessible on the market, easy to get unlike the carp pituitary hormone which is much more expensive and difficult to procure.

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DIVERSITY AND DISTRIBUTION OF PARASITES IN SOME FRESHWATER FISH FROM ROMANIAN SECTOR OF PRUT RIVER

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Abstract

Fish parasites represent a major part of aquatic biodiversity. The aim of present investigation was to assess the diversity and distribution of parasites from some fish species from the Prut River. Fish were sampled from three station on Prut River (Rădăuți, Drânceni, Oancea) during the year 2020. The different types of fishes from 5 families Cyprinidae, Esocidae, Percidae, Siluridae, Cobitidae have been examined for analysis of the distribution of parasites from these fish, in order to complete the existing data on metazoan parasites of freshwater fishes in the Romanian sector of Prut River. Parasitological investigations were performed on fresh samples by classic methods and the obtained results were expressed in grades of prevalence and intensity. In the analysed fish, were identified 12 parasitic species belonging to 6 systematic groups: Protozoa, Monogenea, Trematoda, Cestoda, Nematoda, Anelida. The prevalence of the parasitosis varied among examined fish species. The ciliated protozoa and monogenic worms were the most commonly identified parasites, but the intensity of parasitism was low. The effects of parasites on fish hosts in the natural environment may be difficult to isolate and quantify.

Key words*: ecto parasites, endoparasites, freshwater fish, Prut River.*

INTRODUCTION

The Prut River basin is shared by Ukraine, Romania, and Moldova. Its source is in the Ukrainian Carpathians. Later, the Prut forms the border between Romania and Moldova. The diversity of aquatic ecosystems and the richness of freshwater fish species are features of the Prut River. Like other living organisms, fish have parasites either external or internal which cause a host of pathological debilities. Freshwater fish species may be definitive and intermediate hosts of parasites with larvae and mature stages infecting a variety of vertebrates, including humans (Djikanovic et al., 2012). Fishes are susceptible to all the phyla of parasites including annelids and arthropods and can affect the fish biology which tends into diseases, mortality, disordered growth pattern, and ultimately makes the loss to fish values (Lafferty, 2008).

The authors of several studies have revealed large parasitic fauna in freshwater fishes (Cojocaru, 2010, Ejere et al., 2014) ranging from ectoparasites (Kostoingue et al., 2001; Oniye et al., 2004) to endoparasites (Kumar, et al., 2012; Cakic et al., 2008) which can affect fish health, growth and survival. Studies on parasitic communities of wild fish populations increase understanding of the parasite-host-environment interactions, because parasites may be indicators of environmental conditions and of their hosts (Hoshino et al., 2014). The parasites of fish can reflect the life habits of the fish, including their interactions with the benthic, planktonic and fish communities (Landsberg et al., 1998). Parasite species richness and abundance can differ geographically for the same host species and it can be influenced by the ecosystem characteristics and its trophic diversity (Vales et al., 2010).

Our team analyzed the fish community like *Silurus glanis, Stizostedion lucioperca, Cyprinus carpio, Abramis brama,* from the Prut River (Frumușița station, Cotul Chiului area) and the parasites identified do not affect the health status of their hosts (Docan et al., 2019).

This type of information is poorly studied in fish species from Prut River, in particular, and therefore, become the general targets of this study by focusing on the fish types, abundance, and prevalence of parasitic infestation. In this paper we present an analysis of the distribution of parasites from some fish species, in order to complete the existing data on metazoan parasites of freshwater fishes in the Romanian sector of Prut River.

MATERIALS AND METHODS

The fishes were collected randomly between the period of summer (April 1st to July 30th) 2020, from three stations on Prut River (Rădăuți, Drânceni, Oancea). The different types of fishes belong from 5 families: *Cyprinidae, Esocidae, Percidae, Siluridae, Cobitidae*. The scientific fishing activity from every area was carried out over a length of 2 km, with the fishing net wall. The fish were weighed (g) and their total length was measured (cm). Fish were transported in Research Centre MoRAS-UDJ Galati laboratory (http://moras.ugal.ro) and in ICDEAPA Galați laboratory (https://asas-icdeapa.ro/) where parasitological analyses were carried out.

The sampled fish were examined for both ectoparasites and endoparasites using standard parasitological procedures. The external surface of the fish was examined thoroughly using a hand lens for macroscopic ectoparasitic species, including crustaceans and hirudineans. Smear of scrapings from the skin, fins and gills were also examined for ectoparasites. Each fish was sectioned dorso-ventrally and the alimentary canal, liver, kidney, swim bladder and spleen were examined for endoparasites. Parasites were identified to family, genus or species level when possible.

The taxonomic classification and identification of the observed parasites were done on the basis of Munteanu, 2005, Bauer, 1984, 1985, 1987. For isolation, selection and identification of the parasite fauna of freshwater fish from Prut River, we used a Zeiss microscope. The extent of parasite infection was expressed in terms of an individual host as the intensity of infection (the number of individual parasites of a particular species harboured) and in terms of host populations as the prevalence (the proportion of hosts harbouring at least one individual parasite of a particular species) (Bush et al., 1997).

RESULTS AND DISCUSSIONS

The data on freshwater fish parasites are important for the evaluation of health conditions and the general influence of the level of parasitism on the community structure (Djikanovic et al., 2012).

The results of the parasitological examination are presented synthetically in tables 1, 2 and 3: the parasite and their habitat, the prevalence and mean intensity of infestation, in the three analyzed stations.

In the upper sector of the Prut River, Rădăuți station were captured 9 species grouped into 3 families: *Cyprinidae, Esocidae, Percidae*, respectively: *Cyprinus carpio* Linnaeus, 1758*, Carassius auratus gibelio* Bloch, 1782*, Hypophthalmichthys molitrix* Valenciennes, 1844*, Abramis brama danubii* Linnaeus, 1758*, Rutilus rutilus carpathorossicus* Linnaeus, 1758*, Scardinius erythrophtalmus* Linnaeus, 1758*, Sander lucioperca* Linnaeus, 1758*, Acerina cernua* Linnaeus, 1758*, Esox lucius* Linnaeus, 1758.

No infectious and fungal pathogens were identified. In this preliminary study the parasites will be declared by ectoparasite or endoparasite due to the general targets of this study to concentrate on the prevalence of parasitic infection.

Of the total fish species studied, 61% have weak polyparasitosis, of which: 47% ciliate products (*Trichodina domerguei, Apiosoma piscicola*), 43% monogenic worm products (*Dactylogirus vastator and Diplozoon paradoxus*), 5% cestode worm products (*Cysticercercus* sp.) and 5% annelid worm products (*Piscicola geometra*).

The results presented in Table 1 show that ectoparasites predominate in the analyzed fish. Ectoparasites found on body surface and gill, are *Trichodina domerguei, Apiosoma* sp., *Dactylogyrus vastator* and *Diplozoon paradoxus*. In our study, only one specimen of *Cysticercus sp.*was found in a liver, surrounded by a thin connective tissue capsule. This encapsulation of nematode larvae has been

observed in several cyprinids (Moravec, 1994). Cestode and anellida parasites occurred rarely in our samples. Adult worm of *Piscicola geometra* represented the only anellid species found in *Esox lucius.*

Table 1. Prevalence and intensity of metazoan parasites of fish from the Prut River, Rădăuți station

Systematic group	Species of parasite	Fish host	Parasite habitat	N/n	P%	MI
Protozoa/ Ciliata	*Trichodina domerguei*	*Carasus auratus gibelio*	gills	10/4	40	8.25
		Sander lucioperca	gills	6/1	16.66	13
	Apiosoma piscicola	*Acerina cernua*	gills	4/1	25	7
		Hypophthalmichthys molitrix	gills	6/2	33.33	5.5
Monogenea	*Dactylogyrus extensus*	*Carasus auratus gibelio*	gills	10/3	30	4,25
		Abramis brama	gills	8/3	37.5	4.66
		Cyprinus carpio	gills	10/4	40	5.75
	Diplozoon paradoxum	*Abramis brama*	gills	8/2	25	4.5
		Rhutilus rhutilus carpathorossicus	gills	5/1	20	6
		Schardinus erhythrophthalmus	gills	5/1	20	4
Cestoda	*Cysticercus* sp.	*Abramis brama*	liver	8/1	12.5	3
		Sander lucioperca	liver	6/1	16.66	2
Anellida	*Piscicola geometra*	*Esox lucius*	skin	5/1	20	3

N = total number of examined fish specimens
n = total number of infected fish specimens.
P% = prevalence.
MI = mean intensity.

In the middle sector of the Prut River (Rădăuți station) were caught 7 fish species belonging to the families *Cyprinidae* and *Percidae*: *Carassius auratus gibelio, Cyprinus carpio, Abramis brama danubii, Rutilus rutilus carpatorossicus, Scardinius erythrophtamus, Barbus barbus, Sander lucioperca*, which were parasitologically examined.

Of the examined species (Table 2), 57% had weak polyparasitosis, of which: 45% products of ciliated protozoa (*Trichodina* sp.), 35% products of monogenic worms (*Dactylogirus vastator and Diplozoon paradoxus*), 6% products of trematodes (*Neascus cuticola*), 6% products of molluscs (*Glochidia* sp.), 8% produced by cestodes (*Ligula intestinalis*). Prevalence of ectoparasites and endoparasites form freshwater fish species had different values. Regarding the number of parasites belonging to a species, identified in a certain host, there were differences between the studied fish species.

In the C*iprinids* species, the monogenic worm *Dactylogyrus extensus* was identified on the branchial scrapes, the parasitic intensity being reduced to 5-10 specimens/fish. Chubb (1977) had earlier identified temperature as the most important single factor controlling the seasonal prevalence of dactylogyrids. Monogenean trematodes, as flatworms, commonly invade the gills, skin, and fins of freshwater fish from most families of *Teleostei* (Whittington et al., 2000). Monogeneans worms have direct life cycles and they have specificity for the host. *Dactylogyrus extensus*, in the massive invasion, was found to be fatal to both young and adult fish (Munteanu et al., 2003).

Grossly examination of *Rhutilus rhutilus* revealed the presence of some blackspots in the skin on only one individual fish. The low level of *Posthodiplostomum cuticola* could be seen as a positive sign for a fishery because it shows that there is substantial habitat for aquatic gasteropods to survive, and they are an important part of the food chain in fisheries (Munteanu et al., 2003).

Plerocercoids of *Ligula intestinalis* were observed in the body cavity of *Cyprinus carpio* and *Rhutilus rhutilus,* but with low mean intensity. Ligulids have a complex life cycle involving copepods, fishes and birds. It is known to affect especially *Alburnus escherichii, Leuciscus cephalus, Tinca tinca, Cyprinus carpio and Rutilus rutilus,* which are members of the *Cyprinidae* (Înnal, 2007). Molluscae and cestode parasites occurred rarely in our samples. *Glochidia* sp. were found only in two host species, from Drânceni station,

that infected the fins and gills parts (*Abramis brama* and *Barbus barbus*). The presence of these larval stages of unionid mussels in fishes reflects the presence of adult bivalves at that sampling site. All parasite species found in our study are common and most of them occur in many fish species in the Prut River basin.

Table 2. Prevalence and intensity of metazoan parasites of fish from the Prut River, Drânceni station

Systematic group	Species of parasite	Fish host	Parasite habitat	N/n	P%	MI
Protozoa	*Trichodina domerguei*	*Carasus auratus gibelio*	gills, skin	9/3	33.33	9.66
		Sander lucioperca	gills	5/2	40	6,5
Monogenea	*Dactylogyrus extensus*	*Carasus auratus gibelio*	gills	9/4	44,44	5,25
		Abramis brama	gills	7/3	42.86	4.33
		Cyprinus carpio	gills	8/3	37.5	5.66
	Diplozoon paradoxum	*Abramis brama*	gills	7/2	28.6	6.5
		Rhutilus rhutilus carpathorossicus	gills	5/1	20	3
		Schardinus erhythrophthalmus	gills	5/1	20	2
Trematoda	*Posthodiplostomum cuticola*	*Rhutilus rhutilus carpathorossicus*	skin	5/1	20	9
Cestoda	*Ligula intestinalis*	*Cyprinus carpio*	body cavity	8/3	37.5	5.66
		Rhutilus rhutilus carpathorossicus	body cavity	5/2	40	2,5
Mollusca	*Glochidia* sp.	*Abramis brama*	gills, fins	7/1	14.28	4
		Barbus barbus	gills	4/1	25	3

The situation of parasitic agents identified in the fish analyzed on the Prut river, Oancea station, shown in the Table 3, highlights that 78% of fish species have weak polyparasitosis: 25% ciliate (*Trichodina domerguei* and *Apiosoma piscicola*), 25% monogenic worms (*Dactylogyrus extensus* and *Diplozoon paradoxus*), 25% digenic worms (metacercaria larva: *Neascus cuticola* and *Diplostomum spathaceum*), 12.5% nematodes (*Hepaticola* sp.), 12.5% cestodes (*Caryophilaeus* sp.). *Hypophthalmichthys molitrix* was the weakest parasitic species.

The proportion of ecto- and endoparasite specimens infecting fish species was similar in the three analyzed stations.

The ciliated protozoa of *Apiosoma piscicola* were observed in the gills of *Acerina cernua* and *Hypophthalmichthys molitrix* from this station. The pathogenicity of *Apiosoma species* is insufficiently known; ultrastructural observation on attached *Apiosoma* did not reveal any interference with the host cell serving as substrate or peripheral tissue response (Loom, 1973).

Two adults of cestode *Caryophyllaeus* sp. infected the intestine of a *Cyprinus carpio*. Elevated *C. fimbriceps* infection in ciprinids samples may have been caused by a high proportion of oligochaeta species, the intermediate hosts of this parasite, in fish diet.

Chunchukova, 2010 show that cestode species that refer to *A. brama* from Bulgarian part of Danube River belong to order *Caryophyllidea*.

Like the metacercaria larva of *Diplostomum* sp. was identified only in one specimen of *Rhutilus rhutilus.*

A single specimen of nematode *Hepaticola petruschewskii* was found, in the liver of a *Cyprinus carpio* and *Abramis brama*. Nematodes occur worldwide particularly the species utilizing fish as intermediate or transient hosts and can infect all of their organs.

In general, endo-parasites of fish influence fishes negatively in several ways and represent a possible threat to the sustainability of fisheries (Paperna, 1996).

The endo-parasites like tapeworms, nematodes, or acanthocephalans infect the internal organs of fish with their intermediate stages (larvae) and sometimes encysting in various host tissues or most adults mainly affect the digestive systems of their hosts (Luque et al., 2004).

Table 3. Prevalence and intensity of metazoan parasites of fish from the Prut River, Oancea station

Systematic group	Species of parasite	Fish host	Parasite habitat	N/n	P%	MI
Protozoa	*Trichodina domerguei*	*Carasus auratus gibelio*	gills, skin	8/3	37.5	10.33
		Sander lucioperca	gills	5/1	20	12
	Apiosoma piscicola	*Acerina cernua*	gills, skin	4/1	25	8
		Hypophthalmichthys molitrix	gills	3/1	33.33	7
Monogenea	*Dactylogyrus extensus*	*Carasus auratus gibelio*	gills	8/3	37.5	7.66
		Abramis brama	gills	7/2	28.57	6.5
		Cyprinus carpio	gills	8/4	50	6.25
	Diplozoon paradoxum	*Abramis brama*	gills	7/1	14.28	7
		Rhutilus rhutilus carpathorossicus	gills	4/2	50	4.5
		Schardinus erhythrophthalmus	gills	4/1	25	3
Trematoda	*Diplostomum spathaceum*	*Rhutilus rhutilus carpathorossicus*	eyes	4/1	20	2
Cestoda	*Caryophyllaeus fimbriceps*	*Cyprinus carpio*	intestine	8/4	50	5.25
		Rhutilus rhutilus carpathorossicus	intestine	4/1	25	3
Nematoda	*Hepaticola petruschewskii*	*Cyprinus carpio*	liver	8/1	12.5	1
		Abramis brama	liver	7/1	14.28	1

Although the parasitism is common in fish, parasitic diseases are triggered only in environmental conditions that facilitate the multiplication of parasites; therefore, clinical parasitosis is quite rare in freshwaters river. The presence of parasites can provide information about the state of the environment: the ciliates and nematodes should be sensitive indicators of eutrophication and thermal effluent, while digeneans and acanthocephalans should make good indicators of heavy metals and human disturbances (Lafferty 1997).

The establishment of only one intestinal parasite in two cyprinid fishes (*Barbus cyclolepis* and *Squalius orpheus*) indicated poor species diversity within the studied freshwater habitat and negative impacts on the ecosystem (Chunchukova et al., 2020)

CONCLUSIONS

The results from this study show that there is no difference in value prevalence and intensity between the three Prut River stations. But, the total parasite load of analyzed fish was relatively low compared with that observed in the other similar studies (Docan, et al., 2019). Of the eleven parasites identified in fish from the Prut River, the species *Dactylogirus vastator* and *Diplozoon paradoxum* have the highest frequency being found in 37.5% of the species caught. On the other hand, the species *Neascus cuticola* had the lowest frequency being present in 2.5% of captured species.

All captured species had gill parasites but *Abramis brama* was the species in which most parasitic taxa were identified.

In conclusion, none of the parasites collected from analyzed fish species are novel species to the Romanian sector of Prut River. Indeed, all of the observed parasite species are commonly found in native fishes from this river. The abundance of parasites varied among the fish species and station of study, with cyprinids species hosting the richest parasite community.

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ESTIMATION OF GROWTH PARAMETERS AND MORTALITY RATE OF PONTIC SHAD (*ALOSA IMMACULATA*, BENNETT, 1835) IN THE ROMANIAN SECTOR OF THE DANUBE RIVER, KM 169 - KM 197

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Abstract

Alosa immaculata represents one of the most appreciated fish, mainly due to the high nutritive quality of meat. This study aimed to investigate the age, structure, growth and mortality parameters for Pontic shad population in the Romanian sector of the Danube River (km 169- km 197). Sampling was carried out from March to June 2020 during the migration season. According to the age, distribution varied from 2 to 7 years, being dominated by 4-year-old fish. Using the ELEFAN program in the FiSAT II computer package, the growth parameters based on length-frequency analysis were: FL_{∞}=43.05, and K=0.51 year^{-1}. The length-weight relationship (L - W) was $W = 0.069 \times L^{2.400}$ (R^2 = 0.77). Total mortality (Z) had a value of 2.32, while the natural mortality (M) was 0.77 year^{-1} at an annual average temperature of 14.52˚C. The fishing mortality was computed as F = Z – M and had a value of 1.55 year^{-1}.

Key words*: Alosa immaculata, growth, weight, age, mortality, von Bertalanffy equation.*

INTRODUCTION

Alosa immaculata lives in the Black Sea and Azov Sea and migrates into the Danube and in others rivers tributary to the Black Sea and Azov Sea, Dnieper, Dniester, Don and Kuban for spawning. The migration starts in spring when the water temperature reaches 3.0-7.5°C, peaks in April-May when the water temperature reaches 9-17°C and ends in June-July, at 22-26°C (Schmutz, 2006; Năvodaru, 1997; Năvodaru, 1998). The Pontic shad migrates to spawn at three years, but according to Năvodaru (1998), frequently are found fish even at 4-5 years at the first reproduction. In Romania, most of the spawning occurs between kilometers 180 and 500 of the Danube River.
Unfortunately, the population registered a declining trend and, according to the IUCN Red List of Threatened Species, the species is classified as vulnerable (VU) (Freyhof and Kottelat, 2008). Between the major threat to the species, overfishing, pollution, climate change, and dam construction which has led to the loss of large areas of spawning grounds, conducted to a decrease of Pontic shad stocks (Kottelat & Freyhof, 2007). Knowledge of more information related to the age structure, growth parameters, and mortalities rates of a fish population offers the possibility to gather information about fish stocks, which are needed to understand the fish population response to environmental changes and the stock dynamics (Tribuzio et al., 2009; Wells et al., 2013). Also, this information is useful for the implementtation of fisheries management policies for sustainable fishery (Evans et al., 2015). Therefore, understanding the dynamics population of the Pontic shad is crucial for stock assessment and conservation.
In this study, population parameters such as the growth, mortality, and exploitation rate for Pontic shad caught in the Romanian sector of the Danube River, km 169 - km 197, were investigated.

MATERIALS AND METHODS

Study area. The fishing area was situated between km 169 of the River (Brăila) and km 197 of the River (Gropeni) (Figure 1). From the studied data, it seems that this sector is the most important for the reproduction of Pontic shad.

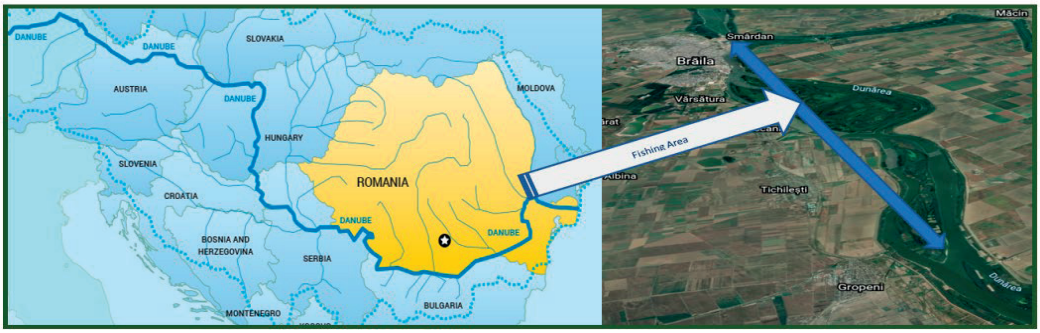

Figure 1. Reasearch area - km 169 (Brăila) and km 197 (Gropeni- Brăila)
Source: https://sos.danubis.org/eng/country-notes/romania/and google maps

Data collection. Fish samples were collected from scientific and commercial fishing. The fishing gear used was the shad gill nets, with a 30-32 mm mesh size. Samples were collected in the year 2020 during March - June 2020. In total, it was collected and sampled a number of 685 fish.

All fishes were measured for total length with an ichthyometer (±1 mm precision) and weighed with an electronic scale (±0.01 g precision).

At the same time, ten scales were removed from the anteromedial part of the body above the lateral line. The age was determined by reading the rings' annual growth on the scales using a stereo microscope with 1×10 magnification (Bagliniere et al., 1992; Yilmaz and Polat, 2002) (Figure 2).

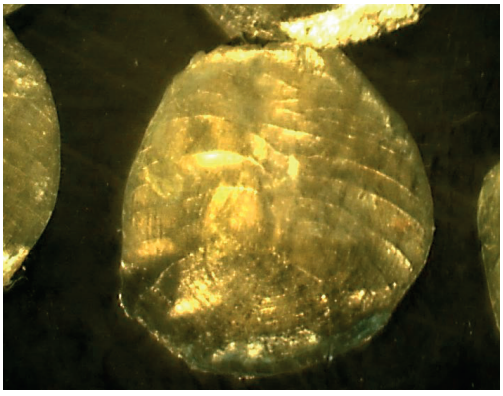

Figure 2. Age determination of Pontic shad (original photo)

Data analysis. For data analysis, we used the software package FiSAT II (FAO - ICLARM Stock Assessment Tool) and Microsoft Excel 2019.

The length-weight relationship (L-W) for the whole population, females, and male, was estimated using the equation $W=a\times L^b$, where: W - total body weight (g), L - total length (cm), "a" -intercepts and "b" the slope.

Also, in order to evaluate the status of the "well-being" of the Pontic shad population, we calculate the Fulton coefficient, $K = (W/L^3) \times 100$ where: K = coefficient of Fulton; W=fish individual weight; L = length of the fish body (Ricker, 1975).

The growth and mortality parameters. The growth parameters (L_∞, k, t_0) were calculated for the whole population by the length-frequency analysis using the ELEFAN model. The t_0 was estimated using the following equation according to Pauly (1979):

$\text{Log}(-t_0) = -0.392-0.275\text{Log}L_\infty - 1.038\text{Log}K.$

To realize the length distribution for the study period, all the length measurements were grouped into 0.5 cm length classes. The resulting length-frequency distribution was then used to estimate the population parameters like growth and mortality rates.

The total mortality rate (Z), natural mortality rate (M), fishing mortality rate (F), and rate of exploitation (E) were estimated according to Pauly (1980) and Pauly (1983).

Total mortality (Z) was computed using the length converted catch curve analysis method in the FiSAT II computer software package.

The natural mortality (M) determination was calculated with the empirical formula

developed by Pauly (1980): Log(M) = -0.0066-0.279 log (L∞) + 0.6543 log(K)+0.4634 log(T), where: K and L∞ are the growth parameters from Von Bertalanffy Growth function, T°C - is the average temperature from the Danube, which in our study was 14.50°C.

The *mortality determination through fishing (F)* has resulted from the difference between total mortality (Z) and natural mortality (M) (F = Z-M) (Gayanilo et al., 2003; Sparre and Venema, 1992). Also, we calculate the *exploitation rate (E)*, which is the report between fishing mortality and total mortality (E = F/Z) (Ricker, 1975). If the exploitation rate is under 0.5, the stocks are easily exploited, but if the values of E are 0.5-1, the stocks are heavily exploited.

RESULTS AND DISCUSSIONS

Length-frequency distribution and age structure. Of the 685 fish caught, 509 were females (74.31%) and 176 males (25.69%). Total length ranged from 23.9 cm to 41.5 cm, with significant differences between females and males ($p<0.05$).

The results are in line with those reported for Pontic shad in a previous study carried out by Ibănescu et al. (2017), on the same fishing area in 2009 (total length was between 24 and 39 cm, with an average value of 31.11 cm, while the individual weight was between 100 g and 400 g with a mean value of 276.72 g).

The females have a maximum total length of 41.5 cm and weight of 695 g, while the male maximum total length was 41 cm and weight of 565 g. However, the statistical analysis (T-Test) revealed significant differences between the total length and weight of females and males ($p<0.05$) (Table 1).

Information regarding the sex ratio (total numbers of females/total number of males) is important for understanding the relationship between fish and the reproductive potential of a population (Vicentini and Araujo, 2003). The female: male ratio was 1:0.34. Our results are in line with those reported for this species (1:0.51) by Năstase et al. (2018) for the Danube River, but lower than the sex ratio (1:0.62) reported by Țiganov et al. (2018) for the Black Sea.

The age structure of the *fish* was determined based on growth rings from scales and consisted of six age groups ranging from 2 to 7 years. The majority of the individuals caught during the migration of the year 2020 were four years old (41.90%), followed by six years (19.85%) and five years old (15.62%), three years (13.14%), seven years (8.32%), while only 1.17% of the population was two years old.

Regarding the sex structure population from 2020, dominated was females aging four years old (28.90%), followed by six years (19.71%) and five years (14.01%). The male population was represented mostly by fish of four years old (12.99%), followed by three years (9.78%) (Table 2).

Analyzing the structure of the population by ages and sexes, statistical differences ($p<0.05$) were observed between the length and weight of the two sexes.

Table 1. Values of the length and weight in Pontic shad

Sex	Fish number	Total length (cm)			Weight (g)		
		Min.	Average	Max.	Min.	Average	Max.
Female	509	23.9	33.10±3.19	41.5	200	318.18±97.13	695
Male	176	20.5	29.65±4.34	41	80	244.43±91.95	565
Both sexes	685	20.5	32.21±3.82	41.5	80	299.22±101.04	695

Note: n = the number of fish; SD - standard deviation

Table 2. The frequency, mean total length (cm), and weight (g) of Pontic shad during 2020 migration

Age	Female						Male					
	Freq. (N)	Fish (%)	Total length ±SD (cm)	c.v. (%)	Weight ±SD (g)	c.v (%)	Freq. (N)	Fish (%)	Total length ±SD (cm)	c.v. (%)	Weight ±SD (g)	c.v (%)
2	1	0.15	30	-	220	-	7	1.02	27.05±4.25	15.71	155.57±68.76	44.19
3	22	3.36	28.92±3.33	11.51	230.86±41.84	18.12	68	9.78	25.97±3.42	13.17	180.86±41.81	23.11
4	198	28.90	32.37±2.99	9.23	298.17±93.46	31.34	89	12.99	32.93±2.71	8.22	274.78±75.09	27.32
5	96	14.01	33.47±2.90	8.66	324.48±95.25	29.84	11	1.61	36.80±1.7	4.62	416.36±28.73	6.90
6	135	19.71	33.91±3.25	9.58	346.96±103.55	28.43	1	0.15	41	-	565	-
7	57	8.32	35.04±2.03	5.79	359.67±80.20	22.29	0	0	0	0	0	-

Note: n = the number of fish; SD - standard deviation

The length-weight relationships and Fulton coefficient. To evaluate the fish condition, we calculate the L-W relationship for the whole population and separately for females and males. These relationships in fishes can be affected by habitat, temperature, seasonal effect, gonad maturity, degree of stomach fullness, food availability, fish health, general fish condition (Wootton, 1990; Battes et al., 2008). The relationship between the total length and weight (Lt-W) for the Danube shad population during the study period was determined as $W = 0.069 \times L^{2.40}$, for females was $W = 0.044 \times L^{2.52}$ and for males was $W = 0.137 \times L^{2.19}$.

In our study, the "b" values of the whole captured population, females and males, displayed negative allometric growth, meaning that the increase in length was faster than in weight (Figures 3-5).

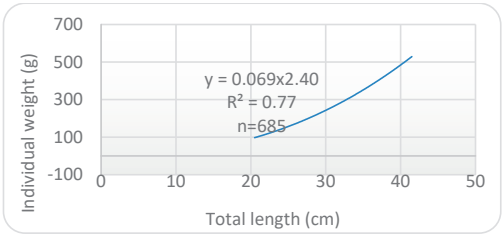

Figure 3. Length-Weight relationship for the females and male's population

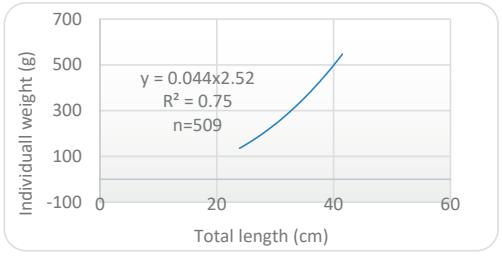

Figure 4. Length–Weight relationship for the female's population

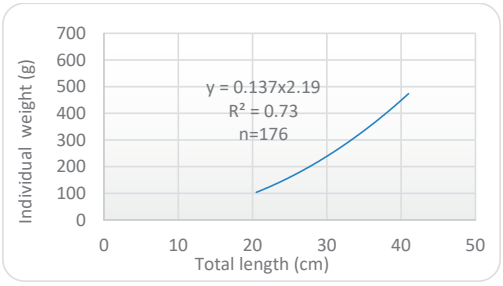

Figure 5. Length–Weight relationship for the male's population

However, the results of this study were similar to the general study trend shown by Ibănescu et al. (2017) ($W = 0.052 \times L^{2.487}$) for Pontic shad, on the same fishing area, for the year 2009. Also, Năvodaru (1997) obtained negative allometric growth (b = 2.45) for Pontic shad, for the Danube River.

Higher values were obtained by the Țiganov et al. (2018) for the Black Sea coast ($W = 0.0057 \times L^{3.134}$).

The Fulton coefficient calculated for the whole population was 0.88 ± 0.17, 0.86 ± 0.13 for females, and 0.93 ± 0.26 for males. A lower Fulton coefficient for females is explained by the fact that during the spawning period, females lose weight.

Năvodaru and Năstase (2014) reported a Fulton coefficient for the Pontic shad in the period 1988-2014 equal to 1.42.

According to Năvodaru (1997), the Fulton coefficient values for Pontic shad tend to decrease with migration distance to the Danube River and reproduction due to the energy consumed for migration and spawning.

Of the 685 individuals captured during March-June 2020, 48.91% were captured in May, 43.21% in April, and only 4.08% and 3.80% in March and June, respectively (Figure 6).

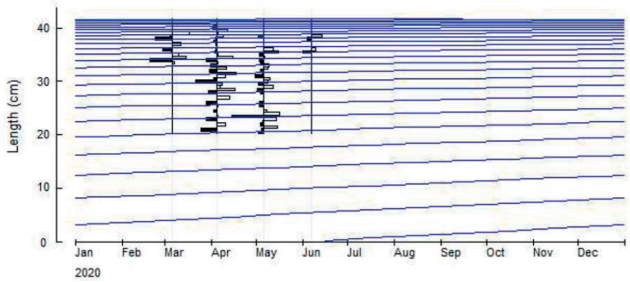

Figure 6. Length frequency distribution output from FISAT of Pontic shad fitted with growth curves

Growth parameters. The growth parameters ($L\infty$- asymptotic length, K- growth rate, and t_0- the age at zero-length) are useful in assessing the growth rates between and within individuals inhabiting various environments. For growth parameter estimation, the data set of 685 individuals was used to calculate Von Bertalanffy's growth parameters ($FL\infty$, K, t_0) which were presented in Table 3.

The asymptotic length obtained in our study ($L\infty$ = 43.05 cm) was very close to Ibănescu et al. (2017) ($L\infty$ = 40.43 cm) for Pontic shad for the year 2009, for the same Danube sector km 169 (Smârdan - Brăila) and km 197 (Gropeni-Brăila). Țiganov et al. (2018) obtained an $L\infty$ = 41.5 for Pontic shad (data obtained after a study period of two years, 2012-2013).

Table 3. The Von Bertalanffy growth parameters of Pontic shad

Species	Parameters	Estimated
Alosa immaculata	FL∞ (cm)	43.05
	K (per year)	0.51
	t_0	-0.53

Regarding the constant growth K, the present study revealed a value of 0.51 year^{-1}. Our value is lower than those reported by Ibănescu et al. (2017) (K = 0.90 year^{-1}). Then, the t_0 was calculated as - 0.53 years using the empirical formula presented in the materials and methods section.

Mortality and estimation rate. The mortality coefficient (Z) was estimated using the length converted catch curve, and the values were 2.32 Natural mortality (M) calculated was 0.77, and fishing mortality (F) was 1.55. The value of the exploitation rate (E = 0.67) is over the optimum level of exploitation (E = 0.50), suggesting that in this Danube sector, the Pontic shad population is overexploited by fishing or by poaching (Figure 7).

It can be observed that in our study, the estimated mortality rates were found to be higher than estimates from other scientific studies regarding the Pontic shad, and the fishing mortality rate (F) was higher than the natural mortality (M) (Table 4).

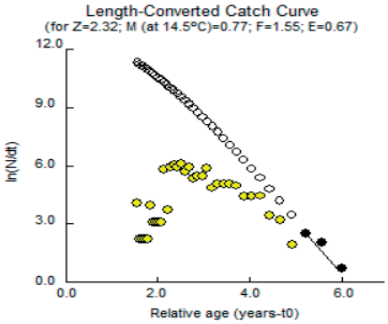

Figure 7. FiSAT II output of linearized length-converted catch curve for Pontic shad

Table 4. Mortality rates of *Pontic shad* population

Z	M	F	E	Year	Area	Reference
1.54	0.58	0.95	0.61	2009	Danube River, km 170-197	Ibănescu et al., 2017
1.71	0.58	1.13	0.66	2012	Black Sea	Tiganov et al., 2018
1.71	0.63	1.07	0.62	2013	Black Sea	Tiganov et al., 2018

CONCLUSIONS

From the studied population of Pontic shad from Danube km 169 (Smârdan - Brăila) and km 197 (Gropeni- Brăila), it can be concluded:

- ✓ Migration in the year 2020 was dominated by females and males aging 4-years.
- ✓ The length-weight relationship of Pontic shad, followed a negative allometric growth, the increase in length being greater than in weight.
- ✓ The fishing mortality rate (F) was higher than the natural mortality rate (M), indicating a fishing pressure on the Pontic shad population in this Danube sector.
- ✓ The value of exploitation rate was (E) was 0.67, indicating that the stock of this species is over exploited.

It could be concluded that the Pontic shad stock in this Danube sector is in a situation of over exploitation, and for the management purpose, the current exploitation rate should be reduced under 0.5. However, changes in the population structure and stock size must be monitored continuously, and some measures should be taken to reduce the pressure on fish stocks.

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GROWTH PERFORMANCE AND CONDITION FACTOR OF *OREOCHROMIS NILOTICUS* SPECIES FEED WITH A DIET WHICH INCLUDE SOME PHYTO-ADDITIVES

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Abstract

The purpose of this research was to evaluate the growth performance of Oreochromis niloticus reared in a recirculating aquaculture system in case of inclusion in feed of some phytoadditives. The experiment was conducted on 98 days and the biometric measurements were performed at the beginning (I.), at the end (F.), but also at an intermediary moment (INT.-after20 days of the experiment). The experimental variants were: V1-control, V2-1% Rosmarinus officinalis, V3-1% Hippophae rhamnoides and V4-1% Zingiber officinale. At the end of the experiment the best values of IBG (1.95 g/ind.-Int.; 8.50 g/ind.-F.), SGR (1.28%/day-Int.; 0.76%/day-F.), FCR (1.37 g/g-Int.; 1.63 g/g-F.) and PER indicator (1.93 g/g-Int.; 1.61 g/g-F.) were registered in case of variant V3. Also, in same variant, were observed a reduction of the variability of body mass and total length of fish. In the variants in which phytobiotics were administered, were obtained a better condition factor (V1-2.04; V2-2.11; V3-2.07; V4-2.09) at the end of the experiment. In conclusion, the results shows that the administration of sea buckthorn in a long period of time, has the best effect on growth performance of Oreochromis niloticus species.

Key words*: condition factor, growth performance, Nile tilapia, phytobiotics, recirculating aquaculture system.*

INTRODUCTION

In recent decades, a large part of studies has been focused on the use of medicinal herbs in the light of numerous advantages, such as sustainability and less side effects, over other immunostimulants (Mohammadi et al., 2020).

A wide range of medicinal plants show potential effects on growth and survival properties of aquatic organisms. Whole plant, parts of plant (leaf, root or seed) or extract compounds have been used as feed additives in aquaculture (Hai, 2015). The growing interest of using herbal immunostimulants in aquaculture has increased worldwide because they are easy to prepare, cheap, and they contain natural organic compounds that do not cause any threat to fish health or to human health (Talpur et al., 2013; Hai, 2015).

Generally, the primary effects of medicinal plants are to improve feed efficiency and/or daily gain (Adekunle & Oladoye, 2015), but also can be a good alternative to replace antibiotics and chemicals to prevent and control diseases (Harikrishnan et al., 2010; Vaseeharan & Thaya, 2014; Syahidah et al., 2015).

There are various reports of herbal fish diets promoting growth performance (Kim et al., 2000; Ji et al., 2007).

In aquaculture sector, the use of medicinal plants (phytochemicals) has increased significantly over the past decade for different purposes such as sex reversal compound (Gholipour et al., 2011), growth enhancer (Turan and Akyurt, 2005; Banaee, 2010; Banaee et al., 2011; Ahmadi et al., 2012; Asadi et al., 2012), immunostimulant and antipathogenic (Yılmaz et al., 2013).

Ginger (*Z. officinalis*) is generally considered as a safe herbal medicine and perennial herbaceous plant, is a part of the Zingiberaceae family (Weidner & Sigwart, 2000). They are polyphenol compounds (6-gingerol, shogaols and zingerone, alkaloids, flavonoids, polyphenols, saponin, steroids, tannin, fiber, carbohydrate, vitamins, carotenoids and minerals), which have a high antioxidant activity (Hori et al., 2003; Otunola et al., 2010; Shirin & Prakash, 2010).

Supplementing ginger in fish diets may enhance growth and will signify change in magnitude and their body composition (Talpur et al., 2013).

Sea-buckthorn (*Hippophae rhamnoides*) is a plant rich in vitamins (vitamin C, vitamin E) and carotenoids (like β-carotene and lycopene B2) and secondary plant metabolites like flavonoids (quercetin and kaempferol) which are abundant in fruit pulp and seeds (Kagliwal et al., 2012). In fish diets, sea buckthorn it is used successfully for improving disease resistance and growth performance (Todoran, 2015).

The sea buckthorn berry flour is like a fruit, its seeds and leaves are rich in nutrients and bioactive components such as vitamins, amino acids, lipids, carotenoids, xanthophyll, phenols and flavonoids and have a higher content of essential oils (Repyakh et al., 1990; Bekker & Gluschenkova, 1997; Ranjith et al., 2006; Singh et al., 2006; Yang, 2009), making it a very beneficial feed supplement for laying hens.

The sea buckthorn and its by-products are examined in recent years as a supplement in animal nutrition. Moreover, the sea buckthorn plays an important role in improving the efficiency of feed and has been used as an alternative feed, particularly in poultry, to maintain their production, performance and high-quality yield (Mohamed et al., 2018).

Sea buckthorn (*Hippophae rhamnoides*), as a functional plant homologous to medicine and food, is crammed with a variety of nutrients and biological substances, such as organic acids, polysaccharides, unsaturated fatty acids, and various amino acids required by the human body (Pichiah et al., 2012; Tamchos & Kaul, 2019). Although several studies have reported that sea buckthorn extract has the positive effect of lowering plasma cholesterol, increasing intestinal probiotics, improving lipid metabolism enzyme activity, enhancing antioxidant ability, and reducing the incidence of chronic diseases (Yuan et al., 2011; Pichiah et al., 2012; Kwon et al., 2017; Hao et al., 2019), but studies using sea buckthorn itself are limited.

Rosemary (*Rosemarinus officinalis*), belonging to the Lamiaceae family, is well known for its antioxidative properties, and is also used in several pharmaceutical applications (Cheung & Tai, 2007). Biologically, rosemary extract improved feed conversion, efficiency of broilers fed diet supplemented with such herb (Ghazalah & Ali, 2008). Cagıltay et al. (2013) reported that the parallel to the concentration of rosemary in fed has decreased crude fat and increased amount of crude protein. Also, there have been a few studies on the antioxidative effect of rosemary in fish (Perez-Mateos et al., 2002; Valeria et al., 2010; Alvarez et al., 2012). The growth performance (SGR and WG) and feed utilization (FCR and FER) of the fish increased with the increase in the rate of rosemary in the feed ($p<0.001$) (Karatas et al., 2020).

The antioxidant capacity of rosemary is attributed to three phenolic diterpenoids (carnosic acid, carnosol and rosemaric acid), but many other components (rosmanol, epirosmanol, isorosmanol, rosmaridiphenol, rosmadial, rosmariquinone, carvacrol, carvone, cymene, cineole, fenchone, limonene, terpinene and thymol) are expected to contribute to its antioxidative and antimicrobial properties (Fu et al., 2007).

The aim of this study was to evaluate the growth performance of *Oreochromis niloticus* reared in a recirculating aquaculture system in case of inclusion in feed of some phyto-additives.

MATERIALS AND METHODS

Description of the RAS Pilot Station.

The present study was conducted in RAS pilot station within "Dunărea de Jos" University of Galați, during a 98 days experimental period. The experimental intensive production system was designed and configurated according to the indications presented by Cristea (2008). The detailed designed of the aquaculture intensive production system is described in Figure 1.

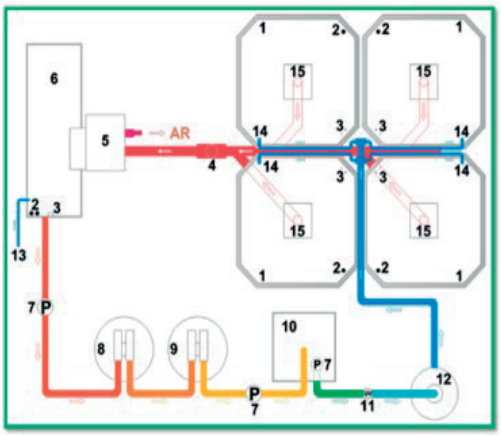

Figure 1. The design of RAS pilot station: rearing units - No. 1; nitrogen compounds sensors - No. 2; water level sensors - No. 3; RAS outlet structure - No. 4; mechanical drum filter - No. 5; sump - No. 6; pumps - No. 7; sand filter - No. 8; activated charcoal filter - No. 9; biological trickling filtration unit - No. 10; sterilization UV filter - No. 11; oxygenation unit - No. 12; automatically fresh water inlet No. 13; rearing units water inlet/outlet structure - No. 14, 15 (Petrea et al., 2020)

Design experimental

The biological material consisted in a total number of 168 individuals of *Nile tilapia*, with an initial average weight of 280.06 ± 54.02 g/fish, respectively with an initial total length of 24.25 ± 1.38 cm/fish, that were randomly distributed in four rearing units.

The experiment was conducted on 98 days and the biometric measurements were performed at the beginning (I.), at the end (F.), but also at an intermediary moment (INT. - after 20 days of the experiment).

The experimental variants were organized as follows: V1 - control, V2 - 1% rosemary (*Rosmarinus officinalis*)/kg feed, V3 - 1% sea buckthorn (*Hippophae rhamnoides*)/kg feed and V4 - 1% ginger (*Zingiber officinale*)/kg feed. These phytobiotics were purchased from a Plafar market, like dried plants, after which they were grounded and used as powder.

The additivation of fish feed with these plants was made according to the indications presented by Mogodan et al. (2020).

Fish were fed with SOPROFISH pelleted feed, with 38% crude protein and 7% crude fat. The feed biochemical composition was related by Antache et al. (2013). Fish were fed four times per day with a daily ration of 2% from fish body weight.

During the experimental period the monitored water quality parameters were kept within acceptable limits for the optimal growth of the *Oreochromis niloticus* species.

The main physico-chemical water quality parameters were monitored daily: oxygen (mg/L) with TriOxmatic 700IQ WATT (W) sensor, temperature (°C) with sensor TrioxiTherm WATT, pH with WAT Sensolyt 700 IQ (SW) sensor and the concentration of $N\text{-}NO_2$ (mg/L), $N\text{-}NO_3$ (mg/L) and $N\text{-}NH_4$ (mg/L) was also monitored twice a week using Spectroquant photometer, Nova 400.

Technological indicators

The analysed technological indicators were:

- Individual biomass gain:

IBG = (Bf)-(Bi)/fish number [g/fish], (1);

with:
Bf - final fishbiomass,
Bi - initial fish biomass.

- Specific growth rate:

SGR = 100 x (ln Bf - ln Bi)/t [% fish biomass/day], (2);

with:
Bf - final fish biomass,
Bi - initial fish biomass,
t - duration of the experiment.

- Feed conversion ratio:

FCR = F/IBG [kg feed intake/kg fish biomass gain], (3);

with:
F - feed intake,
IBG - individual biomass gain.

- Protein efficiency ratio:

PER = IBG/(F*CP/100) [kg/kg], (4);

with:
IBG - individual biomass gain,
F - feed intake,
CP - crude protein.

- Condition factor:

$K = W/L^3 \times 100$, (5);

with:
W - body weight,
L - body length.

- Variation coefficient:

$CV_{BW \text{ or } TL}$ = (Dev. St. / Avg W (or L)) x 100 [%], (6);

with:
Dev. St. - standard deviation,
Avg BW or TL - fish body weight or length.

Statistical methods

For the statistical analysis presented in this paper was used the software IBM SPSS Statistics 20. To determine significant differences among groups was used the one-way analysis of variance (ANOVA); $p<0.05$ was considered as significant. The homogeneity of variance was tested by using Levene's test (F value).

RESULTS AND DISCUSSIONS

When it was done the fish distribution in all four experimental variants has been taken into account the homogeneity of fish in terms of body weight (Levene test $p>0.05$).

The variability of fish body weight (g) and total length (cm) is expressed by the coefficient of variability (CV - %). The evolution of the variability coefficient of body weight (CV_{BW}) and of the total length (CV_{TL}) throughout the research period can be observed in Figure 2, respectively in Figure 3.

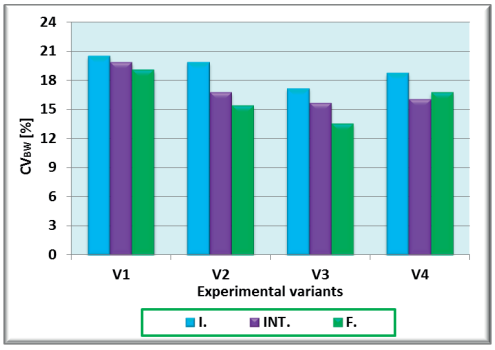

Figure 2. Evolution of variability coefficient of body weigth (CV_{BW}) throughout the experiment
Note: I. - initial, INT. - intermediary, F. - Final

During the experimental period a reduction in body weight and total length variability in all experimental variants was observed. However, at the level of body weight, this is more evident in the variants in which phyto-additives (V2, V3, V4) were administered after 98 days compared to the values recorded at the beginning of the experiment. In conclusion, this shows us a better grouping of the body weights around the average value in V2, V3 and V4 variants. In the control variant we can say that at the end of the experiment the heterogeneity of the studied group increased.

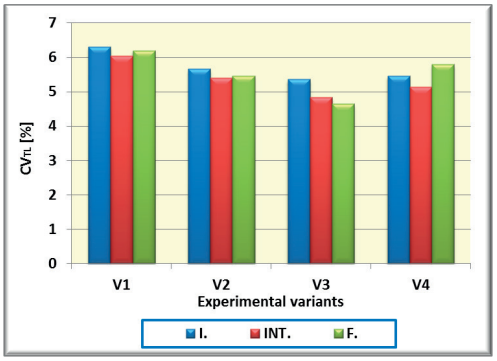

Figure 3. Evolution of variability coefficient of body weigth (CV_{TL}) throughout the experiment
Note: I. - initial, INT. - intermediary, F. - Final

The normality of fish body weight distribution values is presented using histograms. The evolution of the normality of distribution during the experiment can be observed in the Figures 4, 5 and 6.

Thus, the initial mean of fish body weight did not show significant differences by statistically point of view between the experimental variants, these values being grouped by the Duncan test in a single subset of data ($p>0.05$).

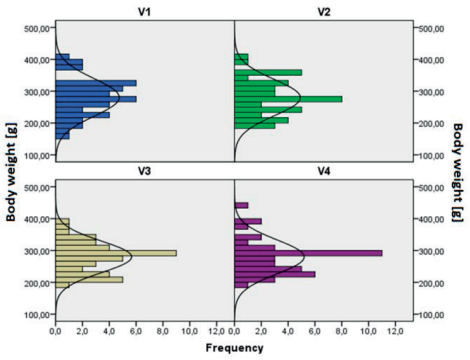

Figure 4. Histogram of the individual body weight at the beginning of the experiment (I.)

The individual mean body weight recorded at the beginning of the experiment for each experimental variant was: 280.40 ± 57.53 g/fish in V1 variant; 279.83 ± 55.58 g/fish in V2 variant; 280.05 ± 48.13 g/fish in V3 variant and 279.98 ± 52.51 g/fish in V4 variant.

Regarding to the individual average of total length between the experimental variants, no significant differences were registered ($p>0.05$), the variants being homogeneous. The

average values was 24.36 ± 1.54 cm in V1 variant, 24.26 ± 1.37 cm in V2 variant, 24.24 ± 1.30 cm in V3 variant and 24.15 ± 1.32 cm in V4 variant.

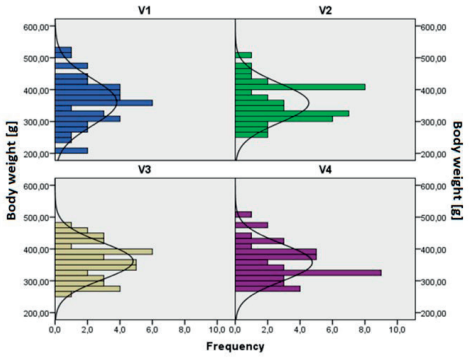

Figure 5. Histogram of the individual body weight at the intermediary moment (INT.)

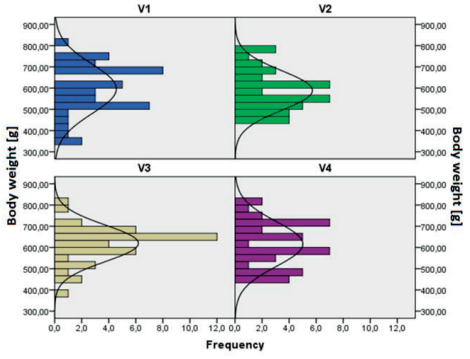

Figure 6. Histogram of the individual body weight at the end of the experiment (F.)

Following the somatic measurements performed during the experiment (INT. and F.), the boxplots corresponding to body weight and total length were also made.

The values of the median, minimum, maximum and quartile for the individual body weight and total length at the beginning of the experiment are presented in the boxplots from Figures 7, respectively 8.

Both after the intermediary measurements and at the end of the experiment a constant increase of the individual average body weight was observed, especially in the variant in which sea buckthorn (V3) was administered, but an increase was also observed in the variant in which ginger (V4) was administered, but was lower than in variant V3.

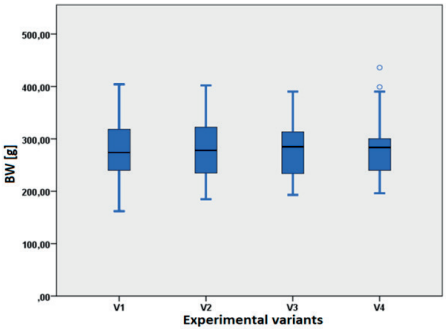

Figure 7. Boxplot of initial body weight (g)

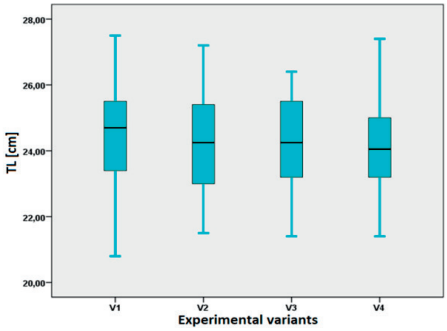

Figure 8. Boxplot of initial total length (cm)

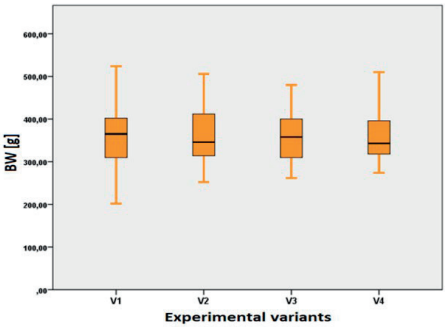

Figure 9. Boxplot of body weight (g) at intermediary moment

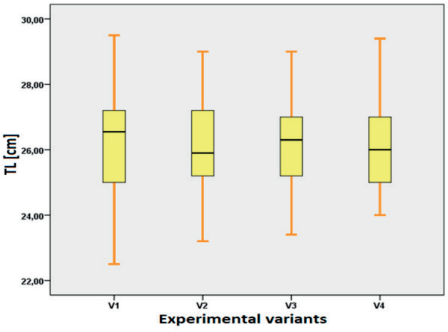

Figure 10. Boxplot of total length (cm) at intermediary moment

However, during the experimental period the increase of mean body weight in variant V3 was not significant compared to the results obtained in the other experimental variants (INT. - $p>0.05$; $p = 0.985$; F. - $p>0.05$; $p = 0.456$). Also, during the experiment, no significant differences were found in the individual total lengths of fish ($p>0.05$) (INT. - $p = 0.875$, F. - $p = 0.330$).

The boxplots of fish body weight for the intermediary moment and for the end of the experiment are presented in Figures 9 and 11, and for the total lengths the boxplots are showed in Figures 10 and 12.

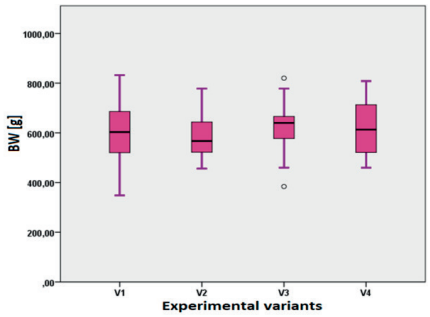

Figure 11. Boxplot of body weight (g) at the end of the experiment

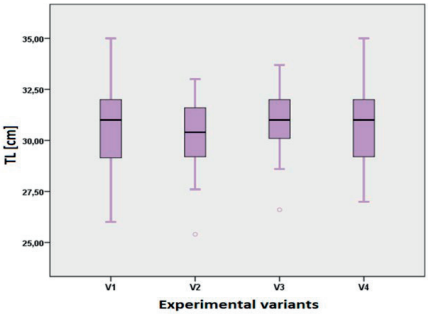

Figure 12. Boxplot of total length (cm) at the end of the experiment

The correlation between body weight and total length for the Nile tilapia exemplars was determined from each experimental variant. Therefore, the Pearson coefficient was calculated (Table 1).

Pearson coefficient indicates the degree of association between two variables, in our case it is represented by the fish total length and body weight. Thus, from the values obtained for the Pearson coefficient it can be seen that both at the beginning of the experiment and in the other experimental stages (INT. and F.) between the two variables there was a strong positive relationship, because the values of R were registered between 0.75 and 1.

Table 1. Values of Pearson coefficient during the experimental period

Experimental variants	Pearson coefficient		
	I.	INT.	F.
V1	0.904	0.854	0.864
V2	0.915	0.867	0.842
V3	0.883	0.841	0.822
V4	0.889	0.889	0.792

In the same time, the regressions were performed with which we can determine allometric factor "b" through which we can see if the fish has grown more in mass or in length. The allometric factor “b” and the determination coefficient “R^2” during the entire experiment for each experimental variant are presented in Figures 13, 14, 15 and 16.

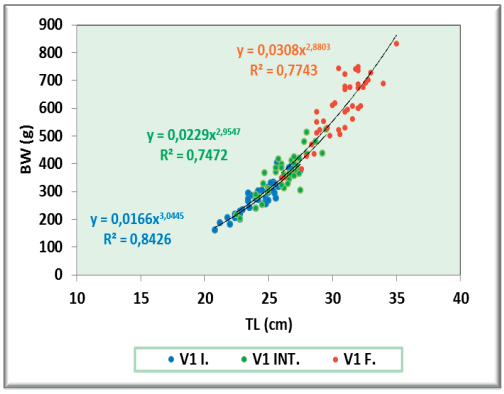

Figure 13. Power regression of individual fish length-weight from V1 variant during the experimental period

The allometric condition factor “b” shows us that during the experiment a negative allometry was registered. The values obtained show that the fish increased more in length than in body weight, because the value of "b" is less than 3. Following the analysis of the determination coefficient “R^2”, a good correlation was found between the total length and the body weight of the specimens, respectively the proportion in

which the increase of the body mass can be attributed to the increase in length.

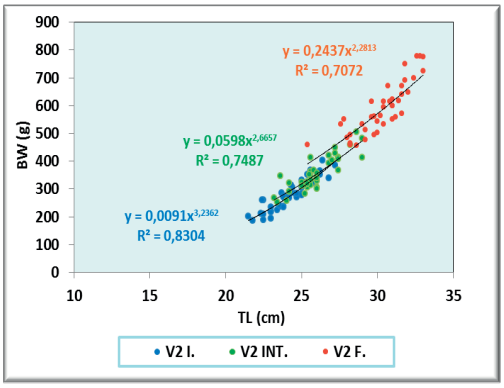

Figure 14. Power regression of individual fish length-weight from V2 variant during the experimental period

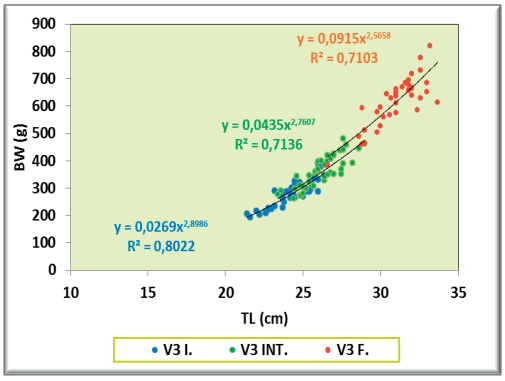

Figure 15. Power regression of individual fish length-weight from V3 variant during the experimental period

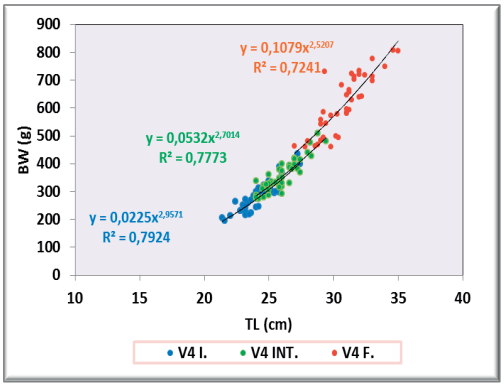

Figure 16. Power regression of individual fish length-weight from V4 variant during the experimental period

Therefore, it varied at the beginning of the experiment from a minimum of 79.2% (V4) to a maximum of 84.3% (V1). Among the variants in which phyto-additives were administered only in variants V3 and V4 was observed an increase more in body weight than in length compared with V2 variant fact also given by the value of the determination coefficient (Figures 13-16).

In aquaculture, the condition factor (Fulton`s coefficient - "K") is used in order to compare the "condition", "fatness" or well-being of fish. It is based on the hypothesis that a better physiological condition is reflected by a heavier fish of a particular length (Metaxa et al., 2018).

Table 2. Condition factor "K"at Nile tilapia during the experimental period

Experimental variants	Condition factor		
	I.	INT.	F.
V1	1.92±0.17	1.99±0.21	2.06±0.21
V2	1.94±0.17	2.02±0.18	2.11±0.21
V3	1.95±0.16	2.00±0.17	2.07±0.17
V4	1.97±0.17	2.02±0.16	2.10±0.21

Moreover, the condition factor is at the same time a measurement which shows the fact whether the fish is fed enough (Mert et al., 2008).

In variants in which the phyto-additives were administered, the highest values of the condition factor "K" were registered, which shows a good condition of the fish. But, the differences were statistically insignificant ($p>0.05$) compared to the control variant both at the intermediary moment and at the end of the experiment (Table 2).

Regarding the technological growth indicators, was registered a better growth performance in the variants in which sea buckthorn (V3) and ginger (V4) were administered compared to the other experimental variants both in the intermediary moment (INT.) and at the end of the experiment (F.) (Table 3).

It was observed that the best evolution of the stocking density (SD - kg/m^3), but also of the total biomass gain (TBG - kg/m^3) was registered in the variant in which sea buckthorn was administered (V3), followed by V4 variant in which ginger was administered. The individual average of body weight recorded the best values also in the V3 variant (INT. - 362.10 ± 56.66 g/fish; F. - 620.20 ± 84.40 g/fish) (Figures 9 and 11).

If at the intermediary moment the total biomass gain did not show visible changes with the administration of phytobiotics, at the end of the experiment the changes are much more obvious. In V3 variant was observed an increase with 2.09% at intermediary moment, respectively with 7.32% at the end of the experiment compared to the control variant (V1). Ginger administration in fish feed led to a reduction of the total biomass gain with 3.14% at intermediary moment, but and an increase with 6.31% at the end of the experiment compared to the control (V1).

Regarding to the rosemary administration, it contributed to a reduction of the total biomass gain with 2.62% in intermediary moment, respectively with 2.27% at the end of the experiment compared to the control variant (V1). Thus, the highest total biomass gain was recorded in the variant in which sea buckthorn (*Hippophae rhamnoides*) was administered, followed by ginger (*Zingiber officinale*).

The most suggestive technological indicators for growth performance show that the food conversion ratio (FCR) is indirectly proportional with the specific growth rate (SGR) and the protein efficiency ratio (PER). Their best values were registered in the variant in which sea buckthorn was administered in fish feed, being followed by those obtained in the variant with ginger administration and in the control variant. The administration of rosemary did not lead to an improvement in growth performance, fact evidenced by the results obtained from the SGR, FCR and PER determination.

Table 3. Growth performance indicators for each of the experimental variants

Technological indicator	Experimental period	Experimental variants			
		V1	V2	V3	V4
Fish stocking density (kg/m^3)	I.	29.44	29.38	29.41	29.4
	INT.	37.87	37.59	38.02	37.54
	F.	59.72	58.93	62.02	61.69
Total biomass gain (kg/m^3)	I.- INT.	8.43	8.21	8.61	8.14
	INT. - F.	21.85	21.34	24	24.15
	I. - F.	30.28	29.55	32.61	32.29
Individual biomass gain – IBG (g/fish)	I. - INT.	1.91	1.86	1.95	1.85
	INT. - F.	5.66	5.64	6.02	5.95
	I.-F.	7.92	7.74	8.5	8.42
Specific growth rate - SGR (%/day)	I.- INT.	1.26	1.23	1.28	1.22
	INT. - F.	0.58	0.58	0.63	0.64
	I. - F.	0.72	0.71	0.76	0.75
Feed conversion ratio - FCR (g/g)	I.- INT.	1.4	1.43	1.37	1.44
	INT. - F.	1.87	1.88	1.73	1.7
	I. - F.	1.74	1.76	1.63	1.64
Protein efficiency ratio - PER (g/g)	I.- INT.	1.88	1.84	1.93	1.82
	INT. - F.	1.41	1.4	1.52	1.54
	I. - F.	1.52	1.5	1.61	1.61

Note: I. - beginning of the experiment, INT. - intermediary moment, F. - final of experimental period.

Regarding to the effect of sea buckthorn on technological indicators of growth performance Csep and Bud (2010) reported similar results at *Cyprinus carpio* species. The research carried out by them consisted in the existence of three experimental variants: L1 - control variant, L2 - variant in which the diet was supplemented with 1% sea buckthorn/kg feed and L3 - variant in which the carp diet was supplemented with 2% sea buckthorn/kg feed. The results obtained by these showed that the administration of sea buckthorn in a concentration of 1%/kg feed led to the improvement of growth performance to a greater extent than in the variant L3, respectively L1 (Csep & Bud, 2010).

The ginger administration in 1% concentration per kg of feed contributed to a better optimization of growth than that obtained in the control variant and in the variant in which rosemary was administered, but lower than that obtained in variant in which sea buchthorn was administered. Better results of the feed conversion ratio and an increase of body weight, compared to the control variant, were also registered in the case of the diet

supplemented with ginger in case of rainbow trout (Nya & Austin, 2009; Gabor et al., 2011). Another study provides evidence that dietary administration of ginger in 0.8% concentration for 60 days can modulate growth performance at *Labeo rohita* species (Sukumaran et al., 2016).
The growth enhancement following ginger administration in fish feed can be attributed to stimulated secretion of intestinal proteases by the host, thus improving digestion and absorption of proteinous components of the feed (Mohammadi et al., 2020). Besides, ginger rhizomes as a rich source of proteinase, enhance proteins digestion and amino acid absorption in the gastrointestinal tract (Hashim et al., 2011). Ginger also has positive effect on intestine probiotic bacterial flora and aid in gaining even more nutrients (Ali et al., 2008).
Concerning to the supplementation of the diet with 1% rosemary/kg feed, a reduction of the technological indicators of growth performance in Nile tilapia was observed compared to the results obtained in the control variant at the end of each experimental period (INT. and F.). At the *Dicentrarchus labrax* species, the same aspect of decreasing the values of growth indicators was observed in the case of rosemary administration in 1% concentration in feed compared to the control variant (Yilmaz et al., 2012). Instead, Yilmaz et al. (2011) showed that the addition of rosemary extracts promoted growth and enhanced some nonspecific immunity indicators at tilapia, *Oreochromis mossambicus* and study of Turan & Yigitarslan (2016), established the efficacyty of rosemary extract feed additives as a growth promoter in *C. gariepinus*.

CONCLUSIONS

The determination of growth performance indicators, such as the specific growth rate, the feed conversion ratio, the protein efficiency ratio and the biomass gain, indicated that the best values were recorded in the variant in which sea buckthorn was administered, followed by the variant in which ginger was administered. But, the effect of ginger on growth was superior to the effect of rosemary.
Although, the experiment was carried out over a longer period of time (98 days), the research showed that the administration of rosemary did not contribute to the improvement of growth performance at Nile tilapia in our case.
The weight-length relationship, fish distribution and the condition factor values, presented in this research, provides useful information in terms of fish production technology and fish population dynamics during a growth production cycle. Following the analysis of the variability coefficient, a reduction of body weight variability was found in variants V2, V3 and V4, especially at the end of the experiment, respectively after 98 days of experiment. Also, a better condition factor was registered in variants in which were administered phyto-additives (V2, V3 and V4).
In conclusion, the results shows that the administration of sea buckthorn in a long period of time, has the best effect on growth performance of *Oreochromis niloticus* species.

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EFFECTS OF DIETARY VITAMIN E AND SAGE (*SALVIA OFFICINALIS* L.) ON GROWTH PERFORMANCE OF KOI CARP (*CYPRINUS CARPIO* L., 1758)

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Abstract

The present research aims to evaluate the impact of both dietary vitamin E and dietary sage supplementation on growth performance of koi carp fingerlings, reared in a partial recirculating aquaculture system. Koi carp specimens with an average individual biomass of 5.1 ± 0.4 g were equally distributed, as follows: V1 - diet supplemented with 1% vitamin E (V1), respectively, V2 - diet supplemented with 1% sage. A 5% BW (Body weight) daily feeding ratio was applied. During the 49 days trial, individual biomass measurements were performed at initial, after 8, 15, 25 and 37 days, as well as at the final stage of the experiment. The specific growth rate indicates a superior fish production at V2 (3.30%BW/day), compared to V1 (2.75%BW/day). Also, better FCR values are recorded at V2 (1.28), compared to V1 (1.62). The Protein efficiency ratio (PER) results indicate a better ability of V2 koi carp specimens to utilize proteins (1.39), compared to V1 specimens (1.10). Thus, dietary supplementation with 1% sage has a superior effect on growth performance of koi carp fingerlings, compared to 1% vitamin E dietary supplementation.

Key words*: koi carp, sage, vitamin E, dietary supplementation, growth performance.*

INTRODUCTION

Plants and their extracts are known to possess many bioactive components such as tannin, alkaloids, and essential oils which have both antimicrobial and antioxidant activities. These bioactive components exert their beneficial effects by manipulating the intestinal microflora and improving digestibility (Asheg et al., 2014). Plant-derivatives, or the so-called "phytogenics" and plant extracts "phytobiotics" which are mainly obtained from aromatic plants and their essential oils, have been greatly utilized to enhance the growth performance of fish (Abdel-Latif et al., 2020).

Certainly, the use of immunostimulants as functional additives is acknowledged to improve the non-specific defence mechanism in fish, so giving resistance to infections (Cristea et al., 2012). Among the natural phytobiotic products with the potential of growth-promoting activity is sage (*Salvia officinalis*). The sage is one of the largest genera of the family *Lamiaceae*. It is widely distributed in the temperate, subtropical, and tropical regions all over the world (Sharifi-Rad et al., 2018).

The vitamin E plays a significant role in the health and growth of fish by improving their immune responses as well as their resistance to stress and disease (Naderi et al., 2017).

According to other authors (Cristea et al., 2012), various types of feed additives enhance the digestibility and/or utilization efficiency of nutrients, including exogenous enzymes, stimulators of enzyme secretion, compounds that aid in the digestive process by improving absorption, mobilization and transport of nutrients, feeding stimulants that reduce feed/nutrient waste, prebiotics, probiotics, and botanical extracts that modulate gut microflora.

The aim of the present study is to evaluate the impact of both dietary vitamin E and dietary sage supplementation on growth performance dynamics of koi carp fingerlings, reared in a partial recirculating aquaculture system.

MATERIALS AND METHODS

Experimental design

Two experimental diets were used: V1 - diet supplemented with 1% vitamin E (V1), respectively V2 - diet supplemented with 1%

sage. The feed proximate analysis revealed a value of 56% protein and 15% lipids.
The introduction of vitamin E and sage into fish feed was made by applying the following described protocol:
• a mixture of phytobiotic/vitamin E and gelatin (2% concentration) was made
• the mixture was sprayed uniform over the feed surface, while assuring a continuous shaking,
• the final mixture is dried at 25°C, for 2 hours and administrated to fish biomass as described in the feed administration protocol.
The experiment was conducted at the MoRAS Research Center - Food Science and Engineering Faculty, "Dunarea de Jos" University of Galati, during a period of 49 days. After acclimation, fish were stocked in a partial recirculating system with consists in a series of rectangular rearing units and water conditioning unit (aeration unit, mechanical and biological filtration). In order to maintain the water quality parameters within an optimal range for koi carp growth, a daily exchange rate of 30% was applied. Fish were divided into 2 treatments (with replicates) and fed with the experimental diets, by applying a 5% BW daily feeding rate. Feed was administrated manually, 4 times per day, at 9:00, 11:00, 13:00 and 15 h, during a 7 weeks experimental period.

Biological material and water quality
The koi carp (*Cyprinus carpio* L.) fingerlings (5.1 ± 0.4 g), obtained through artificial reproduction in the spring of 2020 year were acclimated to experimental rearing conditions for 2 weeks, during which fish were fed with commercial diet (56% crude protein).
The water dissolved oxygen (DO) concentration, pH and temperature (°C) were measured daily, by using a HQ40d Portable Multi-Parameter.

Growth performance and feed utilization parameters
A number of four intermediary biometric and biomass measurements were made in order to upgrade the quantity of daily administrated feed. The analysed technological indicators were as follows (Petrea et al., 2019):
Individual biomass gain: IBG = (Bf)-(Bi)/ fish number [g/fish], with Bf – final fish biomass; Bi – initial fish biomass (1);
Relative growth rate: RGR = ((Bf- Bi)/t)/Bi [g/g/day], with Bf - final fish biomass; Bi – initial fish biomass, t - duration of the experiment (2);
Specific growth rate: SGR = 100 x (ln Bf - ln Bi)/t [% fish biomass/day], with Bf - final fish biomass, Bi – initial fish biomass, t - duration of the experiment (3);
Feed conversion ratio: FCR = F/IBG [kg feed intake/kg fish biomass gain], with F - feed intake, FBG – individual biomass gain (4);
Protein efficiency ratio: PER = BG/(F*CP/100) [kg/kg], with FBG - individual biomass gain, F - feed intake, CP - crude protein (5).
Variation coefficient: $CV_{w/L}$ = (Dev. St./Avg w/L) x 100 [%], with Dev. St. - standard deviation, $Avg_{w/L}$ - fish body weight/length

Data analysis
For statistical analysis was used the IBM SPSS Statistics 20 for Windows and statistical differences between treatments were tested using T test ($\alpha = 0.05$) after a normality test (Kolmogorov-Smirnov). Comparisons between variants were assessed using post-hoc Duncan test for multiple comparisons (ANOVA).

RESULTS AND DISCUSSIONS

Water quality
The water parameters were within the optimal range for koi carp growth, as mentioned by Boyd and Tucker (2012), at both experimental variants, as follows: temperature ranged between 19-23.5°C, the DO ranged between 5.2-7.5 mg/L at V_1, respectively 4.7-7.4 mg/L at V_2, and pH ranged between 6.9-8.1 upH at V_1, respectively 6.7 - 7.9 upH at V_2.
The pH and DO variation for V_1 and V_2, respectively, are presented in Figure 1 and Figure 2. Not significant differences ($p>0.05$) were recorded between both experimental variants in terms of DO, pH and temperature. However, the vitamin E diet experimental variant (V1) had registered better values related to technological water quality, compared to sage diet experimental variant (V2).
It is observed that the dissolved oxygen concentration and pH had a decrease in the last part of the experiment period, which indicates the limitations of the production system in terms of maintaining water quality in terms of increasing the amount of food administered.

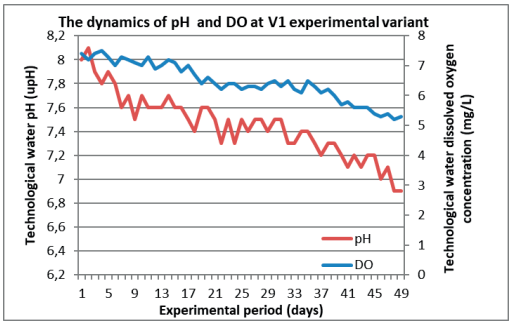

Figure 1. The dynamics of pH and DO for V_1

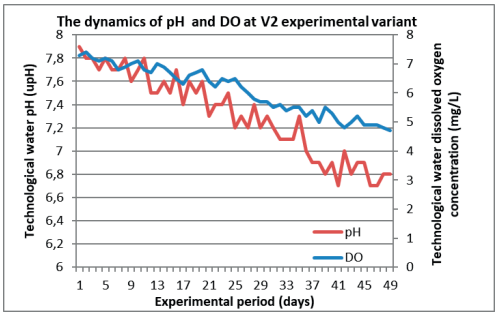

Figure 2. The dynamics of pH and DO at V_2 experimental variant

The effects of experimental diets on the growth performance indices and feed utilization parameters are presented in Table 1.

There were no significant differences between the fish specimens in terms of initial body weight (IBW), at the start of the feeding trial ($p>0.05$). The growth performance indicators (Table 1) reveled no mortalities during V_2 and 10% in varianta V_1, therefore confirming the good results registered in terms of water quality. The average specific growth rate indicates a superior fish production at V_2 (3.30% BW/day), compared to V_1 (2.75% BW/day) experimental variant (Table 1).

However, from the perspective of feeding strategy efficiency, the average food conversion ratio (FCR) indicates better values for V_2 experimental variant (1.28 g feed/g biomass gain), compared to V_1 (1.62 g feed/g biomass gain) (Table 1). The average protein efficiency ratio (PER) registered higher values at V_2 experimental variant (1.39), compared to V_1 (1.10), revealing the ability of fish organism to utilize proteins, which positively affects growth rate (Table 1).

Table 1. Growth performance indicators for each of the experimental variants

Nr. crt.	Indicator	Period	V_1 (vit. E)	V_2 (Sage)
1.	Experimental period (days)	Initial - INT.1	8	8
		INT.1-INT.2	7	7
		INT.2 - INT.3	10	10
		INT.3 - INT.4	12	12
		INT.4 - Final	12	12
		Initial - Final	49	49
2.	Survival (%)	Initial - Final	90	100
3.	Individual average biomass (g/fish)	INITIAL	5.1	5.2
		INT.1	6.7	6.9
		INT.2	8.2	8.8
		INT.3	10.7	12.3
		INT.4	14.3	17.6
		FINAL	19.6	26.1
4.	Individual average length (cm/fish)	INITIAL	7.0	7.2
		INT.1	7.6	8.1
		INT.2	8.3	8.9
		INT.3	9.4	10.3
		INT.4	10.1	11.1
		FINAL	11.6	13.6
5.	Individual biomass gain (g/ex)	Initial - INT.1	1.6	1.7
		INT.1-INT.2	1.6	1.9
		INT.2 - INT.3	2.4	3.5
		INT.3 - INT.4	3.6	5.3
		INT.4 - Final	5.3	8.4
		Initial - Final	14.5	20.9
6.	Relative growth rate - RGR (g/g/day)	Initial - INT.1	0.039	0.042
		INT.1-INT.2	0.034	0.04
		INT.2 - INT.3	0.030	0.039
		INT.3 - INT.4	0.029	0.036
		INT.4 - Final	0.031	0.04
		Initial - Final	0.015	0.016

Nr. crt.	Indicator	Period	V_1 (vit. E)	V_2 (Sage)
7.	Specific growth rate (%BW/day)	Initial - INT.1	3.36	3.63
		INT.1-INT.2	3.03	3.52
		INT.2 - INT.3	2.59	3.31
		INT.3 - INT.4	2.45	3.01
		INT.4 - Final	2.62	3.26
		Initial - Final	2.75	3.30
8.	Feed conversion ratio - FCR (g feed / g biomass gain)	Initial - INT.1	1.29	1.19
		INT.1-INT.2	1.48	1.25
		INT.2 - INT.3	1.69	1.28
		INT.3 - INT.4	1.75	1.38
		INT.4 - Final	1.62	1.26
		Initial - Final	1.62	1.28
9.	Protein efficiency ratio - PER (g/g)	Initial - INT.1	1.38	1.50
		INT.1-INT.2	1.20	1.43
		INT.2 - INT.3	1.06	1.40
		INT.3 - INT.4	1.02	1.30
		INT.4 - Final	1.10	1.42
		Initial - Final	1.10	1.39
10.	Feed protein (%)	Initial - Final	56	56
11.	Daily feeding ratio (% BW)	Initial - Final	5	5
12.	Weight variation coefficient - CVw (%)	FINAL	6.52	7.25
13.	Length variation coefficient - CVL (%)	FINAL	2.89	3.42

By analyzing the variation coefficients, it can be stated that both experimental variants had registered a high homogeneity degree in the first part of the experimental period (Figures 3 and 4).

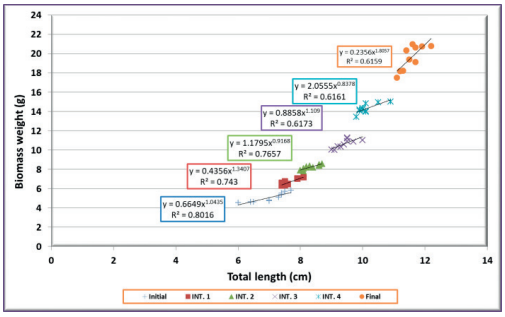

Figure 3. Total Length-Weight relation for V_1 biomass, during the experimental period

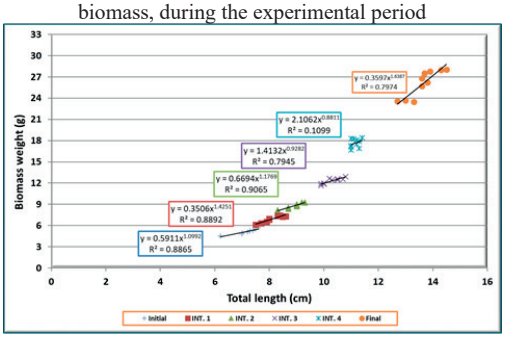

Figure 4. Total Length-Weight relation for V_2 biomass, during the experimental period

However, the V_1 experimental variant revealed a significant decrease on the homogeneity degree after the first 15 experimental days, compared to V_2 experimental variant, which continued to register high homogeneity until the last part of the experimental period (first 37 days) (Figures 2 and 3).

The condition status of biological material was evaluated by using the allometric condition factor F ($F = W/L^b$, where b = allometric exponent, experimentally determined).

The allometric exponent "b" has its values under three units at both variants, during the entire experimental stage (Figures 2 and 3), fact which reveals a faster growth in length rather than weight.

Also, the K condition factor registered lower average values at the end of the trial at V_1 (K = 0.236), compared to V_2 (K = 0.315).

Nowadays, there is an increasing interest in the usage of dietary phytobiotics as natural growth promotors and immunostimulants in practical fish diets to improve growth, health status, immune responses, and protection against bacterial diseases (Abdel-Latif et al., 2020).

Furthermore, phytoadditives appear to be reasonable alternative solutions to substitute synthetic antimicrobials used in aquaculture without any undesired effects upon recent related studies (Daood, 2011).

The present study confirms the beneficial effects of sage use, as observed by Salomon et al. (2020) who evaluated the growth response in juvenile gilthead sea bream (*Sparus aurata*) fed with a functional diet containing a medicinal plant leaf extract from sage (*Salvia*

officinalis) and lemon verbena (*Lippa citriodona*) and subliniated the beneficial effects of the dietary administration.
According to other authors (Cristea et al., 2012), sustainable aquaculture depends on perfectly balance between health and growth condition of fish. Thus, according to other study (Abd-El-Rhman, 2009), certain phytobiotics as propolis-ethanolic-extract were found to promotes the growth of intensive aquaculture fish species, as Nile tilapia. Therefore, the used of phytobiotics and vitamins promotes growth and therefore, maximize productivity and income, especially in intensive and semi-intensive production fish farms. However, a proper period for the administration must be identified and suitable phytobiotics should be selected since, some authors (Kono et al., 2000) reported experienced a reduction growth performance when green and ground tea extracts were added in fish diet.

CONCLUSIONS

The partial recirculating aquaculture system used in this present study managed to maintain water quality within the optimal range, even if a 5% BW feeding ratio was applied. However, if feeding ratio will increase, the water conditioning units must be upgraded, or water exchange rate must increase. The results presented in current research revealed that the supplementation with 1% sage has a superior effect on growth performance of koi carp fingerlings, compared to 1% vitamin E dietary supplementation. Also, the use of sage in fish diet can help define the concept of circular economy given that sage biomass could be obtained in an integrated aquaponic production system that uses nutrients provided by aquaculture effluents as main source for plant biomass growth.
However, further research is required for better understanding the mechanisms of improving fish health and productivity by dietary sage administration. Also, the optimal period for the administration of sage into koi carp diet must be identified in order to integrate this information into a long-time rearing technology.

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COMPARATIVE MORPHOMETRIC ANALYSIS OF RUSSIAN STURGEON (*ACIPENSER GUELDENSTAEDTII*) MALE AND FEMALE INDIVIDUALS AT THE AGE OF SEVEN YEARS

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Abstract

Seven-year-old male (n = 25) and female (n = 25) Russian sturgeons (Acipenser gueldenstaedtii), grown under the same conditions on a super-intensive cage farm, 36 plastic and 7 meristic traits are studied and 7 morphometric indices are calculated. Female fish had significantly (P <0.001) better fattening than males, their body was more compact (P <0.001) and thicker (P <0.01). In females, the ratio of antiventral distance, back thickness and body girth to the total body length is higher. In male fish, the ratios of the head lengths, the caudal stalk, the ventral fin, the anal fin height and the ventro-anal distance to the total body length are relatively larger. In female fish, the height and length of the space behind the eye are greater, they have a wider mouth, a larger eye and a wider snout. In males, the long-headed index is higher (P <0.05), the snout and the distance from its end to the mouth are longer, the lower lip interruption is larger. Female fish have significantly fewer ventral bone scutes the left (P <0.05) and the right (P <0.01) sides of the body.

Key words*: aquaculture, morphometric features, sturgeon.*

INTRODUCTION

The situation with natural Sturgeon populations raises serious concerns (Sandu et al., 2013, etc.). Vasileva (2015) emphasizes that a complex approach is needed in the conditions of constant reduction of natural Sturgeon stocks, including increasing the scale of natural and artificial reproduction, formation of productive herds in controlled conditions and Sturgeon aquaculture development. A number of authors note the importance of creating *in vivo, ex sit*u genetic collections of Sturgeon (Morev, 1999; Friedrich, 2018, etc.). Existing sturgeon *ex-situ* live gene banks are used for Sturgeon rehabilitation programs (Halasi-Kovacs, 2019).

Initially, Sturgeon conservation programs included mainly artificial reproduction of special sturgeon hatcheries. At the same time, the work of the latter is increasingly difficult due to the lack of necessary sexually mature individuals from natural populations (Ruban et al., 2015; Salmanov et al., 2016). Sturgeon aquaculture plays an important role in reducing the anthropogenic pressure on natural populations, as a subsector providing desirable delicacies for humans, as well as an important element of endangered species rescue programs (Nikolova, 2019; Chandra and Fopp-Bayat, 2021).

The goals of commercial Sturgeon aquaculture and activities related only to *ex situ* concervation differ in nature (Reinartz et al., 2016), but available Sturgeon species aquaculture herds can also be a source of valuable genotypes for restocking programs.

Sturgeon aquaculture is developing at a good rate, with developed technologies for breeding, rearing, feeding, etc. At the same time, appropriate zootechnical approaches for breeding are still to be introduced everywhere in Sturgeon breeding. It is necessary to establish the phenotypic values of biological characteristics of populations and herds at the beginning of each selection activity. Different methods are used to characterize fish, one of the most widely used being morphometric analysis (Pavlov, 2012; Svirsky and Skirin, 2005, etc.). Morphometric parameters and morphophysiological indices have been studied in different species, breeds and populations (Morev, 1999; Khosrow and Amirkolaie, 2010; Treer et al., 2000, etc.); in fish of different sexes and from separate ecosystems (Coban et al., 2011; Jawad et al., 2017; Khristenko & Kotovska, 2017; Vélez-Arellano et al., 2017).

Russian sturgeon is one of the most important species for Sturgeon aquaculture in a number of countries around the world (Kim et al., 2019; Nikolova, 2019; Sergeev, 2020). When farmed on industrial farms, the fish are under unusual conditions. In this regard, the regularities of development in the conditions of real specific industrial technologies are of interest (Nikolova et al., 2018).

We set ourselves a goal to make a comparative morphometric characteristic of Russian sturgeon of different sexes on the same age grown on an industrial cage farm located in South-Eastern Bulgaria.

MATERIALS AND METHODS

The study was carried out with Russian sturgeon (*Acipenser gueldenstaedtii*) male and female individuals at the age of seven years from a net-cage farm, located in a warm water reservoir. According to its type, the reservoir refers to large and deep ones. Its area is 16.07 km^2, the volume is 532.9 x 10^6 m^3. The reservoir is located in South-East Bulgaria, at 41°37' N latitude and 25°20' E longitude. It falls into the South Bulgarian climate zone, East Rhodope climate region. The average altitude is about 280 m. Fish of different age groups were grown in separate net-cages. The cages were 8 × 8 m in size, the water depth being 6 m. Each cage had a double polyamide net.

Feeding was done with commercial granulated sturgeon feed (Table 1). Twenty five Russian sturgeon fish of different sexes were randomly selected for morphometric analyzes at the end of the vegetation period (in November). The mean body weight of females was 5000.1 ± 140 g and that of males -4000.5 ± 100 g.

Classical methods developed for the study of alive hydrobionts were applied for the study of sturgeon species (Pravdin, 1966; Krilova & Sokolov, 1981; Morev, 1999; Svirski & Skirin, 2005, etc.).

The studied indicators and codes for their designation are presented in Table 2.

A measurement scheme proposed by Krilova & Sokolov (1981) specifically for sturgeon and their hybrids was used (Figure 1).

Table 1. Composition of the commercial feed

Indices	Value	Indices	Value
Protein, %	46	Vitamin A, IU.kg^{-1}	10 000
Fat, %	15	Vitamin C, mg.kg^{-1}	520
Crude fibre, %	1.4	Vitamin E, mg.kg^{-1}	200
Ash, %	6.5	Vitamin D3, IU.kg^{-1}	2 303
Total P, %	1.03	Gross energy, MJ.kg^{-1}	21.0
Ca, %	1.4	Digestible energy, MJ.kg^{-1}	19.2
Na, %	0.3%		

Table 2. Metric and meristic features used in the study

Features	Code
Total body weight, g	BW
Metric body features	
Total length, cm	TL
Fork length, cm	FL
Standart length, cm	SL
Antidorsal distance, cm	AD
Antiventral distance, cm	AV
Antianal distance, cm	AA
Maximum body width, cm	SC
Maximum body height, cm	H
Minimum body height, cm	H1
Tail stalk length - from the end of the anal fin to the roots of the middle rays of the caudal fin, cm	PL1
Tail stalk length - from the end of anal fin to the end of the middle rays of the caudal fin, cm	PL2
Dorsal fin length, cm	LD
Dorsal fin height, cm	HD
Anal fin length, cm	LA
Anal fin height, cm	HA
Pectoral fin length, cm	LP
Abdominal fin length, cm	LV
Pecto-ventral distance, cm	PV
Ventro-anal distance, cm	VA
Maximum body girth, cm	CC
Metric head features	
Head lenght, cm	C
Snout length, cm	R
Maximum head height (before the 1st dorsal bony scute), cm	HC
Minimum head height (above the eye), cm	HCO
Behind eye area length, cm	CP
Horizontal eye diameter, cm	O
Inter orbital distance, cm	IO
Maximum head width, cm	BC
Distance from the beginning of the snout to a line passing through the middle of the front barbels` roots, cm	RC
Distance from the end of the snout to the mouth cartilaginous arch, cm	RR
Distance from the middle barbels` roots to the mouth cartilaginous arch, cm	RL
Longest / lateral / barbel`s length, cm	LC
Snout width at the middle barbels` roots, cm	SRC
Snout width at the mouth cartilaginous arch, cm	SRR
Mouth width, cm	SO
Lower lip`s break width, cm	IL
Meristic features	
Number of dorsal bony scutes	SD
Number of lateral bony scutes from the left side of the fish	SL1
Number of lateral bony scutes from the right side of the fish	SL2
Number of ventral bony scutes from the left side of the fish	SV1
Number of ventral bony scutes from the right side of the fish	SV2
Number of rays in the dorsal fin	D
Number of rays in the anal fin	A

Measurements of individual body parts are made with a caliper with an accuracy of 0.1 mm, a strip measure with an accuracy of 1 mm (for body girth measurements) and a graduated ichthyological board with an accuracy of 1 mm for measuring lengths, thicknesses and body heights.

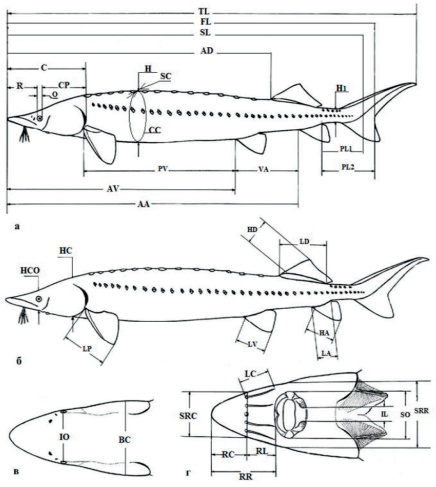

Figure 1. Sturgeon fish measurements scheme (Krylova and Sokolov, 1981, Svirski and Skirin 2005)

Morphometric indices were calculated on the basis of morphometric measurements (Table 3). For statistical data processing IBM SPSS Statistics 21 was used.

Table 3. Morphometric indices

Indices	
CFF	Fulton's coefficient [(BW/SL^3)*100], %
IC	Condition index [BW/(SL*H*CC) *100], %
ICR	Modified Fulton's coefficient by Jones et al., 1999 (acording Richter et al., 2000) [BW/(SL^{2}H)*100]
IHB	High-backed index (SL/H)
IBB	Broad-backed index [(SC/SL)*100], %
ILH	Long-headed index [(C/SL)*100], %
IH	Hardness index [(CC/SL)*100], %

RESULTS AND DISCUSSIONS

Metric features of the body in seven-year-old Russian sturgeons of different sexes are shown in Table 4.

Total length (TL) at the age of seven years ranged from 93 to 106 cm in females, and 90-107 cm in males. In the natural area of Russian sturgeon (Caspian Sea) in six-summer-old fish the total length (TL) varies in the range of 70-80 cm, and with age there is a big difference in linear parameters between the fish who live in the sea (which are smaller), and migrated to freshwater (males with TL - 124 - 126, and females - 141-146 cm) (Gritsenko et al., 2006). In another study of Russian sturgeon from the Caspian region, the fish had a TL of 1220.53 ± 14.31 mm (Birstein et al., 2005).

Table 4. Metric features of Russian sturgeon body at the age of seven years, cm

Features	Sex	X	Min.	Max.	± Sx	CV
TL	F	99.20	93.00	106.00	0.71	3.26
	M	97.50	90.00	107.00	0.84	3.96
FL	F	86.20	81.30	97.40	0.78	4.15
	M	84.80	79.00	93.00	0.68	3.65
SL	F	80.70	77.20	85.40	0.53	3.03
	M	77.00	74.00	87.10	0.65	3.74
AD	F	62.10	58.80	66.20	0.48	3.56
	M	60.30	56.10	66.1	0.53	4.00
AV	F	53.90	50.50	57.30	0.46	3.94
	M	50.90	46.6	55.00	0.42	3.78
AA	F	67.20	63.60	71.00	0.52	3.52
	M	65.30	59.10	71.00	0.55	3.86
SC	F	9.85	8.50	10.70	0.12	5.35
	M	9.20	8.20	10.30	0.12	5.74
H	F	12.30	11.00	13.60	0.14	5.06
	M	12.00	10.7	13.70	0.15	5.92
H1	F	3.59	3.25	4.22	0.06	7.07
	M	3.56	3.10	3.94	0.04	5.35
PL1	F	8.39	6.90	9.45	0.14	7.64
	M	9.66	8.25	11.80	0.18	8.40
PL2	F	13.7	11.80	14.70	0.17	5.66
	M	15.1	12.70	17.20	0.23	6.86
LD	F	10.00	8.75	10.80	0.12	5.69
	M	10.10	8.65	12.1	0.22	9.82
HD	F	9.18	7.20	11.10	0.22	10.80
	M	9.36	6.80	12.10	0.30	14.80
LA	F	5.54	4.05	6.90	0.15	12.10
	M	5.70	4.20	11.1	0.29	23.60
HA	F	9.03	7.10	10.50	0.21	10.6
	M	10.00	8.17	11.40	0.21	9.58
LP	F	11.70	10.40	13.10	0.16	6.44
	M	11.60	8.45	14.50	0.37	14.6
LV	F	7.67	7.10	9.10	0.17	10.20
	M	8.34	6.12	10.10	0.27	15.0
PV	F	36.50	32.60	40.00	0.42	5.30
	M	33.30	30.40	39.50	0.47	6.54
VA	F	13.90	11.10	15.80	0.25	8.37
	M	15.00	11.50	20.50	0.43	13.10
CC	F	37.30	34.10	40.00	0.42	5.12
	M	33.80	30.20	37.00	0.37	5.02

In Podushka's (1988) study of Russian sturgeon from Don river TL varied from 108 to 210 cm, with an average length of 161.3 ± 1.22 cm in female fish and 130.9 ± 1.11 cm in males.

A study of morphological variability allows to assess the rate of reaction in a particular species, its adaptive capacity (Romanov and Skirin, 2011). This is especially important for farmed fish.

The measured body lengths in both sexes have a slight variation (<10%) (Table 4). The same goes for the minimum and maximum heights;

body width and girth. Higher levels of variation were found with respect to fin sizes. The variation ranged from low (in the length of the dorsal and pectoral fins) to medium (<25%), with the highest values found in the length of the male Russian sturgeons anal fin. The variation in ventro-anal distance of male fish, is relatively higher (over 1.5 times).

Metric features of the fish head of both sexes are shown in Table 5. The metric features of the head are an important part of the morphometric analysis in sturgeons. Salmanov et al. (2016) note that in sturgeons, the indicators characterizing the size of head parts should be considered as species-specific differences. It is important for each study to be performed with fish at known age. Svirski and Skirin (2005) point out that the metric features of the head can change significantly in the process of ontogenesis, and it is these features that show allometry with an increase in the size and age of the fish. Svirsky (1968) and Ruban (1999) in a study of Amur and Siberian sturgeons found that head indices differed significantly in young and mature individuals.

Table 5. Metric features of Russian sturgeon head at the age of seven years, cm

Features	Sex	X	Min.	Max.	± Sx	CV
C	F	16.50	15.60	17.80	0.12	3.27
	M	16.80	15.50	17.90	0.16	4.25
R	F	5.25	4.70	6.25	0.09	7.71
	M	6.40	4.71	7.80	0.15	10.9
HC	F	8.78	7.80	9.80	0.15	8.03
	M	8.56	7.50	13.30	0.25	13.6
HCO	F	5.68	5.23	6.70	0.07	5.86
	M	5.35	4.75	5.85	0.06	5.47
CP	F	10.30	9.70	11.10	0.10	4.53
	M	9.50	8.80	10.30	0.09	4.22
O	F	1.60	1.20	2.10	0.05	15.60
	M	5.35	1.20	2.38	0.06	5.47
IO	F	6.07	5.71	6.45	0.04	3.07
	M	6.26	5.80	6.82	0.06	4.35
BC	F	7.75	7.22	8.30	0.06	3.64
	M	7.89	7.35	8.53	0.07	3.97
RC	F	1.52	1.20	1.90	0.04	12.60
	M	2.62	1.46	3.58	0.10	17.60
RR	F	5.51	4.50	6.30	0.09	7.49
	M	6.87	4.97	7.95	0.16	11.0
RL	F	3.90	3.20	4.80	0.09	10.70
	M	4.50	3.70	5.20	0.09	9.67
LC	F	2.83	1.95	3.20	0.07	11.20
	M	2.99	1.70	3.88	0.11	17.6
SRC	F	4.75	4.10	5.77	0.09	8.59
	M	4.87	4.45	5.85	0.07	6.76
SRR	F	8.06	7.70	8.80	0.07	4.24
	M	7.78	7.05	8.58	0.08	4.58
SO	F	5.95	5.50	6.70	0.06	4.94
	M	5.21	4.82	5.90	0.07	5.88
IL	F	1.46	0.80	2.15	0.07	21.30
	M	1.61	1.30	2.10	0.05	14.60

We have not found significant variation of both sexes in metric features of the Russian sturgeon head. The variation in eye diameter of female fish is at medium levels (15.60%).

Table 6. Individual measurements to the absolute length ratio of seven year old Russian sturgeon body, %

Features	Sex	X	Min	Max	±Sx	CV
FL/TL	F	86.88	83.05	96.44	0.57	3.00
	M	87.02	84.42	96.13	0.50	2.62
SL/TL	F	81.32	78.48	84.84	0.32	1.83
	M	82.20	79.47	89.28	0.44	2.44
AD/TL	F	62.54	59.05	65.68	0.37	2.72
	M	61.96	58.82	70.17	0.51	3.75
AV/TL	F	54.28***	51.46	58.21	0.39	3.33
	M	52.23***	49.95	58.01	0.42	3.67
AA/TL	F	67.69	64.00	72.21	0.39	2.62
	M	67.06	64.51	73.81	0.43	2.97
SC/TL	F	9.92**	9.14	10.84	0.10	4.72
	M	9.46**	8.45	10.50	0.14	6.72
H/TL	F	12.36	11.46	13.89	0.12	4.53
	M	12.28	11.45	15.11	0.19	7.04
H1/TL	F	3.62	3.17	4.18	0.05	6.27
	M	3.66	3.13	4.14	0.05	6.09
C/TL	F	16.61**	15.80	18.05	0.12	3.25
	M	17.22**	15.66	19.34	0.18	4.83
PL1/TL	F	8.45***	7.42	9.36	0.12	6.34
	M	9.92***	8.67	11.86	0.18	8.40
PL2/TL	F	13.78***	12.65	15.22	0.15	4.96
	M	15.50***	12.83	17.24	0.21	6.08
LD/TL	F	10.12	9.26	10.95	0.11	4.90
	M	10.40	9.11	11.75	0.18	8.12
HD/TL	F	9.24	7.14	10.57	0.19	9.46
	M	9.60	6.98	12.11	0.29	13.75
LA/TL	F	5.59	4.01	7.09	0.16	12.75
	M	5.88	4.24	12.33	0.34	26.81
HA/TL	F	9.10***	7.29	10.38	0.20	9.83
	M	10.30***	8.51	11.93	0.20	8.82
LP/TL	F	11.83	10.62	13.05	0.17	6.63
	M	11.94	8.58	15.00	0.39	15.04
LV/TL	F	7.73*	6.48	8.88	0.15	9.16
	M	8.56*	6.30	10.36	0.29	15.31
PV/TL	F	36.75***	33.57	40.48	0.42	5.23
	M	34.15***	31.92	40.10	0.45	6.10
VA/TL	F	13.98**	11.67	15.54	0.23	7.60
	M	15.40**	12.57	21.13	0.41	12.19
CC/TL	F	37.60***	35.30	41.01	0.32	3.85
	M	34.75***	31.44	39.23	0.41	5.40

Differences between the values within the feature are significant: ****P<0.001, **P<0.01, *P<0.05*

In male fish, the variation in this indicator is almost three times less. In terms of barbel length, the variation is greater in male fish. The highest value of the coefficient of variation is in the indicator width of the lower lip break in female fish (21.3%), but its value is also within the average level of variation

The comparative analysis of the individual parameters ratio to the total body length (Table 6) shows that there are significant differences between Russian sturgeons of different sexes.

The ratios - AV/TL ($P<0.001$); SC/TL ($P<0.01$); PV/TL ($P<0.001$) and CC/TL ($P<0.001$) are higher in female fish; and C/TL

(P<0.01); PL1/TL (P<0.001); PL2/TL (P<0.001); HA/TL (P<0.001); LV/TL (P<0.05); VA/TL (P<0.001) are smaller.

Significant differences were found between individual sexes and in the relative values of the individual features of the head to its length (Table 7). The values in female individuals are higher at the HCO/C ratios (P<0.001); CP/C (P<0.001); O/C (P<0.05); SRR/C (P<0.001); SO/C (P<0.001); and smaller respectively at R/C (P<0.001); RC/C (P<0.001); RR/C (P<0.001) (Figure 3). The ratio of the lower lip break to the mouth width is less (P<0.001) in female Russian sturgeons.

The findings of Birstein et al. (2005) C/TL, H/TL, AD/TL, AV/TL, AA/TL, LP/TL PV/TL are higher than in our study, while the dorsal and anal fin height and length are smaller.

Table 7. Head metric features to head length ratio of seven years old Russian sturgeon, %

Features	Sex	X	Min	Max	±Sx	CV
% of the head length						
R/C	F	31.84***	28.91	37.88	0.48	6.91
	M	38.08***	29.81	42.90	0.67	8.04
HC/C	F	53.25	48.48	61.30	0.80	6.88
	M	51.20	44.13	82.35	1.71	15.29
HCO/C	F	34.51***	31.89	41.36	0.47	6.27
	M	31.96***	28.70	37.74	0.42	6.01
CP/C	F	62.73***	59.15	67.92	0.46	3.36
	M	56.74***	51.40	63.83	0.71	5.75
O/C	F	9.67*	7.41	12.80	0.31	14.72
	M	8.77*	6.73	14.69	0.35	18.47
IO/C	F	36.84	34.03	38.51	0.24	3.01
	M	37.36	35.48	39.42	0.24	2.93
BC/C	F	47.03	44.90	49.11	0.29	2.81
	M	47.08	45.41	49.12	0.27	2.64
RC/C	F	9.25***	7.46	11.31	0.24	11.97
	M	15.57***	9.01	19.69	0.54	15.89
RR/C	F	33.42***	28.85	36.19	0.50	6.85
	M	40.89***	30.68	44.41	0.78	8.72
RL/C	F	23.68***	20.37	28.48	0.51	9.95
	M	26.81***	23.33	33.21	0.51	8.80
LC/C	F	17.21	12.34	19.87	0.43	11.52
	M	17.78	10.97	22.17	0.61	15.71
SRC/C	F	28.83	24.40	34.35	0.52	8.32
	M	29.05	26.02	34.21	0.43	6.84
SRR/C	F	48.92***	45.56	53.92	0.40	3.75
	M	46.43***	43.52	49.60	0.36	3.58
SO/C	F	36.13***	32.07	39.88	0.37	4.66
	M	31.09***	27.94	34.69	0.39	5.82
IL/C	F	8.84	4.90	12.49	0.39	20.36
	M	9.63	6.88	12.96	0.33	15.68
% of the mouth width						
IL/SO	F	24.48***	13.01	34.40	1.06	19.79
	M	30.94***	21.67	37.37	0.89	13.23

Differences between the values within the feature are significant: ***P<0.001, **P<0.01, *P<0.05*

Salmanov et al. (2016) found similar to our results in seven-year-old Persian sturgeon body propotions of indeterminate sex - AA - 67.4 ± 0.31; AV - 52.4 ± 0.25; AD - 61.2 ± 0.31% of TL. In the same study, the found head proportions were similar to those obtained for female fish in terms of R/C, CP/C, SO/C, and averaged between the two sexes in terms of RC/C and RR/C.

External profiles are used to assess the physique of animals, and in fish they are built on the basis of the ratio of each feature and the total body length. Exterior profiles are used in the characterization of individual Sturgeon species and hybrids (Morev, 1999, etc.). The author notes the importance of studying the phenotypic variability of morphological features in fish, along with biochemical genetic studies.

Graphically presented profiles of body, head and indices of Russian sturgeon of both sexes in our study are presented in Figure 2.

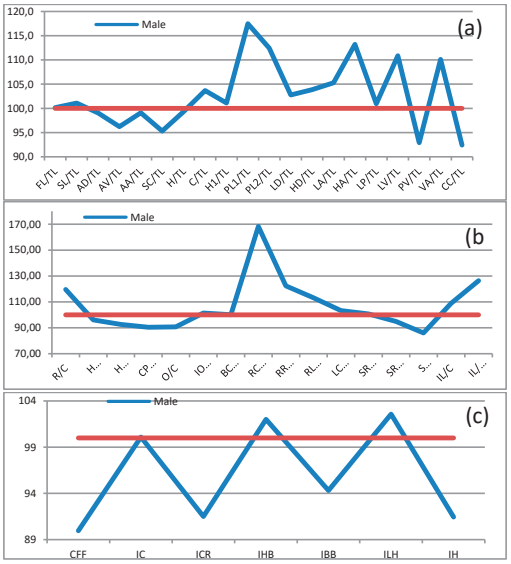

Fig. 2. Exterior profiles of body (a), head (b) and indices (c) in Russian sturgeon of different sexes

We calculated 7 exterior indices based on the measurements (Table 8).

Different coefficients of fatness are used in different scientific studies related to the exterior of fish, which is why we calculated several main indices used in fish farming - CFF, IC and ICR (Table 8). This gives a good opportunity to compare the results obtained by us with those obtained by other authors.

The Fulton's coefficient (CFF) is a classic method for determining the condition of fish by

body weight and standard body length. The coefficient is studied not only in different species, but also in different fish breeds. Thus, Cekov (1985) and Nikolova (2015), in relation to CFF, have found significant breed differences in carp. CFF remains an important index in fish research, despite its shortcomings. Kolisnyk et al. (2014) indicate that CFF shows the ability of fish to absorb available food. McPherson et al. (2011) found that there is no relationship between CFF and mesenteric fat, but there is between the level of fatty acids in muscle.

Table 8. Morphometric indices in a seven-year-old Russian sturgeon

Indices	Sex	X	Min	Max	±Sx	CV
CFF	F	0.97***	0.86	1.11	0.01	6.49
	M	0.87***	0.73	1.05	0.02	10.3
IC	F	13.8	12.80	16.20	0.16	5.38
	M	13.9	11.60	15.40	0.21	7.01
ICR	F	6.40***	5.75	7.28	0.08	5.90
	M	5.85***	5.09	6.87	0.10	8.21
IHB	F	6.59	6.11	7.08	0.06	4.29
	M	6.72	5.44	7.15	0.10	6.49
IBB	F	12.20**	11.00	13.20	0.12	4.44
	M	11.50**	10.10	12.60	0.17	6.68
ILH	F	20.40*	19.10	21.80	0.15	3.33
	M	21.00*	18.60	22.30	0.22	4.77
IH	F	46.20***	43.10	50.10	0.39	3.83
	M	42.3***	37.60	45.40	0.50	5.46

Differences between the values within the feature are significant: ****P<0.001, **P<0.01, *P<0.05*

A number of coefficients have been developed in order to improve the accuracy of calculating the fattening of live fish, which includes more external indicators. Thus, in ICR, body height is taken into account, and in IC - body height and girth.

The data in Table. 8. show similar trends in CFF and ICR comparisons in both male and female Russian sturgeons. These indices demonstrate that female Russian sturgeons have significantly (P<0.001) better fattening than males of the same age under the same breeding conditions (Figure 2).

No significant differences were found between the sexes regarding the IC and the high back index, and the difference in body thickness (P<0.01) was in favor of female fish. The long-headed index was higher (P<0.05) in male fish and the hardness index in female fish (P<0.001).

Meristic signs in Russian sturgeon of different sex are presented in Table 9. The number of dorsal, lateral and ventral bony scutes in Sturgeons is an important taxonomic feature (Podushka, 1988), as the author cites data from Ruban and Sokolov, and Stroganov, that the number of bony scutes can be significantly influenced by environmental conditions, especially the temperature. Romanov and Skirin (2011) found that there is a high level of morphological variability in the number of bony scutes in some Sturgeon species and their hybrids, with a particularly large amplitude of variation in lateral bony scutes.

The study by Romanov and Skirin (2011) shows that in hybrids involving Russian sturgeon, the number of dorsal scutes was 13.46 - 13.86 at a limit of 11-16; the number of the lateral scutes - 40.46-42.06 at limit values 33-49 (CV 6.80-8.38%); the number of ventral scutes - 9.31-9.40 at limit values 7-12 (CV 7.84-8.83%).

Sergeev (2020) points out that there are no big differences in the number of abdominal scutes in a comparative analysis between Russian and Persian sturgeon, there are small differences in the number of dorsal, and clear differences in the number of lateral scutes. In this study, the number of dorsal scutes in Russian sturgeon was 12.5, ranging from 10 to 17; the laterals - 35.03 (from 26 to 48); the ventral ones - 9.61 (from 7 to 12). In Podushka's (1988) study of Russian sturgeon from the Don River, the mean SD number in female fish was 11.57 ± 0.11 (8-15); SL1 31.18 ± 0.31 (20-43); SL2 31.17 ± 0.27 (24-38); SV1 9.76 ± 0.10 (7-12); SV2 9.68 ± 0.09 (7-13), and in males, respectively - SD 11.56 ± 0.12 (9-15); SL1 30.18 ± 0.27 (24-39); SL2 30.31 ± 0.24 (23-38); SV1 9.61 ± 0.10 (8-12); SV2 9.60 ± 0.10 (8-13).

In our study, the number of dorsal scutes in females was from 9 to 13, and in males from 8 to 13, and no significant difference in the indicator was found in fish of different sexes. The coefficient of variation is medium (CV 10.3-10.6%), slightly exceeding the maximum level for a low degree of variation.

The number of lateral bony scutes on the left side of the fish varies from 24 to 30 in females and from 25 to 32 in males. There is a low degree of variation in the indicator (CV 7.6-8.23). A difference was found between the number of lateral scutes on the left and on the right side. Their number in female fish varies from 22 to 31 (CV 7.66%) on the right, and in

males from 25 to 34 (CV 9.08%). Female fish have significantly fewer ventral bony scutes on both the left (P<0.05) and right (P<0.01) sides. The maximum number of ventral scutes in male and female fish on both sides of the body is the same - 11. The minimum number of ventral scutes on the left side in female fish is 7, and in males 8. Eight is the minimum number of scutes on the right side in fish of both sexes. The highest coefficient of variation on the feature was obtained for the left side in female fish (CV 12.30%).

Table 9. Meristic features of seven-year-old Russian sturgeon

Features	Sex	X	Min	Max	±Sx	CV
SD	F	11.30	9	13	0.25	10.30
	M	11.20	8	13	0.26	10.60
SL1	F	27.5	24	30	0.46	7.66
	M	28.10	25	32	0.51	8.23
SL2	F	27.10	22	31	0.46	7.66
	M	28.23	25	34	0.56	9.08
SV1	F	9.14*	7	11	0.25	12.30
	M	9.73*	8	11	0.18	8.50
SV2	F	8.86**	8	11	0.19	10.00
	M	9.68**	8	11	0.20	9.23
D	F	32.20	21	39	0.90	12.80
	M	34.50	23	45	1.12	15.00
A	F	19.90	16	25	0.44	10.10
	M	19.60	16	23	0.44	10.40

Differences between the values within the feature are significant: ***P<0.01, *P<0.05*

Between the sexes of Russian sturgeon no significant differences were found in the amount of rays in the dorsal and anal fins. The amount of rays in the dorsal fin varies from 21 to 39 in female fish, and in the anal - from 16 to 25. In males, respectively, from 23 to 45 and from 16 to 23. The highest variation, within the average, in the number of the rays in the studied fins were found to be dorsal fin in male individuals.

CONCLUSIONS

Comparative characteristics of female and male Russian sturgeon at the age of seven showed that there are differences in morphometric characteristics between the two sexes. The female Russian sturgeon has significantly better fattening (P<0.001); their body is more compact (P<0.001) and thicker (P<0.01). They have a higher ratio of anti-ventral distance, back thickness and body girth to the total body length. The proportions of the head in female individuals are characterized by greater height and length of the space behind the eyes; wider mouth; larger eye and wider snout at the mouth cartilaginous arch. The number of dorsal bony scutes varies from 9 to 13; lateral on the left side - from 24 to 30, and on the right - from 22 to 31; ventral on the left - from 7 to 11, and on the right - 8-11. The number of rays in the dorsal fin is from 21 to 39, and in the anal from 16 to 25. Female fish have a significantly smaller number of ventral bony scutes compared to males from both the left (P<0.05) and the right (P<0.01) side of the body. Male fish have a larger head than females. The ratio of the head length, the caudal stalk, the ventral fin, the anal fin height, the ventro-anal distance to the total body length is greater. In males, the long-headed index is higher (P<0.05), the snout and the distance from its end to the mouth are longer, the lower lip brake is larger. The number of dorsal bony scutes varies from 8 to 13; lateral on the left side - from 25-32, and on the right from 25 to 32; ventral on both sides of the body from 8 to 11. The number of rays in the dorsal fin is from 23 to 45, and in the anal - from 16 to 23.

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OBSERVATIONS REGARDING THE BIOLOGY ASPECTS OF HORSE MACKEREL FROM ROMANIAN COAST BETWEEN 2018-2020

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Abstract

Due to the current situation when we observe numerous climate changes, manifested by rising air and seawater temperatures, there have been observed also, changes in recent years regarding the biology aspects of some fish species from the Romanian Black Sea coast. Trachurus mediterraneus (Steindachner, 1868) - horse mackerel, having economic importance it is necessary to observe its biology in order to develop an appropriate management required by the changes observed at the population level. This paper presents results on temporal variations of total length and weight, age composition, growth parameters and sex ratio of the Mediterranean horse mackerel from the Romanian Black Sea coast. Data were collected from commercial trap net catch and from pelagic trawl expeditions in the period 2018-2020. Significant differences were observed between the length distribution and the stations, with the specification that in 2020 individuals from classes of smaller lengths predominated compared to previous years.

Key words*: growth, morphometrics characteristics, sex ratio, temperature.*

INTRODUCTION

Horse mackerel (*Trachurus mediterraneus*) is one of the main species that have commercial importance and are caught in the Romanian waters. Horse mackerel fishing is conducted with different fishing gears including pelagic trawl, set nets and hand line.

There are a plenty of studies considering this species which is intensively caught in the Black Sea. It is necessary to constantly monitor reproduction, growth, migration, stock size, life span and death rates of horse mackerel population in the Black Sea ecosystem which are of importance for sustainable fisheries.

In this paper growth and other biological variable of the horse mackerel from the Romanian Black Sea coast were reported. The main goal of our study it was to observe the changes that may occur over time and space on different biological parameters.

The relationship between body length and weight are of great importance in fishery biology as they allow estimating fish growth parameters (Gulland, 1983).

Since the growth of teleosts is linked to the foraging behavior (Lloret et al., 2014) and the diet may change temporally, then growth may also vary temporally (Albo-Puigserver et al., 2017).

Growth and sex ratio are important parameters for fisheries management.

MATERIALS AND METHODS

For the purpose of this study, 817 individuals were collected from 15 stations with the trawl and traps net catches along the Black Sea area in the northern area (Navodari, Corbu, Vadu, Periboina, Perisor) and the southern area (Eforie Sud, Costinesti, Vama Veche) between May and November in 2018-2020 (Figure 1).

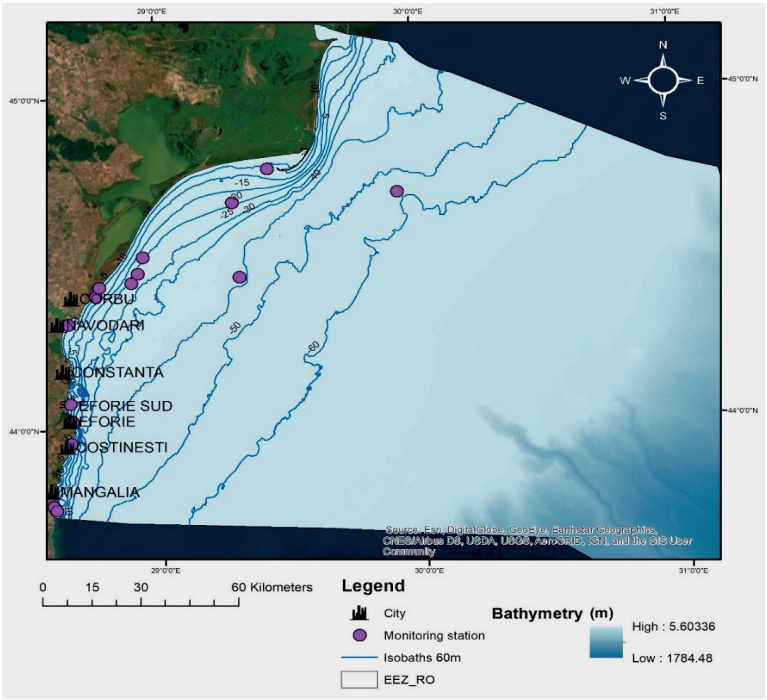

Figure 1. Sampling map

For each specimen, total length (Lt) was recorded (mm) and total weight (Wt) in grams (g). Sex was determined by macroscopic observation of the gonads to all individuals. Sexual maturity stages were assessed according to Nikolsky's scale (1963), and according Follesa, M. C., Carbonara, P., 2019; I: immature, II: resting, III: developing, IV: maturing, V: mature, VI: spent.

The length of a fish is proportional with weight, being strongly connected to development stages (juvenile and adults, and adults different reproduction related stage; size at first maturity, gonad development and spawning) (Serajuddin et al., 2013). Studying the length-weight relationship allows comparing population spatially and temporally (Pandev et al., 1974). Related to biomass data, this relationship may also allow establishing the recruitment yield (Richter, 1958; Beverton et al., 1957) and estimating the biomass of potential exploitable fishes. Length and weight parameters are used in calculating fishing gear selectivity and mainly in sizing the mesh, aiming at improving the catch per unit effort. For age determination, all the 817 individuals were selected from each 1 cm size interval to represent all length groups. The sagittal otolith pairs were removed and cleaned, and stored in dry conditions inside the microplate. Age determination was performed using a stereoscopic zoom microscope under reflected light against a black underground. Opaque and transparent rings were counted: 1 opaque zone, together with 1 transparent zone, was considered the annual macrostructure (Aydin & Karadurmus, 2012). Age estimations were made by 2 independent readers.

The sexual maturity in fish has a great practical importance in the analysis of many population parameters. In general, fish are considered to be mature when they reach the middle of their maximum size (Holden & Raitt, 1974). So, the sex, sex-ratio and reproduction stages were estimated for all the individuals. Identification of sex and sexual maturity stages find their primary application in providing basic knowledge of the reproductive biology of the stock.

Fulton's relative body condition factor (Ricker, 1975) was calculated for 563 individuals using the following formula: $K = (TW \times 100)/TL^3$, with TW = total mass (in grams) and TL = total length (in centimetres).

The non-parametric analyses of variance (Kruskal-Wallis) were performed using the STATISTICA 13.1 in order to analyse differences between years, months, areas and sex.

RESULTS AND DISCUSSIONS

Total length

The mean length of the analyzed individuals was 11.5 ± 2.1 cm. High significant differences were shown between years, followed by differences between months and the lowest ones between sexes (see H tests values in Tables 1 to 3).

The highest mean of length was registered in 2018 (12.3 ± 1.3 cm), and the lowest one in 2020 (10.9 ± 1.2 cm) (Table 1).

The highest values were recorded in May, July and October and the lowest ones in August, September and November (Table 2).

Significant different values were found only between females and juveniles (Table 3).

Table 1. Mean values and standard error of the total length (TL), total weight (TW) and Fulton index (K) by year

Year		TL (cm)		TW (g)		K	
	N	Mean	SE	Mean	SE	Mean	SE
2018	180	12.3	1.3 ^{a}	18.3	6.1	0.9	0.1 ^{a}
2019	322	11.9	1.7 ^{b}	16.2	6.7 ^{b}	0.9	0.1 ^{a}
2020	315	10.9	1.2 ^{c}	11.9	4.2 ^{c}	0.8	0.1 ^{a}
		H = 34.19		H = 58.90		H = 159.65	
		p < 0.001		p < 0.001		p < 0.0001	

N.B. H represents the test statistics of Kruskal-Wallis testing the significance of the differences between years. P = associated p-value. Superscript letters represent post-hoc groups. For each variable values with similar post-hoc letters are not significantly different (P > 0.05). N= number of analysed individuals. SE = standard error.

Table 2. Mean values and standard error of the total length (TL), total weight (TW), Fulton index (K), age (A) and degree of maturity (DM) by month

		TL (cm)		TW (g)		K			Age (y)			DM	
Month	N	Mean	SE	Mean	SE	Mean	SE	N	Mean	SE	N	Mean	SE
May	10	12.97	1.0	20.95	5.78	0.94	0.08	10	1.8	0.8	10	2.9	0.6
June	52	11.8	2.1 ^{bc}	13.5	6.9 ^{bc}	0.80	0.45 ^{a}	52	1.5	1.2 ^{c}	52	2.6	1.1 ^{b}
July	233	12.2	1.7 ^{c}	17.3	8.0 ^{c}	0.91	0.09 ^{c}	233	1.5	1.2 ^{b}	233	2.9	0.9 ^{c}
August	196	10.7	2.0 ^{b}	10.8	6.6 ^{b}	0.78	0.11 ^{c}	196	0.9	1.0 ^{b}	196	2.2	1.0 ^{a}
September	84	10.6	1.5 ^{bc}	11.1	6.1 ^{bc}	0.87	0.06 ^{ab}	84	0.6	0.9 ^{b}	84	2.2	0.8 ^{bc}
October	145	12.3	2.9 ^{a}	18.0	11.1 ^{a}	0.83	0.12 ^{b}	145	1.7	1.4 ^{a}	145	2.9	1.5 ^{a}
November	97	10.8	1.1 ^{a}	10.0	3.1	0.82	0.07 ^{b}	97	0.7	0.8 ^{a}	97	2.2	0.5 ^{a}
		H = 125.23		H = 150.2		H = 265.78			H = 100.82			H = 114.73	
		p < 0.001		p < 0.001		p < 0.0001			p < 0.001			p < 0.0001	

N.B. H represents the test statistics of Kruskal-Wallis testing the significance of the differences between months. P = associated p-value. Superscript letters represent post-hoc groups. For each variable values with similar post-hoc letters are not significantly different (P > 0.05). N= number of analysed individuals. SE = standard error.

Table 3. Mean values and standard error of the total length (TL), total weight (TW) and Fulton index (K) by sex

Sex		TL (cm)		TW (g)		K	
	N	Mean	SE	Mean	SE	Mean	SE
Males	392	11.7	1.8 ^{ab}	11.5	7.8 ^{ab}	0.84	0.11 ^{a}
Females	368	12.0	1.9 ^{a}	15.6	8.4 ^{a}	0.84	0.11 ^{a}
Juveniles	57	7.6	1.0 ^{b}	3.6	1.0 ^{b}	0.83	0.43 ^{b}

The captured individuals were smaller than those identified on the Bulgarian Black Sea coast, up to 19 cm (Yankova, 2013). The horse mackerel samples from the Romanian Black

Sea coast have a growth rate different from those in other areas of the sea, most likely due to different living conditions and availability of food (Bănaru et al., 2009). Inter annual variation may be related to differences in recruitment but also to migrations and common studies should be make in the Black Sea in order to cover the entire migration area of this species.

Total weight

The mean of the weight of the analyzed individuals was 14.3 ± 8.3 g. The highest weight was registered in 2018 (18.3 ± 6.1 g), and the lowest ones in 2020 (11.9 ± 4.2 g) (Table 1). Similarly, to the total length, the highest values were recorded in May, July and October and the lowest ones in August, September and November (Table 2) and significant different values were found only between females and juveniles (Table 3). Studies conducted for horse mackerel taken from the Turkish Black Sea region revealed a spectrum of weight between 3.32 g and 59.98 g (Aydin & Karadurmuş, 2012); so, hight weight were register than those from this study. The length-weight relationship was established using the equation $W = 0.0073 \times L3.0546$ ($R^2 = 0.8726$) (Figure 2). The mean value of *b* (3.0546) did not significantly differ ($P<0.05$) from the standard value of 3.0, implying that the "cube law" could be applied for this species (Ricker, 1973). When the weigh-length exponent *b* is equal to 3.0, the body form maintains a constant proportion to the length and the fish grows isometrically, resulting in an ideal shape (Pauly, 1983). However, when *b* is less than 3.0, the fish shows negative allometric growth, and when the *b* value is greater than 3.0, the fish shows positive allometric growth (Weatherley and Gill, 1987). Thus, the fish are expected to grow proportionally in all directions. Changes in fish weight are generally greater than those in fish length (Ahmed et al., 2011). In general, when the value of *b* exceeds 3.0, fish become fatter, and when the value falls below 3.0, fish become leaner. The value of *b* found in the present study is within the interval of 3 to 3.5 recorded for many fish species by (Froese, 2006). As shown in several studies, when the size of fish increase, more fat is deposited than the formation of other tissues (Salam and Davies, 1994; Salam et al., 2001).

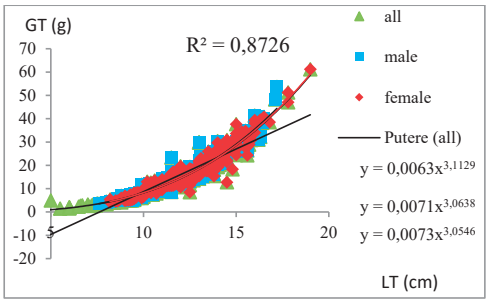

Figure 2. Length - weight relationship of the horse mackerel during the analysed period 2018-2020

Fulton Index

The mean value of the Fulton index of all the analyzed individuals was 0.84 ± 0.15. Higher the Fulton coefficient is and better the relative body condition is. Higher relative body conditions were shown in 2018 and 2019 (0.91 ± 0.1) compared to 2020 (Table 1). Seasonal variations of the relative body condition were highlighted with the lowest values in August and the highest ones in May and July (Table 2). These variations may be related to the life history traits and their environmental condition and diet during the previous months. All, male, female and juveniles have approximately the same body condition (Table 3).

Age

Determining the age of fish is an important element for the study of the population dynamics. Analyzed horse mackerel individuals age varied between 0 and 5 years. The mean age was 1.19 ± 1.2. Seasonal differences were highlighted with the oldest individuals were found in May (1.8 ± 0.8 years) and the youngest in September (0.6 ± 0.9 years) (Table 2).

Individuals with 0+ age predominated in length classes between 51-110 mm, 1 age individuals at 111-120 mm, 2+ age individuals at 121-130 mm, 3+ age individuals at 131-150 mm, 4+ age individuals at 151-170 mm and 5+ age individuals at 171-180 mm (Figure 3).

The mean length and mean weight increased with age from 9.5 + 1.2 cm and respectively 7.4 + 2.9 cm for 0+ age individuals to 17.8 + 1.1 cm and respectively 54.5 + 6.5 g for 5 years individuals (Table 4). Relative body condition factor decreases from 0+ (0.83) to 5 years (0.97), while the degree of maturity increases with age from 1.7 ± 0.7 for 0+ year individuals

to 5.2 ± 0.3 for 5 years individuals. This inverse relation may be related to higher energetic investment for reproduction in larger and older individuals.

Table 4. Mean values and standard error of the total length (TL), total weight (TW), Fulton index (K) and maturity degree by age (years)

Age (y)		TL (cm)		TW (g)		K				DM	
	N	Mean	SE	Mean	SE	Mean	SE		N	Mean	SE
0+	311	9.5	1.2	7.4	2.9	0.83	0.20		311	1.7	0.7
1	209	11.5	0.7	13.6	3.5	0.87	0.11		209	2.5	0.4
2	170	12.7	0.7	17.0	3.8	0.82	0.10		70	2.9	0.5
3	91	14.3	0.6	25.1	4.8	0.85	0.13		91	4.0	0.5
4	33	16.0	0.6	35.4	5.9	0.86	0.08		33	4.7	0.5
5	3	17.8	1.1	54.4	6.5	0.97	0.08		3	5.2	0.3

N.B. N= number of analysed individuals. SE = standard error

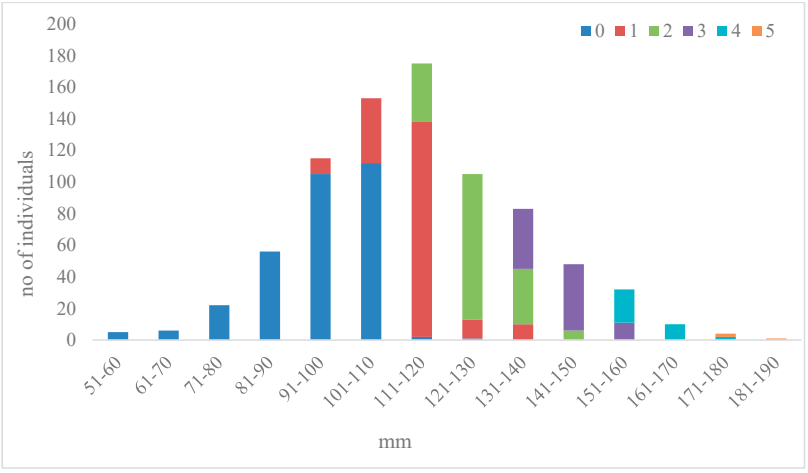

Figure 3. Distribution of *Trachurus meditteraneus* individuals by ages and length classes

Sex determination

Gonads differences between sexes appeared early, at age 1. Mean sex-ratio (males/females) over the study period was 1.06.

This ratio was variable between years (minimum 0.8 in 2018 and maximum 1.5 in 2019).

Males dominated in June, July, October and November while female dominated in May, August and September (Figure 4).

Natural variability of sex-ratio in recruitment but also fisheries pressure on young stages may be responsible of these differences.

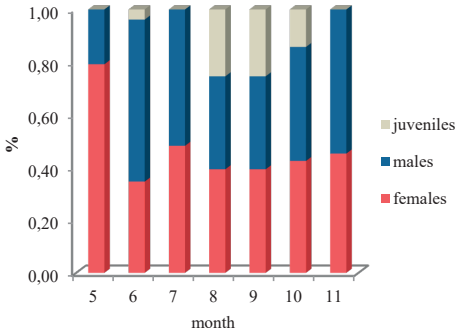

Figure 4. Sex ratio by month of *T. mediterraneus*

Gonadal maturity

Sexual maturity has a practical importance in the analysis of fish population. Generally, fish are considered to be mature when they reach the middle of their maximum size (Păun et al, 2019).

Over the whole studied period, the mean value of the gonadal maturity index of the 817 analyzed individuals was 2.6 ± 1. Significant differences were shown between months with the highest values in May (2.9 ± 0.08) and July (2.9 ± 0.6) when they also had the better relative body condition, while the lowest values were observed in August and September (Table 2).

CONCLUSIONS

In this paper variability of different biological aspects of the horse mackerel from the Romanian Black Sea coast were reported

Regarding the length, the mean of all the analyzed individuals we registered was 11.5 ± 2.1 cm. High significant differences were shown between years; the highest mean length was registered in 2018 (12.3 ± 1.3 cm), and the lowest one in 2020 (10.9 ± 1.2 cm). And significant different values were found between females and juveniles.

The mean weight of the analyzed individuals was 14.3 ± 8.3 g. The highest weight was registered in 2018 (18.3 ± 6.1 g), and the lowest ones in 2020 (11.9 ± 4.2 g).

Relative body condition values were higher in 2018 and 2019 compared to 2020. The relative body condition factor decreased from younger to older individual, while the degree of maturity increased with age. The gonadal maturity analyses showed that the reproduction occurs mainly during summer with a maximum in July. Analyzed horse mackerel individuals age varied between 0 and 5 years; the mean age was 1.19 ± 1.2.

Seasonal differences were highlighted with the oldest individuals that were found in May (1.8 ± 0.8 years) and the youngest in September (0.6 ± 0.9 years).

Mean sex-ratio over the study period was 1.06; this ratio was variable between years.

This study should continue and extended to a longer period. The horse mackerel is an economically species in Romanian waters, therefore, it is necessary to monitor and follow the changes in stock size in term of sustainable fisheries.

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THE INFLUENCE OF CROP DENSITY ON PHOSPHORUS DYNAMICS IN AN INTEGRATED STELLATE STURGEON - SPINACH RECIRCULATING PRODUCTION SYSTEM

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Abstract

In an integrated aquaponic system, the phosphorus present in the technological water can be recycled in the plant biomass, depending on a series of elements related to system design and production technology. The present research aims to identify the influence of crop culture density on phosphorus dynamics in an integrated stellate sturgeon – spinach recirculating production system, by using aquaponic substrate technique. A 44 days experimental trial was performed in order to test three spinach culture densities, in triplicate, as follows: B1H - 59 crops/m^2, B2H - 48 crops/m^2, B3H - 39 crops/m^2. A control variant (with no plants) was used, in order to estimate the spinach biomass capacity to remove phosphorus. Phosphorus removal rate had an upward trend at B1H, B2H and B3H, with an average value of 13.38 ± 5.45 g/m^2/day at B1H, 11.57 ± 5.25 g/m^2/day at B2H and 10.39 ± 4.4 g/m^2/day at B3H. Statistically significant differences ($p<0.05$) were recorded between B1H and the rest of experimental variants. Although the highest phosphorus removal rate was recorded at B1H, some spinach plants manifested signs of stress, fact that might affect the phosphorus removal rate capacity during a longer experimental period.

Key words*: aquaponic, spinach, stellate sturgeon, phosphorus, removal rate.*

INTRODUCTION

In order to maintain both environmental and economic sustainability, a proper waste management must be applied among aquaculture production systems, by identifying new techniques for water treatment. Several studies (Zhang et al., 2021; Robinson et al., 2019) confirmed that solving the phosphorus and nitrogen pollution occurred due to intensive aquaculture practices is the key to maintain the ecosystem health. Thus, both physico-chemical and biological methods can be applied in order to remediate the aquaculture effluents. However, according to several studies (Liang et al., 2020; Jinet al., 2019; Benammar et al., 2015), the physico-chemical methods are less sustainable, from both economic and environmental perspective, compared to biological methods.

Phosphorus is commonly encountered in high concentration in aquaculture effluents and its concentration in water, over the maximum limit, can lead to algae blooms which produce harmful algal toxins.

Integrated multi-trophic aquaculture (IMTA) is considered as a solution for improving the sustainability of aquaculture production systems. The use of aquaponic techniques which consists in the integration of both fish and plants production, can be considered a solution in order to achieve the zero-discharge desideratum. However, according to several studies (Delaide et al., 2017; Groenveld et al., 2019; Jaeger et al., 2019), in most recirculating aquaponic systems, during some periods, up to 20% of wastewater is discharged to maintain water quality. Aquaponics ensure both water treatment by bio and phyto-remediation and improve the fish farm economical performances as a result of commercializing a secondary production - plant biomass. Phosphorus is considered, according to Yang and Kim (2020), a key nutrient that affects agricultural productivity. Also, Daniel et al. (1998) characterized phosphorus as the second most frequently limiting macronutrient for plant growth, making up about 0.2% of a plant's dry weight. Therefore, a balance between phosphorus inputs and outputs must be

assured within an IMTA system based on aquaponics techniques in order to assure both an optimum fish and plants production performance, correlated with low phosphorus discharges. However, some previous studies related to phosphorus dynamics in an IMTA system based on aquaponics techniques (Seawright et al., 1998) had reported that the total amount of phosphorus recovered in fish, plants and solids exceeded the quantity provided through the administrated diet.
Barben et al. (2010) revealed that phosphorus deficiency causes stunted plant growth, whereas phosphorus excess may lead to antagonistic interactions with micronutrients. For fish biomass, phosphorus is an important mineral in nucleic acids and cellular membranes, the main representative of the structural components of the skeletal tissues, and it is directly involved in energy processes (National Research Council, 1993).
The present research aims to identify the influence of crop culture density on phosphorus dynamics in an integrated stellate sturgeon – spinach recirculating production system, by using aquaponic substrate technique.

MATERIALS AND METHODS

Experimental design
A 44 days experimental trial was performed in order to test three spinach culture densities, in triplicate, as follows: B1H - 59 crops/m^2, B2H - 48 crops/m^2, B3H - 39 crops/m^2. A control variant (with no plants, only substrate) was used (Figure 1), in order to estimate the spinach biomass capacity to remove phosphorus.

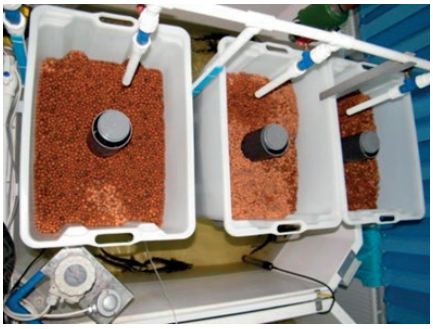

Figure 1: Control variant consist in aquaponic modules with no plants, only substrate - light expanded clay aggregate (LECA)

The detailed description of integrated aquaponic system is presented in a previous study (Petrea et al., 2014). Therefore, the recirculating aquaculture system consists in 4 rearing units and a series of water treatment modules, as follows: sump, mechanical drum filter, UV, oxygenation unit and biological trickling filter. Aquaponic modules, in triplicate, were placed above each of the rearing units and water recirculation was continuously assured during the experimental period by using a recirculation submersible pump, placed in the rearing units, as described in previous study (Petrea et al., 2014). Before starting the experiment, the activation of biological trickling filtration unit was made as described by Petrea et al. (2014).
A number of 184 stellate sturgeons with an average weight of 170 g/exemplar was equally distributed within 4 rearing units and fed with Clasic Extra 1P-41% brute protein and 0.9% phosphorus, by applying a feeding ratio of 1.75% of total biomass weight (BW). Therefore, a daily average input of 6.9 ± 0.52 g phosphorus/day, by fish feed, was assured during the entire experimental period.
Each rearing unit was connected to 3 aquaponic modules filled with LECA, where Matador variety spinach (*Spinacia oleracea*) seedlings were placed according to culture densities previously mentioned (Figure 2).

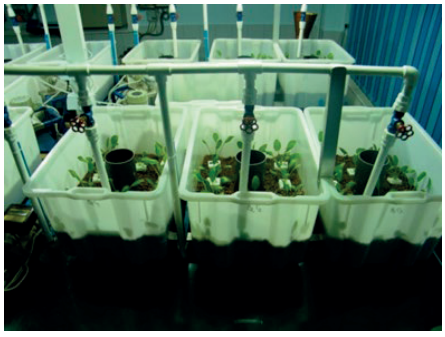

Figure 2. Spinach biomass distributed in each aquaponic module

A constant luminous power of 5800 lm, measured with TESTO 545 light meter, was assured during the entire experimental period.
The hydraulic loading rate (HLR) and hydraulic retention time (HRT) were calculated using the following equations (eq. 1 and eq. 2):

$$HLR = \frac{Q}{S} \quad (1)$$

$$HRT = \frac{S \times h \times p}{Q} \quad (2)$$

where, Q is water flow rate [m^3/s]; S is total surface area of hydroponic module [m]; h is water depth [m] and p is porosity of hydroponic module.

Thus, a constant value of 16 m/day for HLR and 0.48 min for HRT were maintained during the entire experimental period.

Sampling and analysing methods

Technological water analysis was performed by using Spectroquant Nova 400 spectrophotometer, with Merk compatible kits. Samples of water were collected once a week from both the outlet of biological filter (inlet of hydroponic units) and the outlet of each hydroponic unit.

The phosphorus removal rates for each experimental variant were presented as average of the triplicate aquaponic units. The following equation was used in order to determine phosphorus removal rates (eq. 3):

$$PR = [\frac{Q}{V} \times (C_{in} - C_{out}) - \frac{\Delta C_{out}}{\Delta t}] \times d \quad (3)$$

where PR is phosphorus removal rate ($g/m^2/day$), Q is the flow rate (m^3/day), V is the system volume (m^3), C is the concentration of phosphorus (g/m^3), d is the water depth (m) and t is the time (days).

The fish faeces were collected by using a EHEIM water vacuum cleaner provided with a mesh compartment for solids retention, both at the beginning and at the end, but also during the experimental period (2 intermediary determinations). In order to estimate the phosphorus retention in muscle tissues during the experimental trial, samples were collected both at the beginning and at the end of the experimental period. Phosphorus concentration in spinach was determined at the end of the experimental trial, for each of the experimental variant triplicate (B1H1, B1H2, B1H3, B2H1, B2H2, B2H3, B3H1, B3H2, B3H3). The results are presented as 5 samplings average. For determining the phosphorus concentration in fish muscle tissues, spinach and fish faeces, the SR ISO 2294:2009 reference method was used.

Statistical methods

The software IBM SPSS Statistics 20 for Windows was used for the statistical analysis presented in present paper.

The T test ($\alpha = 0.05$) was applied in order to identify the statistical differences between treatments, after the Kolmogorov-Smirnov normality test was performed. The ANOVA test (post-hoc Duncan test) was performed in order to compare variants.

RESULTS AND DISCUSSIONS

Technological water P_2O_5 concentration

The evolution of water P_2O_5 concentration at the inlet and outlet of both mechanical drum filter and biological trickling filter revealed a decrease trend until the end of the second week of the trial (Figure 3). However, a small increase of P_2O_5 concentration can be observed during the last week of the experimental period. This can be due to the accumulation of fish wastes within the integrated system since, according to some studies (Prüter et al., 2020), fish wastes can contain up to 1.7% phosphorus.

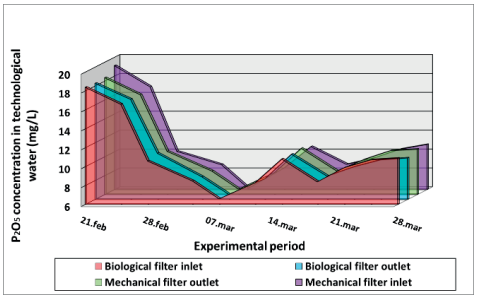

Figure 3. The dynamics of water concentration of P_2O_5 at the inlet and outlet of both mechanical drum and biological trickling filters

According to the integrated system description, the biological trickling filter outlet sampling point can be considered as inlet for all aquaponics modules. The water concentration of P_2O_5 registered in the aquaponics modules sampling points is presented, as triplicate average, in Figure 4.

Therefore, it can be observed that the entire experimental period average concentration of water P_2O_5 was 10.8 ± 3.85 mg/L at the aquaponic units' inlet, while at the outlet the following average concentrations were recorded: 9.52 ± 4.2 mg/L at B1H, 9.49 ± 3.95

mg/L at B2H, 9.72 ± 3.94 mg/L at B3H and 10.81 ± 3.86 mg/L at B4H (control variant), respectively.

The entire experimental period average concentration of water P_2O_5 recorded at the mechanical filter outlet is 10.86 ± 3.86 mg/L, while at biological filter inlet and outlet concentrations of 10.89 ± 3.57 mg/L, 10.75 ± 3.58 mg/L, respectively, were registered.

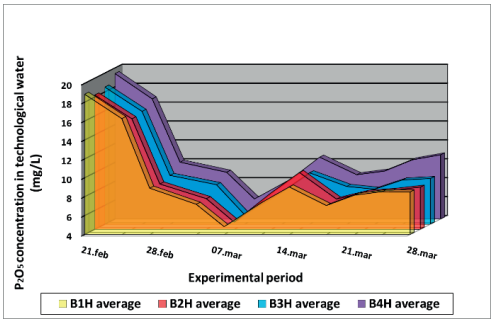

Figure 4. The dynamics of water concentration of P_2O_5 at the aquaponic modules outlet (presented as triplicate average)

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The P_2O_5 concentration in fish wastes and fish muscle tissue

The entire experimental period average concentration of P_2O_5 recorded in fish wastes is 5.47 ± 1.01 g% dry weight (DW). The evolution is related to fish necessity for this nutrient, during their growth period and indicates a decrease trend during the first two weeks of the trial, follow by an upward trend until the end of the trial (Figure 5).

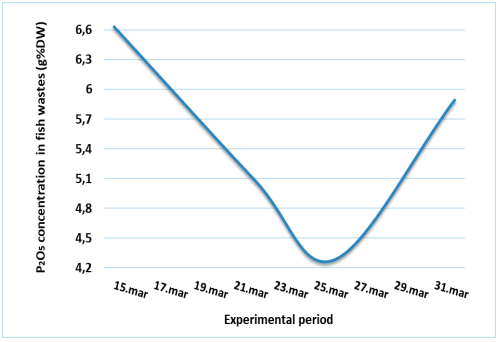

Figure 5. The dynamics of P_2O_5 concentration in fish wastes

However, it is highlighted that, according to some authors (Olson, 1992; Westerman et al., 1993; Naylon et al., 1999), the fish wastes chemical composition may differ significantly, mostly depending on fish metabolism and its body capacity to absorb nutrients. Also, Naylon et al. (1999) stated that a comparison between fish wastes chemical concertation, reported in different studies, is difficult to be performed due to differences between wastes separation time and sampling method.

There is the need of synchronizing both fish and plants biomass in terms of P_2O_5 requirements dynamics, in order to maintain the bio-phytoremediation capacity of the integrated system.

An increase of average P_2O_5 concertation in fish muscle tissue was observed at the end (194.62 ± 12,15 mg/100 g fresh weight - FW), compared to the beginning of the experimental trial (187.92 ± 11.49 mg/100 g FW). Lazzari et al. (2008) pointed out that fish can absorb phosphorus from the water, but due to the low waterborne concentration of this compound, dietary supplementation is necessary. Also, Jahan et al. (2002) revealed that phosphorus retention is also directly correlated with fish growth rate.

The P_2O_5 concentration in spinach leaves

At the end of the trial, the following average P_2O_5 concentrations in spinach leaves were recorded, in triplicate: B1H (B1H1 - 51.09 ± 2.78 mg/100 g fresh weight (FW); B1H2 - 52.39 ± 1.19 mg/100 g FW; B1H3 - 52.57 ± 1.49 mg/100 g FW), B2H (B2H1 - 60.83 ± 1.38 mg/100 g FW; B2H2 - 59.95 ± 1.68 mg/100 g FW; B2H3 - 58.36 ± 1.42 mg/100 g FW) and B3H (B3H1 - 72.44 ± 1.54 mg/100 g FW; B3H2 - 74.73 ± 0.77 mg/100 g FW; B3H3 - 71.6 ± 1.35 mg/100 g FW) (Figure 6).

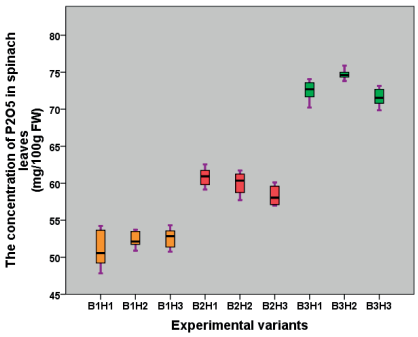

Figure 6. The P_2O_5 concentration in spinach leaves, for each experimental variant, in triplicate

Thus, statistically significant differences ($p < 0.05$) are recorded between the experimental variants. The registered concentrations are superior to those recorded in spinach leaves (29-37 mg/100 g FW) by other authors (Kaya et al., 2001) [252].

The results (Figure 6) emphasizes that the culture density can significantly influence the P_2O_5 concentration of spinach leaves. In order to assure a high phytoremediation capacity of the integrated system, spinach biomass welfare must be maintained at an optimum level. However, signs of stress among spinach biomass were observed at B1H experimental variant, most probably due to high culture density applied (Figure 7).

Figure 7. Visible signs of stress recorded among spinach plants cultured in B1H experimental variant

The P_2O_5 removal rate

The P_2O_5 removal rate had registered a general upward trend in case of B1H, B2H and B3H experimental variants, while a relatively constant trend is revealed at control variant (B4H) (Figure 8).

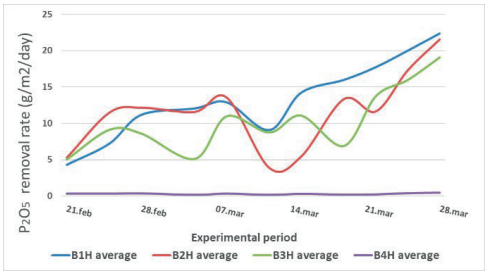

Figure 8. The P_2O_5 removal rate as triplicate average

It can be observed that B2H registered the highest values in terms of P_2O_5 removal rate in the first part of the experimental period (figure 8). However, after the first half of the trial, a considerable decrease of removal rate value is observed (figure 8), most probably due satiety effect in terms of phosphorus. The highest average value for P_2O_5 removal rate is recorded at B1H (13.38 ± 5.45 g/m^2/day), followed by B2H (11.57 ± 5.25g/m^2/day), B3H (10.39 ± 4.4 g/m^2/day) and B4H – control variant (0.29 ± 0.09 g/m^2/day). The distribution of P_2O_5 removal rate values, for each of the experimental variant triplicate, is presented in figure 9.

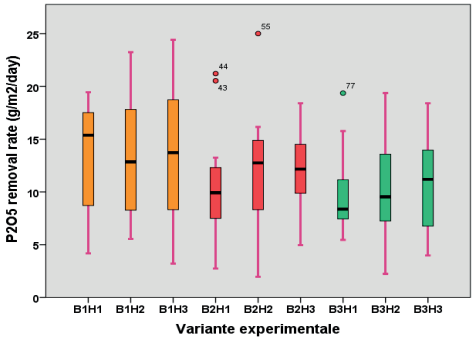

Figure 9. The distribution of P_2O_5 removal rate values, for each of the experimental variant triplicate

Statistically significant differences ($p<0.05$) were recorded between the first three experimental variants (B1H, B2H, B3H) and control variant, as well as between B1H and the rest of the experimental variants. No statistically differences ($p>0.05$) were recorded between the triplicate of each experimental variant.

CONCLUSIONS

The present study revealed that substrate aquaponics techniques is efficient in terms of effluent phosphorus removal from an integrated stellate sturgeon - spinach production system.

Best phytoremediation results were recorded at the experimental variant B1H, where the highest spinach culture density was applied (59 crops/m^2). However, within this variant some spinach plants presented signs of stress, fact that can affect the phytoremediation capacity during a longer experimental period.

Future research must be made in terms of fish feeding rates, fish-plants stocking densities optimisation and, also, the optimization between fish rearing stage and plants stocking density, in order to balance the integrated

system. It is recommended to consider a CPS (conveyor production system) in order to balance the integrated system in terms of phosphorus availability**.**

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THE DYNAMICS OF WILD BOAR HERDS IN THE CONTEXT OF THE APPEARANCE OF A DISTURBING ECOFACTOR

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Abstract

Environmental factors define an ecological potential that ensures a certain biological productivity of the hunting management fund. A first step in establishing the ecological potential of game management funds is to direct efforts to qualitatively and quantitatively assess all factors that influence biological productivity. Monitoring the dynamics of wild boar herds is important because it is necessary to understand the relationships and correlations that are established between different species of animals in the same territory (prey-predator), but also between the studied species and the existence of an optimal ecological factors: biotic, abiotic and anthropogenic, to ensure the viability of the population. In this paper, was made an evaluation of wild boar herds dynamics, recorded between 2015 and 2020, using data from the Ministry of Environment and according to the assessment keys for hunting territories. Statistical elements were established in order to have a more accurate calculation of the wild boar population trend. It is necessary to know the wild boar herds evolution, in order to develop a long-term strategy, in which hunters also have a key role to play in conserving biodiversity.

Key words*: environment, herd dynamics, wild boar.*

INTRODUCTION

The wild boar (*Sus scrofa attila* L.), due to its high breeding potential in a relatively short time, is a large game species that can be a potential danger to both agricultural crops and forest vegetation within the hunting grounds (Cotta et al., 2008.).

The work of developing the ecological diagnosis keys for wild boar took into account this aspect, but also took into account a number of factors that contribute to the differentiation of ecological conditions for this species, given the results of research conducted in a number of European countries, which highlight the importance of climatic factors, the existence of predators, food sources, the occurrence of diseases, human activity (Dardaillon & Beugnon, 1987)

A first group of factors, with the highest score, which have a high influence on the ecology of this game species consists of game culture factors: fields for winter food, distribution and managing food administration, natural numerical ratio predators/wild boar, and number of stray dogs per 1000 ha, as well as from negative abiotic factors, represented by grazing and poaching.

The analysis of this group of factors highlights the fact that the management of hunting funds can have an extremely high influence on the change of wild boar numbers, all the factors listed above can be changed in the desired direction, by appropriate measures.

In the group of factors with average influence on the ecology of wild boar populations we find a series of abiotic factors, represented by: average temperature during calving, average thickness of the snow layer and the size of the snow period, as well as biotic factors, represented by: afforestation percentage, vegetation outside the forest and accessible biomass in winter. This group of factors is characterized by a reduced ability to react to changes that may occur in the management of those hunting funds.

The third group of factors, respectively the factors with relatively low influence, consists of abiotic factors (the average altitude of the land, the amount of precipitation during calving and the hydrographic network), biotic factors, (the percent of classes age of the trees and bushes, existing forest formation, coppices and agricultural crops), as well as negative abiotic factors (the growth of domestic pigs and the

density of the road network). Within this third group of factors, a special situation is represented by the breeding activity of domestic pigs, which can be a source of disease spread among wild boar populations, which can lead to a dramatic reduction in the wild boar number (***Order M.M.A.P. no.393/2002)..

Thus, this factor, of relatively low importance, may take a special importance under certain specific conditions (high densities of wild boar populations, along with the growth of unvaccinated domestic pigs, which can feed on the productive area of hunting funds

The evolution of livestock is influenced by changes in determinants ecological factors, in the geographical distribution. The dynamics of wild boar populations suppose, first of all, an appreciation of the quality of environmental factors as a support for biological productivity, especially if we take into account the fact that the specie is dependent on the existence of balanced ecosystems. The ecosystems have to ensure, in addition to food requirements also the vital spaces necessary for sheltering, breeding and growing piglets.

Ecological environmental factors define, in fact, an ecological potential that ensures a certain biological productivity of the hunting management fund. A first step in establishing the ecological potential of game management funds is to direct efforts to qualitatively and quantitatively assess all abiotic factors that influence biological productivity. Thus, the ecological potential of game management funds must be analyzed in the light of the following components: geology, relief, climatic characteristics, hydro-geomorphological and hydrological elements and soil particularities (Micu, 2004)

MATERIALS AND METHODS

In this paper, was done an analysis of the situation of wild boar herds, in Tulcea County, based on official evaluations, using data from the Ministry of Environment. These evaluations take into account the reports made by the administrators of the hunting funds from Tulcea county, the data being centralized at the ministry level.

Monitoring the dynamics of studied wild boar herds, is important because it is necessary to understand the relationships and correlations that are established between different species of animals in the same territory (prey-predator), but also between the species studied and the existence of an optimal ecological factors. biotic, abiotic and, of course, of an anthropogenic nature, in order to ensure the viability of the populations.

Statistical calculations (mean, standard deviation, mean error, coefficient of variability) necessary to establish the evolution of wild boar numbers were also performed.

RESULTS AND DISCUSSIONS

In Tulcea county, the numbers of evaluated animals, in the period 2015-2020, fluctuated between 269 and 2277 heads (Table 1, Figure 1 - official data taken from the website of the of the Ministry of Environment, Waters and Forests).

Table 1. Total wild boar herds evaluated, in the period 2015-2020, in Tulcea county

Year	Animal number (heads)
2015	1875
2016	1775
2017	1992
2018	2277
2019	334
2020	269
Mean	1420,33
Standard deviation	883.04
Mean error	360.50
Coefficient of variability	62.17

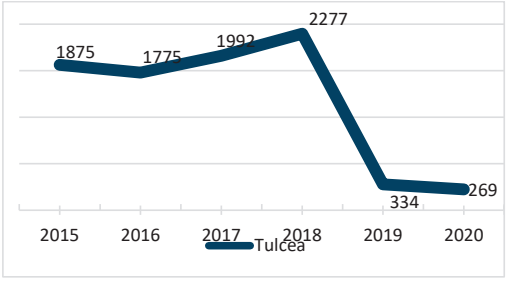

Figure 1. The evolution of wild boar herds, in 2015-2020, in Tulcea County

Statistical analyzes highlight the existence of a high coefficient of variability. This is due to the drastic decrease of the value of the herds evaluated in 2019, the trend maintained in 2020 (269 heads, compared to the highest value, registered in 2018-2277 heads).

Out of the total number of wild boar flocks in Tulcea County, only those registered by the fund managers that appear constantly in the hunting activity, with evaluated and harvested flocks, during the years 2015-2020, were studied (RNP, AJVPS Tulcea, Trei Stejari Association, AV Predeşti Habud, AV San Rafael, AVPS Crângul Slava Rusă and AVPS Mioriţa)

In terms of numbers, as expected, RNP and AJVPS Tulcea dominate, followed by AVPS Mioriţa. The fewest specimens can be found at AVPS Crangul Slava Rusa (Table 2. and Figures 2, 3)

Table 2. The evolution of the wild boar herds, evaluated between 2015-2020, by the main managers of hunting funds from Tulcea county

Year	Manager hunting fund (AJVPS/Association/AV)						
	RNP	Tulcea	Trei Stejari	Predeşti Habud	San Rafael	Crângul Slava Rusă	Mioriţa
2015	1010	202	8	33	25	45	55
2016	978	250	8	35	35	42	60
2017	1097	291	10	40	45	44	65
2018	1128	368	10	40	100	62	70
2019	33	8	10	21	30	15	72
2020	31	16	10	10	25	9	58
Total	4277	1135	56	179	260	217	380
Mean	712.83	189.16	9.33	29.83	43.33	36.16	63.33
Standard deviation	530.21	147.66	1.03	11.95	28.75	20.13	6.80
Mean error	216.45	60.28	0.42	4.88	11.73	8.21	2.77
Coefficient of variability	74.38	78.06	**11.06**	**40.07**	66.35	55.66	**10.73**

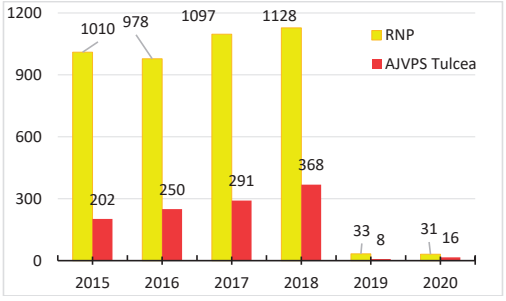

Figure 2. The evolution of the wild boar herds, evaluated between 2015-2020, by the biggest managers of hunting funds from Tulcea County

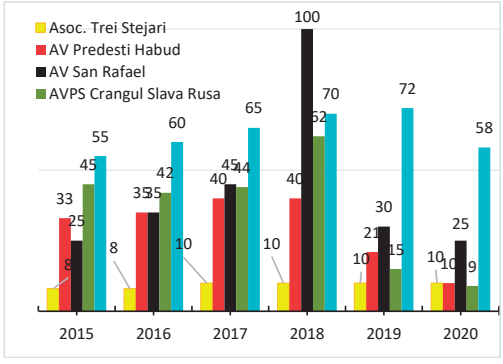

Figure 3. The evolution of the wild boar herds, evaluated between 2015-2020, by the main managers of hunting funds from Tulcea County

Most wild boar specimens were evaluated in 2018, but in 2019 the number of evaluated specimens decreased drastically, in most managers, the same trend being maintained in 2020. At the Trei Stejari Association the number of evaluated specimens remained constant (10 heads) in the last 4 years. At AVPS Miorita, the number increased from 70 heads in 2018 to 72 heads in 2019, and then decreased to 58 heads in 2020.

For the Association of Three Oaks and AVPS Miorita, the coefficient of variability was up to 15% (11.06% and 10.73%, respectively), the evaluated numbers remaining relatively constant throughout the years 2015-2020.

The statistical analyzes highlight the existence of a high coefficient of variability (over 55%) for the herds registered by RNP, AJVPS Tulcea, AV San Rafael and AVPS Crângul Slava Rusă. This fact is due to the decrease of the animal number, established for evaluation for 2019 and 2020, from 1128 to 33 heads and respectively 31 for RNP, from 368 heads to 8 heads, for AJVPS Tulcea, from 100 to 30 heads and respectively 25 for AV San Rafael and from 62 to 15 heads and respectively 9 for AVPS Crângul Slava Rusă.

For AV Predeşti Habud, there was a coefficient of variability of 40.07%, the difference

between the numbers evaluated in 2018 (40 heads) and those in 2019 (21 heads) being 50%, as for those evaluated in 2019 (21 heads) and those of 2020 (10 heads).
This decrease in the evaluated herds in 2020, compared to 2019 and 2018 in particular, is probably due to the rapid spread of swine fever in Tulcea County, which led to a drop in the number of animals existing in this county.
Depending on the evaluated herds there were established over the years the harvesting quotas for wild boar. This were approved and achieved in Tulcea County (Tables 3 and 4. and Figures 4-11).

Table 3. Wild boar quotas approved and achieved in Tulcea County, for the hunting seasons from 2015-2020

Herd size (no.)	Harvest quotas (pcs)				
	2015/2016	2016/2017	2017/2018	2018/2019	2019/2020
Approved	280	339	394	746	286
Achieved	197	232	213	253	66

Table 4. Wild boar quotas approved and achieved in Tulcea County, for the hunting seasons from 2015-2020, for the main hunting fund managers (RNP, AJVPS Tulcea, Asociaţia Trei Stejari, AV Predeşti Habud, AV San Rafael, AVPS Crângul Slava Rusă and AVPS Miorița)

Hunting fund manager	Harvest quotas (pcs)									
	2015/2016		2016/2017		2017/2018		2018/2019		2019/2020	
	Approved	Achieved	Approved	Achieved	Approved	Achieved	Approved	Achieved	Approved	Achieved
RNP	48	30	50	36	43	42	119	100	35	18
AJVPS Tulcea	67	41	87	71	105	71	185	44	28	18
Asociația Trei Stejari	3	0	3	2	5	1	5	0	5	2
AV Predești Habud	10	10	10	8	14	12	24	2	11	0
AV San Rafael	6	2	17	14	23	19	67	21	15	13
AVPS Crângul Slava Rusă	15	4	15	10	15	10	30	24	15	6
AVPS Miorița	24	18	25	24	25	4	46	1	72	2

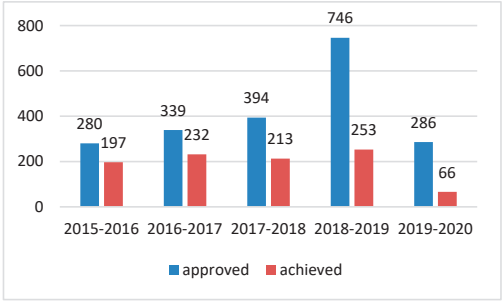

Figure 4. Wild boar quotas approved and achieved in Tulcea County, for the hunting seasons from 2015-2020

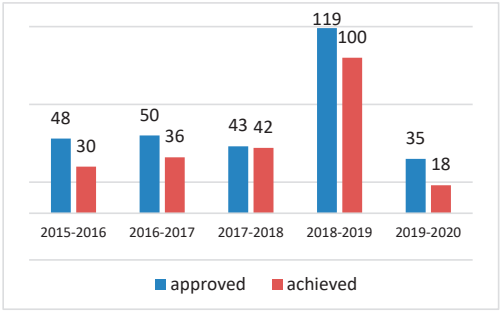

Figure 5. Wild boar quotas approved and achieved by RNP, for the hunting seasons 2015-2020

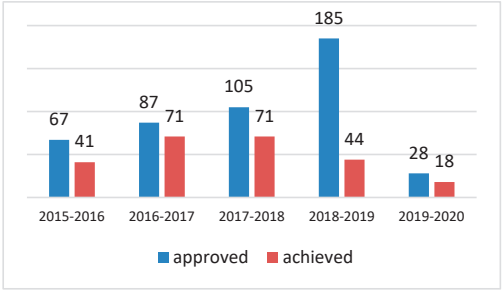

Figure 6. Wild boar quotas approved and achieved by AJVPS Tulcea, for the hunting seasons 2015-2020

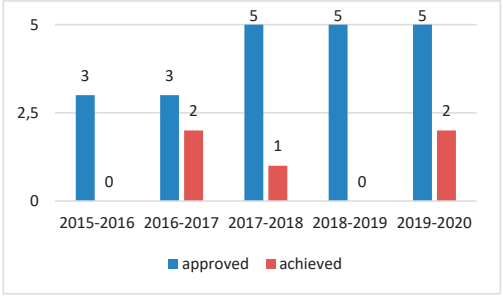

Figure 7. Wild boar quotas approved and achieved by Trei Stejari Association, for the hunting seasons 2015-2020

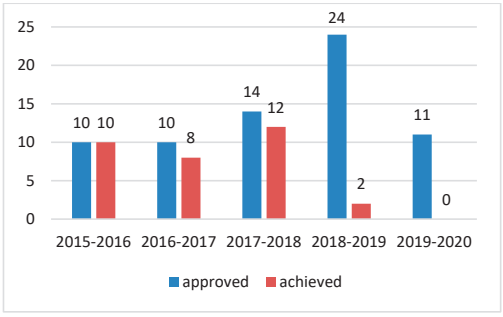

Figure 8. Wild boar quotas approved and achieved by AV Predești Habud, for the hunting seasons 2015-2020

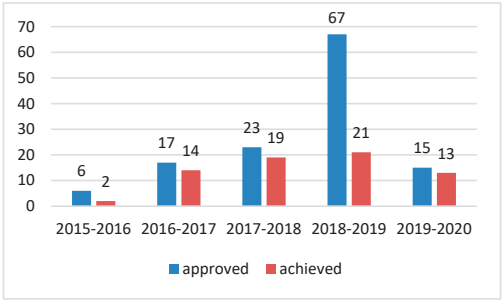

Figure 9. Wild boar quotas approved and achieved by AV San Rafael, for the hunting seasons 2015-2020

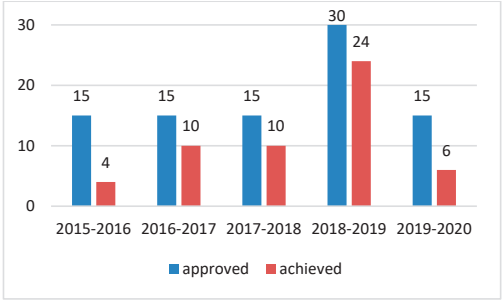

Figure 10. Wild boar quotas approved and achieved by AVPS Crângul Slava Rusă, for the hunting seasons 2015-2020

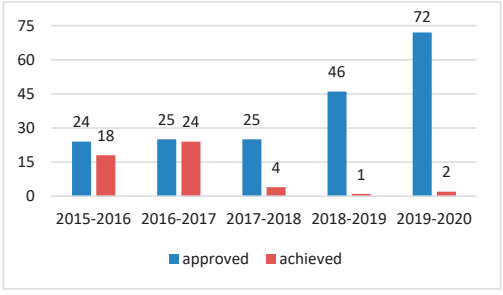

Figure 11. Wild boar quotas approved and achieved by AVPS Miorița, for the hunting seasons 2015-2020

From the analyzed data, it is observed that, both in Tulcea County as a whole, and in the case of each manager taken under discussion, the approved quotas were in a constant increase since 2015, with a significant increasing in the 2018-2019 season, possibly due to the appearance of swine fever. (issuance of Ministerial Order No. 827/23.08.2018 on the approval of measures to control African swine fever, which refers to the "Intervention quota ... is additional to the harvest quota for wild boar species approved by Order of the Minister of Waters and Forests No. 540/2018 regarding the approval of the harvest quotas for some species of hunting interest species, where hunting is allowed, for the hunting period May 15, 2018 - May 14, 2019").

However, it is observed that, for the 2019-2020 season, the approved quotas were drastically reduced compared to the 2018-2019 season for most managers, probably due to the significant reduction of the evaluated herds. The most significant reduction (of 84.86%) is found at AJVPS Tulcea, followed by RNP with 70.58%. The exception was AVPS Miorita, where a higher quota was approved in 2019-2020 compared to 2018-2019.

Regarding the achieved quotas, in none of the analyzed seasons they did not reach the level of the approved quotas, there being managers who had a very low degree of accomplishment. Thus, in 2018-2019 a share of 0% was achieved by Trei Stejari Association, 2.17% by AVPS Miorita, 8.33% by AV Predesti Habud, 23.78% by AJVPS Tulcea, 31.34% by San Rafael. The highest part was achieved in 2018-2019 by the RNP manager, with 84.03%.

In 2019-2020, the lowest quotas were achieved by AV Predesti Habud (0%) and AVPS Miorita (2.77%). The other managers achieved higher quotas than in 2018: 40% at AVPS Crângul Slava Rusă and Asociatia Trei Stejari, 51.42% at RNP and 64.28% at AJVPS Tulcea. The manager of AV San Rafael had the highest share of achievement in 2019-2020, with 86.6%.

CONCLUSIONS

It is certain that in the field the herds remained constant until 2019, when they suffer a dramatic decrease in the number of wild boar

specimens, in some areas of the county, the trend being maintained in 2020. This situation is signaled by most of those who follow the evolution of this species.

An explanation for the low values of quotas achieved in 2018-2019 and 2019-2020 seasons, may exist in the manifestation and rapid spread in Tulcea County of African swine fever, which has decimated a large part of the wild boar herd.

African swine fever (ASF) is a devastating, usually fatal, infectious disease of pigs and wild boars for which there is no vaccine (Popescu & Nicolae, 2020).

Hunters can change things, for better or worse, because they can increase or reduce the spread of the disease.

Hunters can contribute to the spread of the disease by any contact with infected animals and dead bodies (carcasses), contact with any object contaminated with the virus (e.g. clothing, vehicles, other equipment), feeding animals with meat or meat products from infected animals (e.g. unprocessed meat) or scraps containing infected meat (e.g. kitchen waste, pig feed, including eatable offal).

EU and national authorities in the affected countries need to take a wide range of measures to combat and eradicate African swine fever. Cooperation with hunters and their associations is vital. Hunters can and should monitor the health status of wild animals and play a key role in protecting the health of animals, including domestic animals.

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***MMAP Order, no. 540/2018, regarding the approval of harvest quotas for some species of fauna of hunting interest.

***MMAP Order, no. 827 / 23.08.2018, on the approval of measures to combat African swine fever.

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THE EFFECT OF PROBIOTIC BETAPLUS®ULTRA ON HEMATOLOGICAL PROFILE AND IMMUNE RESPONSE OF YOUNG OF THE YEAR *ACIPENSER STELLATUS*

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Abstract

This experiment was carried out to evaluate the effects of the commercial probiotic Betaplus®Ultra on the haematological profile (red blood cell (RBC) counts, haemoglobin (Hb) concentration, haematocrit (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC), glucose and total proteins) and immune response of young of the year Acipenser stellatus. In this sense, a 93-day experiment was carried out with six experimental variants (five different probiotic concentrations: C_1 - 1-1.28×10^{13} CFU, C_2 - 2.56 ×10^{13} CFU, C_3 - 3.84×10^{13} CFU, C_4 - 5.12×10^{13} CFU, C_5 - 6.4×10^{13} CFU and C_0 - control group). All the trials were performed in duplicates. Based on our results, no significant differences ($p>0.05$) were observed in the values of RBC, PCV, MCV and MCHC, while significant differences ($p<0.05$) were registered in the case of Hb and MCH. Regarding the immune response, no significant differences were registered in the values of leukocytes, lymphocytes neutrophils, monocytes and eosinophils ($p>0.05$) and only, thrombocytes and basophiles were significantly influenced ($p<0.05$) by the probiotic concentration. The results suggest that the probiotic Betaplus®Ultra could be used effectively as a probiotic for the use in aquaculture.

Key words*: haematological parameters, immune response, probiotic, sturgeons.*

INTRODUCTION

Sturgeons are among fish with a very high economic value because of their caviar and meat. Among sturgeons, *Acipenser stellatus* is one of the three most important species for caviar (Frimodt, 1995).

Due to overfishing (Aghilinejhad et al., 2018), poaching (Bloesch et al., 2006), pollution or dam construction (Reinartz and Slavcheva, 2016), the sturgeon population experiencing a severe decline (Lenhardt et al., 2006; Bronzi and Rosenthal, 2014; EUMOFA, 2018).

Although considerable efforts have been made during the last years and several conservation measures have been taken, their status continuously decreased and nowadays almost all sturgeon species are listed as critically endangered (Qiwei, 2010).

In this context sturgeon aquaculture can meet the demand of the population for meat and caviar, thus reducing the pressure on natural sturgeon resources (Bronzi and Rosenthal, 2014; Vasilyeva et al., 2015).

To be profitable aquaculture of sturgeon's farms involves the growth of fish in high stocking densities (Rafatnezhad et al., 2008), which can lead to deteriorating of water quality, increase stress and raise susceptibility to a wide range of diseases (Ni et al., 2016; Long et al., 2019).

Therefore, over recent decades, this increase in productivity has been accompanied by the enlarged use of chemicals, especially antibiotics or other substances (Salah and Aqel, 2014). However, there is a real concern regarding the use of antibiotics in aquaculture, because can modify the intestinal microbiota and give rise to resistant bacteria, which could be harmful to aquatic organisms (Dawood et al., 2018), or can give antibiotic resistance at human and cause severe environmental problems (Mo et al., 2017). In Europe, the use of antibiotics is under strict control and

regulatory measures, and only a few antibiotics are approved for use in aquaculture (European Council, 2001).

In this sense, probiotics can be an alternative to substitute antibiotics or other chemicals (Sayes et al., 2018). According to Gatesoupe, 1999, probiotics are defined as microbial cells that are administered in such a way as to enter the gastrointestinal tract and to be kept alive, to improve health. The use of probiotics in aquaculture proved to have several benefits, such as: improving growth performance by stimulating fish appetite (Lara-Flores and Olvera-Novoa, 2013; Opiyo et al., 2019), enhances the immune response and fish welfare (Nayak, 2010), reduce mortality, increased survival, improved resistance against disease (Safari et al., 2016; Hoseinifar et al., 2018), and improves the water quality by modifying microbial communities of water and sediments (Verschuere et al., 2000).

Bacillus genus is among the most frequently used probiotic microorganisms used in aquaculture (Nwanna, 2015). It is proved that *Bacillus subtilis* produce compounds with antimicrobial properties such as antifungal lipopeptides, being very effective to fungal pathogens and a wide variety of microorganisms (Korenblum et al., 2003; Ongena and Jacquea, 2007).

Previous studies were conducted to determine the effects of *Bacillus subtilis* and *Bacillus licheniformis* for several fish species (Azarin et al., 2015; Romanova et al., 2020) but, from our knowledge so far, there is no published information concerning the effects of these probiotics on *A. stellatus* welfare.

Therefore, this study was performed to investigate the effect of probiotic BetaPlus®Ultra on the haematological profile and immune response of stellate sturgeon.

MATERIALS AND METHODS

Experimental design. This study was conducted in a commercial sturgeon farm located in Horia, Tulcea County, Romania. The experimental design was randomized, composed by six treatments in two replications: one control group, feeding continuously only with basal diet (C_0), and five groups feeding with a basal diet supplemented with probiotic in different concentration: C_1 - 1.28×10^{13} CFU (colony-forming units), C_2 - 2.56×10^{13} CFU, C_3 - 3.84×10^{13} CFU, C_4 - 5.12×10^{13} CFU, C_5 - 6.4×10^{13} CFU. The probiotic BetaPlus® (BioChem Co., Germany) was in a powder form and contained a 1:1 ratio of *Bacillius subtilis* (DSM5750) spores, and *Baciulius licheniformis* (DSM 5749) spores.

Water quality parameters included: temperature 23.81 ± 2.43°C, pH 8.41 ± 0.15, dissolved oxygen 6.46 ± 0.32 mg L^{-1}, nitrite ($N\text{-}NO_2^-$) 0.03 ± 0.01 mg L^{-1}, nitrate ($N\text{-}NO_3^-$), and ammonium ($N\text{-}NH_4^+$) 0.22 ± 0.16 mg L^{-1} were in the optimal range for sturgeon's growth, Mims et al., 2002.

Fish. The experiment started with 6000 fish of 35-day post hatched larvae (500 fish per each tank) with a mean individual weight (± SD) of 0.35 ± 0.02 g and a total length between 3-5 cm. The experimental conditions and the growing system were presented in our previous paper (Stroe et al., 2019). The experiment lasted for 93 days and was performed in duplicate.

Collection of the blood samples. At the end of the trial, feeding was stopped for 24 hours and blood samples were collected from seven fish from each experimental variant, from the vein of the caudal region of anesthetized fish. Seven fish per tank (14 fish per treatment) were randomly sampled, anesthetized with 2-phenoxyethanol (8 mL 40 L^{-1} of water for 5 minutes) to reduce handling stress.

For the determination of red blood cells (RBC, 10^6 μl^{-1}), haematocrit (PCV, %), haemoglobin concentration (Hb, g dL^{-1}), blood samples were taken using heparinized syringes, while for the determination of blood glucose (GLU, g dL^{-1}) and proteins (TP, mg dL^{-1}) we used syringes without anticoagulant and allowed to clot for 4 h, then centrifuged in microtube centrifuge at 3,000×g for 5 min.

At the same time, blood smears were made, fixed with absolute Methanol, and allowed to air-dry. The fixed smears were then coloured using May-Grünwald and Giemsa method and examined microscopically using immersion ×100 objective. In total, we analyse 84 blood smears. All areas in each smear were scanned and the percentage of leukocyte cells (monocyte, lymphocyte, neutrophils, and eosinophils) was determined by counting a total

of 200 leukocyte cells, using Zeizz Axio Imager microscope. The absolute number of circulating blood leukocytes and thrombocytes were determinate concerning 1000 erythrocytes and converted to unit blood volume.

Immediately after blood extraction, the red blood cells were counted using a haemocytometer (Improved Neubauer Weber Scientific Ltd.). The haematocrit percentage (PCV, %) was determined using a heparinized haematocrit capillary tube which was filled with blood (30 µl) and centrifuged for 5 minutes at 12000 rpm in a microhematocrit centrifuge. Haemoglobin (Hb, g dL^{-1}) concentration was determined using the cyanmethemoglobin method. For this purpose, 20 µL of whole blood was added to 5 ml of Drabkin's solution and then after an incubation of 30 minutes, the mixture was read at 540 nm. Lately, the haemoglobin (Hb), haematocrit (Ht), and red blood cell (RBC) values were used to calculate the following constants: the mean corpuscular volume (MCV, μm^3), mean corpuscular haemoglobin (MCH, pg), and the mean corpuscular hemoglobin concentration (MCHC, g dl^{-1}).

Statistical analysis. The results are presented as means ± SD, the difference between experimental variants was analysed by ANOVA and statistical assessment of the result was carried out using SSPS software 21 version. The Kolmogorov-Smirnov test was used to confirm the normality and homogeneity variances and if significant differences were found the Post-hoc comparisons were conducted with the help of Duncan's test. The differences were considered to be significant at $p<0.05$.

RESULTS AND DISCUSSIONS

Research presented in this paper is corroborated with those obtained for growth performance. From the analysis of growth performance, it was concluded that fish from the C_2 variant recorded the best growth performance (Stroe et al., 2019). In Table 1 are presented the results of the haematological and biochemical parameters of *A. stellatus* fed with different concentrations of probiotics. From the statistical analysis, significant differences (ANOVA, $p<0.05$) were recorded in the concentration of haemoglobin and MCH. The addition of probiotic BetaPlus®Ultra to the diet had no significant influence (ANOVA, $p>0.05$) on erythrocyte count, haematocrit, the mean corpuscular volume, mean corpuscular haemoglobin concentration, total proteins, and glucose.

The post hoc Duncan test showed that the haemoglobin concentration from the group C_1, C_4, and C_5 was significantly higher than those from the C_0, C_2, and C_3. The increase of Hb in fish blood may indicate the immunostimulant effects and antiinfection properties of this probiotic. Haemoglobin is an indicator for oxygen transportation capacity of fish and according to Talpur and Ikhwanuddin (2012) increase in haemoglobin concentration has an important role in improving the well-being of fish and enhancing the immunity and growth. Increasing haemaglobin after the administration of a mixture of probiotics which contains *Bacillus subtilis* in the diet of *Acipenser baerii* fingerlings was also reported by Sayed Hassani et al., 2020.

Regarding the MCH values, the post hoc analyses showed three different groups. The lowest MCH concentration was obtained in the C_3 and C_4 ($p>0.05$), followed by the variant C_2 and C_1 ($p>0.05$) and C_5, C_0 respectively ($p>0.05$).

The blood biochemical indicators did not show significant differences (ANOVA, $p>0.05$) between the experimental variants. Although the TP did not register significant differences, an increase of their values was observed in the C_2 variant, where also was obtained the best growth performance. Some authors suggested that TP decrease under long-term exposure to stressful conditions (Sala-Rabanal et al., 2003). However, all the haematological results obtained by us for *A. stellatus* are in line with those obtained by other authors (Table 2).

Microscopic examination of blood smears shows no morphologic changes among leukocytes. After microscopic examination of blood smears, it was observed that lymphocytes dominated in comparison with the other types of leukocytes, being present in a very large number (Figure 1).

Table 1. Haematological and biochemical parameters of *A. stellatus* fed different concentrations of probiotics

Experimental variants	RBC, (10^6 μL^{-1})	PCV (%)	Hb (g dL^{-1})	MCV (μm^3)	MCH (pg)	MCHC (g dL^{-1})	TP (g dL^{-1})	GLU (mg dL^{-1})
C_0	0.71±0.11^a	23.43±2.76^a	6.24±0.79^b	333.37±54.14^a	88.70±13.81^b	26.7±2.01^a	4.41±0.14^a	58.11±6.13^a
C_1	0.95±0.26	24.14±2.27	6.60±0.48	270.22±73.32	74.07±20.67	27.41±1.72	4.37±0.66	56.14±6.71
C_2	0.90±0.17	24.00±1.53	6.32±0.36	275.46±51.99	72.17±11.46	26.38±1.60	4.66±0.18	55.90±4.63
C_3	1.00±0.23	23.43±2.15	5.95±0.40	245.87±64.16	62.42±15.57	25.53±2.08	4.38±0.17	56.95±5.43
C_4	1.01±0.21	25.29±2.56	6.81±1.54	258.68±49.07	69.02±15.35	26.71±3.57	4.45±0.17	52.71±3.30
C_5	0.85±0.23	26.14±1.86	7.54±0.98	331.03±109.73	93.62±26.31	28.96±4.30	4.16±0.20	57.00±4.96
ANOVA	0.11	0.17	0.03	0.10	0.02	0.31	0.14	0.52

Note: Values are presented as mean±SD;

Table 2. Reference values of haematological and biochemical parameters of sturgeons

References	RBC, (10^6 μL^{-1})	PCV (%)	Hb (g dL^{-1})	MCV (μm^3)	MCH (pg)	MCHC (g dL^{-1})	TP (g dL^{-1})	GLU (mg dL^{-1})
Grecu et al., 2019	0.555÷1.23	14.46±3.69	5.37±0.69	176.21±40.03	-	-	-	-
Khara et al., 2013	1.36±0.86	6.55±0.46	26.6±0.86	517±11 (fl)	126±0.22	24.5±0.34		
Dorojan et al., 2015	-	-	-	-	-	-	1.96±0.25	44.00±2.00

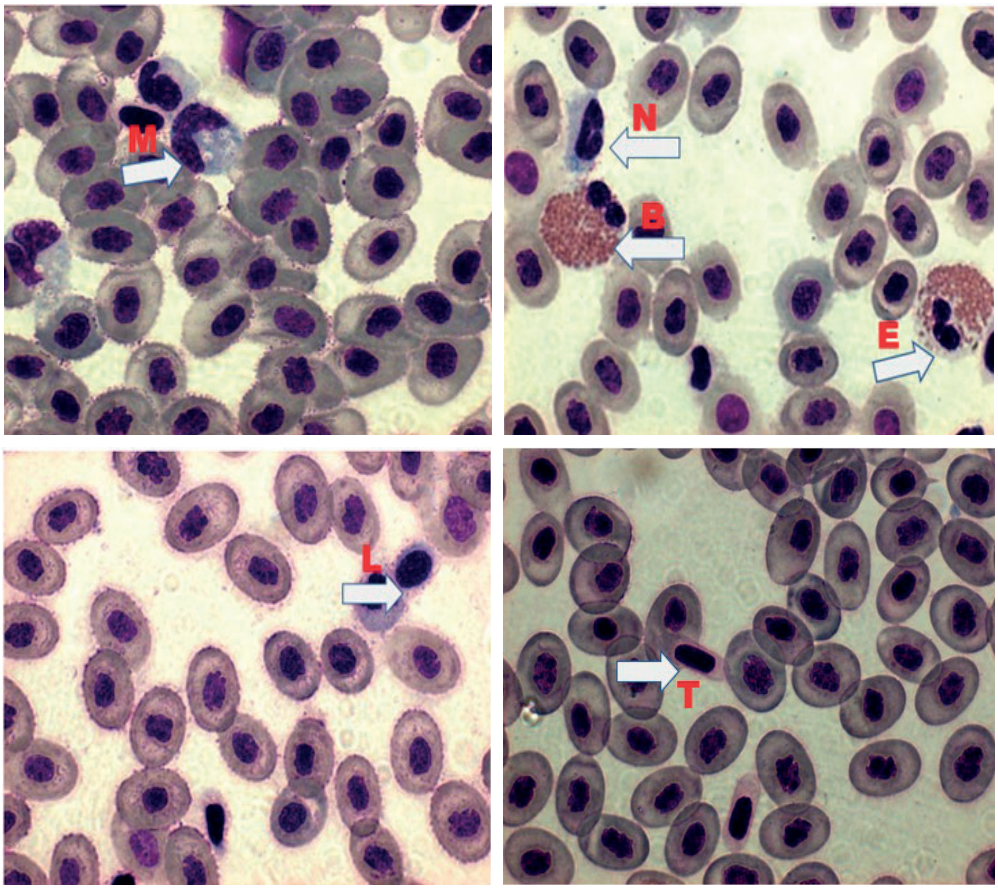

Figure 1. The microstructure of peripheral blood cells of *A. stellatus* ×100
(M - monocytes; N - neutrophils; B - basophils; E - eosinophils; L - lymphocytes; T - trombocytes)

Leucocytes formula of the stellate sturgeon reflects the prevalence of the percentage of lymphocytes reported on all leukocytes, followed by neutrophils, eosinophils and monocytes (Table 3). The statistical analysis ANOVA, revealed no significant differences ($p>0.05$) in the percent of lymphocytes, monocytes, neutrophiles and eosinophils, while

significant differences ($p<0.05$) were recorded in the percentage of basophils.

Regarding the absolute number of leukocytes (Table 4), no significant differences were registered in the values of leukocytes, lymphocytes neutrophils, monocytes, and eosinophils ($p>0.05$) and only, thrombocytes and basophiles were significantly influenced ($p<0.05$) by the probiotic concentration.

The innate cellular immune system is formed by a series of cells which are vital to the survival of the host (Nakandakare et al., 2013). Generally, fish thrombocytes are involved on the animal's defence mechanism, in phagocytosis, blood clotting, and other possible immunologic functions (Nagasawa et al., 2014; Bozzo et al., 2007).

Table 3. The relative number of leucocytes at the end of the experimental period

Relative number (%)	Experimental variants						
	C_0	C_1	C_2	C_3	C4	C_5	ANOVA
Lymphocytes	86.99±2.49	89.91±4.77	91.32±1.66	90.67±2.73	87.09±3.75	88.41±4.10	0.18
Monocytes	0.58±0.08	0.35±0.04	0.16±0.25	0.33±0.25	0.29±0.43	0.35±0.36	0.70
Neutrophiles	7.71±1.89	4.59±2.25	4.58±1.61	4.83±0.98	4.58±1.27	6.31±3.22	0.05
Eosinophils	4.47±1.8	3.71±1.97	3.47±1.15	3.75±2.32	3.47±3.19	4.59±1.88	0.45
Basophiles	0.25±0.27	1.54±0.95	0.48±0.41	0.42±0.25	0.48±0.26	0.63±0.46	0.001

Note: Values are presented as mean±SD;

Table 4. The absolute values of leucocytes at the end of the experimental period

Absolute number ($x10^3$ μL^{-1} blood)	Experimental variants						
	C_0	C_1	C_2	C_3	C_4	C_5	ANOVA
Leukocytes	38.17±7.59	58.04±20.90	59.03±16,46	47.51±19,52	47.20±11.92	52.96±28.31	0.43
Lymphocytes	33.23±6.81	51.47±15.53	53.93±15,11	46.82±25,10	40.73±9.13	47.19±25.86	0.42
Monocytes	0.21±0.23	0.29±0.54	0.11±0,19	0.15±0,15	0.15±0.25	0.18±0.27	0.25
Neutrophiles	2.96±1.03	2.87±2.51	2.71±1,39	2.24±0,90	3.28±1.28	2.50±1.13	0.92
Eosinophils	0.48±0.23	0.49±0.31	0.46±0.16	0.31±0,17	0.61±0.53	0.58±0.39	0.65
Basophiles	0.02±0.03	0.18±0.12	0.07±0.06	0.04±0,03	0.03±0.03	0.07±0.06	0.002
Thrombocytes	9.95±2.02	20.48±16.28	22.82±11.07	31.79±9.42	30.65±10.09	13.53±8.86	0.006

Note: Values are presented as mean±SD;

Increasing of fish thrombocytes in variants where probiotic was administrated can suggests an improvement of immune system of fish. Stimulation of the immune response of sturgeons through dietary supplements represents a great interest for aquaculture (Seyed et al., 2014). Improvement of fish immune response after dietary supplementation with *Bacillius subtilis* was also reported in the case of Persian sturgeon (*Acipenser persicus*) fingerlings (Darafsh et al., 2020). The authors reported a significant impact on the RBC count, PCV, and percentage of neutrophils. Also, Faeed et al. (2016) showed that out of all the blood parameters, only haematocrit and MCV are significantly increased when *Enterococcus faecium* is added to the diets of *Sander lucioperca* as a probiotic. According to Kumar et al. (2008) probiotics interact with the immune cells such as monocytes and neutrophils cells to enhance innate immune responses.

CONCLUSIONS

According to the results obtained from the hematological examination of *A. stelatus*, it can be concluded that the addition of feed with the commercial probiotic BetaPlus®Ultra could improve both the haematological and immunological responses. Obviously, these probiotics boost the immune system to defend the body against pathogenic organisms, but further researches must focus on the effect of this probiotic to digestive enzymes, and challenge test in order to validate the effectiveness of this probiotic on fish resistance to disease.

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A CASE REPORT ON FISH TUBERCULOSIS ("FISH HANDLERS' DISEASE") IN RAINBOWFISH (FAM. MELANOTAENIIDAE) AND ROSY BARB (*PETHIA CONCHONIUS*)

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Abstract

Fish mycobacteriosis is caused by atypical Mycobacterium species, some of which can be transmitted to humans. Often, M. marinum, M. fortuitum and M. ulcerans have been described as with the potential to cause fish-borne zoonoses. M. fortuitum and M. marinum are most commonly reported in tropical fish. These are Gram-positive, aerobic, non-motile rods. Humans may become infected by contact with the diseased or infected fish or with contaminated aquarium water, via lacerated or abraded skin. We were called by a client to see some ornamental fish about which the client complained of being off feed for several days, lethargic, in poor condition, showing dropsy, scale loss and abnormal swimming. We asked about the history of these fish, we inspected the fish and tested the water. Three fish (two rosy barbs and one rainbowfish) showing overt signs of the disease, were retained for further investigations. Following euthanasia of the fish, we carried out post-mortem and histopathological examinations. Following the investigation the fish were confirmed infected with atypical Mycobacterium spp.

Key words*: fish tuberculosis, rainbowfish, rosy barb.*

INTRODUCTION

The ornamental fish market is a big industry, involving over 120 exporting and/or importing countries all over the world. It is valued at around US$15 - 30 billion per year. Over 2,500 freshwater and marine fish species are involved in this commercial activity, with over 60% freshwater fish traded globally (Dey, 2016). The United States is the world's largest importing market of ornamental fish, with about 10 million marine fish species imported annually (Biondo & Burki, 2020; Rhyne et al., 2017), of US$ 42.9 million worth (Dey, 2016). Singapore, Indonesia, Thailand, Hong Kong, the Philippines, Malaysia, Japan, Colombia, Peru and Brazil are catered to this market. With over 160 million ornamental fishes kept by hobbyists in the United States [Dey, 2016], ornamental fish is one of the most common pets in the country. With US$ 29.5 million worth imports, in 2014 (Dey, 2016), the United Kingdom was the second world's importer of ornamental fish. Other importing countries holding stable shares in the market are Germany, Singapore, Japan, China and Hong Kong, France, the Netherlands, Italy, Malaysia, Canada and Belgium. Like Singapore, Germany, Hong Kong, Malaysia and the Netherlands are important trading hubs, re-exporting a major portion of their imports (Dey, 2016).

One of the problems associated with this trade is the lack of appropriate disease monitoring and reporting systems, such as those implemented for the terrestrial livestock trade and husbandry. Humans act as the main connecting interface between domestic and wild habitats facilitating the introduction of pathogens existing in the wild, but new to domestic animal populations, including of pathogens with zoonotic potential (Wobeser, 2006). Compared to their terrestrial counterparts, aquatic animals seem to be involved in a smaller number of zoonotic reported cases. As in the case of zoonoses from terrestrial animals, zoonotic pathogens of fish can transmit to humans through ingestion of, or contact with these pathogens (i.e. via broken, abraded or chapped skin), which may be present on the skin, fins, gills of the infected fish, as well as through contact with, or

consumption of the infected or contaminated fish (food-borne zoonoses), (Evans et al., 2009; Haenen et al., 2013). Some diseases can also be contacted through accidental ingestion of the water contaminated with faeces, skin mucus or other physiological products of the infected aquatic animals.

Fish mycobacteriosis is caused by atypical *Mycobacterium* species. These are Gram-positive, acid-fast, aerobic, non-motile rods, which can be cultured on blood agar or specialized Löwenstein-Jensen or Middlebrook media (Stephenson et al., 2019; Radomski et al., 2010; Griffith et al., 2007; CLSI, 2008; Saitoh et al., 2000). Growth of these mycobacteria is slow. It may take between 2 weeks and several months for the colonies to develop.

Mycobacterium marinum, *M. fortuitum* and several other *Mycobacterium* species are the cause of common, chronic, severe granulomatous systemic disease in ornamental fish and other aquatic animal species.

Any species of fish, amphibians, reptiles, birds, and mammals, can be considered susceptible. These *Mycobacterium* spp. mildly psychrophilic and mesophilic organisms and typically cause infections in the extremities of man, where temperature is generally lower than 33°C.

Other *Mycobacterium* spp. are described as nontuberculous mycobacteria (NTM) and exclude *Mycobacterium tuberculosis* complex and *Mycobacterium leprae* from the group. There are approximately 150 species of NTM (Bi et al., 2015), with *M. marinum*, *M. fortuitum*, and *M. ulcerans* discussed most commonly as fish-borne zoonoses (Boylan, 2011).

These pathogens have proven a zoonotic potential, causing granulomatous nodules at the site of entry, on hands and arms (hence the disease' names, "fish handlers' disease" or "fish tank granuloma"). Humans are typically infected by contamination of lacerated or abraded skin with aquarium water or fish contact. Other sources of infection are spine punctures, hand scratches on fish tanks, mouth-siphoning fish tanks, splinters from fish net handles, etc. The localized granulomatous nodule usually forms at the site of infection, most commonly, on hands or fingers, approximately 6-8 weeks after exposure to the organism.

In healthy children, lymphadenitis caused by the infection with *M. haemophilum* has been also reported in some cases involving zebrafish (Lindeboom, 2011; Whipps, 2007). In immuno-compromised patients, atypical mycobacteria can cause systematic disease, pneumonia and osteomyelitis.

In tropical fish, the most commonly found mycobacteria are *M. fortuitum* and *M. marinum*. In fish, the infection may be clinical (acute or chronic) or sub-clinical (inapparent), the latter being more prevalent in these fish.

Although any species of fish, can be infected with the bacteria, only those more susceptible to the disease will develop the clinical form. Many aquarium fish and other species may have the infection with no clinical signs. There are also fish which develop the chronic form, remaining carriers for long time. In all these instances, the bacteria is shed into the water, where it can resist for long time, due to its thick walls and capacity to stick to the biofilm.

Stress and overpopulation are usually favouring factors for the bacteria to spread into an aquatic system (Alexander et al., 2021). There are no effective clinical tests to identify the infected fish, nor are there effective treatments or vaccines available.

Fish with mycobacteriosis may present with multiple and varied clinical signs, such as exophthalmia, lethargy, scale loss, abdominal distention, poor body condition, and skin ulcers.

Those fish with sub-clinical (inapparent) mycobacteriosis shed the bacteria without overt disease signs, for several weeks or months. Other fish may undergo acute infections, shedding the bacteria prior to the onset of the clinical symptoms. The faecal-oral route is the main route of transmission among fish. Fish may get infected also via ingestion of infected tissues.

However, release of infectious organisms from infected gill tissue or ulcerated skin lesions are also potential dissemination routes, through which the *Mycobacterium* spreads within the water (Niemeyer-Corbellini et al., 2017).

Poor body condition and abnormal swimming, as well as scale loss, skin ulcer, pigment changes and dropsy are commonly seen in those highly sensitive fish.

Since mycobacteria can resist for months in aquarium water, and because usual disinfect-tants, such as dilute sodium hypochlorite and quaternary ammonia compounds are not effective in the case of atypical mycobacteria,

contaminated water remains the most common source of infection for both susceptible fish and humans (Boylan, 2011).

Biofilms protect the atypical mycobacteria making it resistant to these disinfectants (Boylan, 2011). To properly disinfect an aquatic system containing the mycobacteria it priorly requires removal of the biofilm by mechanical scrubbing, to allow disinfectants to effectively kill the mycobacteria. In the case, the most effective disinfectants are phenols, high concentrations of alcohol, and strong sodium chlorite solutions. Safety measures for disposal of contaminated aquarium water should also be accounted for.

As nontuberculous mycobacteria can be found in freshwater fish and their products, it requires appropriate handling and treatment before consumption (Lorencova et al., 2013).

This paper will describe a case of fish tuberculosis (fish mycobacteriosis) in a multi-species freshwater tropical fish tank.

MATERIALS AND METHODS

The fish were being kept in a well tank (Figure 1) of 600 liters. In the tank, there were around 70 to 80 fishes. The fish species present in the aquatic system were: rummynose tetra, zebra Danio, silver dollar, rainbowfish, harlequin rasbora, rosy barb, black neon tetra, neon tetra, black phantom tetra, serape tetra and silver tip tetra.

Figure 1. The fish tank

To test the water tank we used the Sera test kit. The water was tested for pH, ammonia, nitrite, nitrate, KH and temperature. CO2 was derived from tables.

To euthanize the sampled fish, we used immersion of the fish in a bucket of water with iso-eugenol (Aqui-S®). Ten minutes after the respiratory (opercula) movements stopped, we proceeded to the necropsy and collected samples for histo-pathology. For the latter stage, we used light microscopy.

RESULTS AND DISCUSSIONS

One rosy barb had been put by the owner in a bucket with water, for a closer inspection.

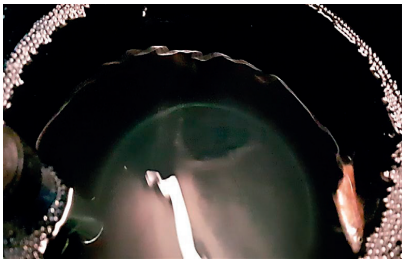

Figure 2. Rosy barb, disoriented

In that fish, the owner reported incoordination of swimming (flashing) and loss of appetite (Figure 2). We further noticed membranous faecal string (diarrhoea) and dyspnoea in that fish Water testing (Figure 3) indicated that the water parameters were close to optimal (Figure 4).

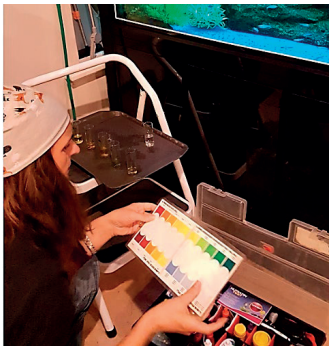

Figure 3. Testing of the tank water

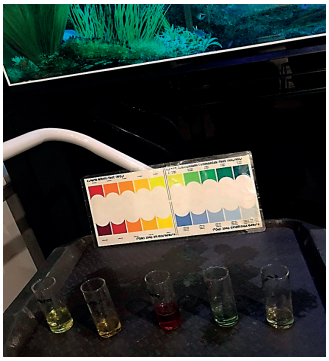

Figure 4. Water testing -the main parameters tested were close to optimum levels

Following inspection of the aquasystem, we noticed a few other rosy barbs and rainbow fishes showing more or less overt signs as those displayed by the previously inspected fish. In addition to these signs, the affected fish were also showing abdominal distention, loss of scales, skin ulcers and haemorrhages. The bucket fish and two additional diseased fishes from the tank (one rosy barb and one rainbow fish), were captured, euthanized, and were then subjected anatomo-pathological and histo-pathological examinations (Figure 5).

Figure 5. Rosy barb, abdominal distention, loss of scales and haemorrhage

The granulomatous inflammation of various organs was consistently seen in all the euthanized fish. The clinical signs previously seen in these fish correlated with the organ most severely affected. The kidney and heart of the fish with dropsy were most affected by the nodules. In the fish with spiraling swimming (the rosy barb in the bucket, Figure 2) the granulomatous lesions were also present in the brain. The other rosy barb, sampled from the fish tank, was having systemic mycobacteriosis, with the nodules affecting most organs, including the intestines (Figure 6).

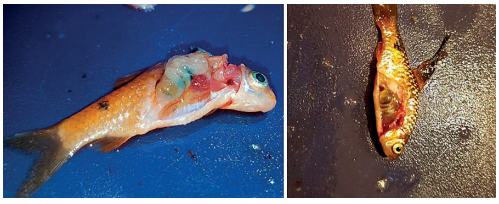

Figure 6. Mycobacteriosis-like granulomatous nodules in rosy barb

The histo-pathological exam revealed the presence of multiple granulomatous inflammatory reactions in the skin, spleen, kidney, heart and brain (Figures 7-9).

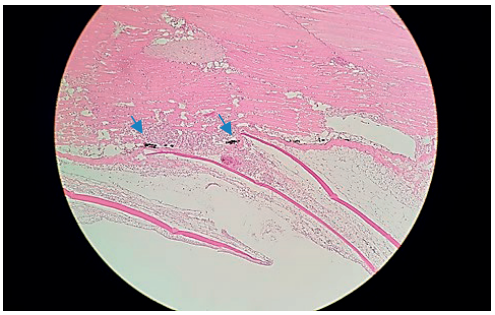

Figure 7. Ulcerative dermatitis and granulomatous inflammation, infection with Mycobacterium (rosy barb)

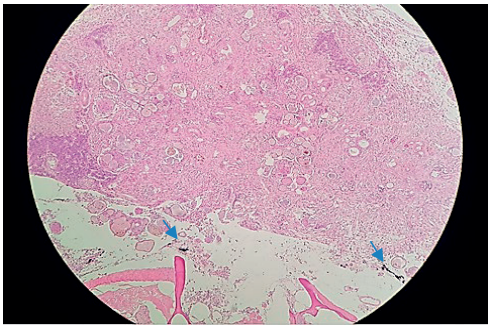

Figure 8. Granulomatous inflammation, infection with Mycobacterium in kidney (rainbowfish)

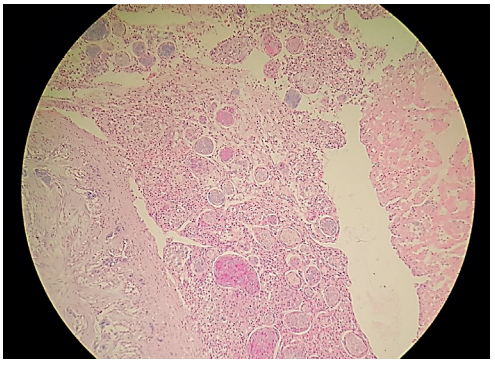

Figure 9. Granulomatous inflammation, infection with Mycobacterium in heart (rosy barb)

CONCLUSIONS

Since from the eleven species kept in the tank only rainbowfish and rosy barb (sub-tropical species) were showing clinical signs, it can be concluded that these two species were more susceptible to the disease than the tropical fish with which they shared the tank, i.e. rummynose tetra, zebra Danio, silver dollar, harlequin rasbora, black neon tetra, neon tetra, black phantom tetra, serape tetra and silver tip

tetra. It is also likely that the fish were immuno-supressed due the water temperature, which had been set to 30°C to accommodate the requirements for the tropical fish in the tank, but which was higher than normal for them.

The owner was advised to do frequent water changes, gradually reduce the water temperature from 30°C to 24°C, avoid as much as possible stress in the fish, and reduce the density of the fish in that tank.

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NEW DATA ON THE PARASITES AND THE PARASITE COMMUNITIES OF *CHONDROSTOMA NASUS* (LINNAEUS, 1758) FROM THE DANUBE RIVER, NORTHWESTERN BULGARIA

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Abstract

In the spring of 2020, ecoparasitological studies were conducted on 30 specimens of common nase (Chondrostoma nasus (Linnaeus, 1758)) from the freshwater ecosystem Danube River near Kudelin village, northwestern Bulgaria. Two parasite species were determined. One species - Proteocephalus torulosus (Batsch, 1786), juvenile, belongs to the class Cestoda. The other one - Contracaecum sp., larvae, belongs to the class Nematoda. C. nasus is a new host for P. torulosus in the studied area. In the study, Contracaecum sp., larvae was a core parasite species (P% = 90.00) in the component community of C. nasus from the Danube River (Kudelin). For Contracaecum sp. larvae, the highest mean intensity (MI = 18.15) and the highest mean abundance (MA = 16.33) were found.

Key words*: Bulgaria, Chondrostoma nasus, Danube River, parasites, parasite communities.*

INTRODUCTION

The Danube River crosses the territory of ten countries in Europe (Sakan et al., 2011), connecting many cities located along it, including four European capitals – Vienna, Bratislava, Budapest, and Belgrade (Baltălungă & Dumitrescu, 2008). The river is 2,857 km long (Mocanu et al., 2020) and reaches the Black Sea (Sakan et al., 2011). The Danube River is characterized by having a great variety of fish, over 100 species (Ibănescu et al., 2020). The ichthyofauna of the Bulgarian section of the river is also rich (www.bd-dunav.org). The fish parasites are also an important element of biodiversity in freshwater ecosystems (Scholz, 1999). Various authors present data on parasites on *Chondrostoma nasus* (Linnaeus, 1758) from the Danube River basin (Djikanović et al., 2012; Djikanović et al., 2013; Marković & Novakov, 2015, etc.). Parasitological studies of common nase from the Bulgarian section of the Danube River are few (Kirin et al., 2013; Zaharieva & Kirin, 2020b; Zaharieva & Zaharieva, 2020a; 2020b). Various authors have conducted investigations on parasites of different fish species (bleak, *Alburnus alburnus*; freshwater bream, *Abramis brama*; barbel, *Barbus barbus*)) from the Danube River's Bulgaria section (Chunchukova & Kirin, 2017; Chunchukova et al., 2017; Chunchukova & Kirin, 2018; Chunchukova et al., 2018; Chunchukova & Kirin, 2020; Zaharieva & Kirin, 2020a; Zaharieva & Zaharieva, 2020c; 2020d). The present study aims to provide new data on the parasites and the parasite communities of common nase (*Chondrostoma nasus* (L., 1758)) from the Danube River near the Kudelin village, northwestern Bulgaria.

MATERIALS AND METHODS

Thirty specimens of *Chondrostoma nasus* (Linnaeus, 1758) were collected from the Bulgarian section of the Danube River after the river enters Bulgaria's territory, close to the village of Kudelin. The Kudelin village is located on the Vidin area territory, in northwestern Bulgaria, about 1.5 – 2 km from the Bulgarian-Serbian border (Figure 1). The fish were caught on the base of a fishing permit issued by the Executive Agency of Fisheries and Aquaculture. The scientific name of the species is presented by Froese & Pauly (2019). For all caught specimens, maximum length and a maximum height of the body, as well as weight (L, H and G), were recorded (Table 1).

Table 1. Length, height and weight (L, H and G) of *Chondrostoma nasus* from the Danube River (Kudelin)

CHONDROSTOMA NASUS **(N = 30)**	**MIN-MAX**	**MEAN ± SD**
L (cm)	30.9-38	33.45 ± 1.96
H (cm)	6.5-9.8	8.09 ± 0.75
G (g)	280-506	369.33 ± 55.04

Figure 1. Danube River (Kudelin) (www.icpdr.org)

All 30 specimens of common nase were subjected to ecoparasitological examination by the methods specified by Petrochenko (1956); Zashev & Margaritov (1966); Kakacheva-Avramova (1983); Bauer (Ed.) (1987); Moravec (2013). For each identified parasite species, mean intensity (MI), mean abundance (MA), and prevalence (P%) were recorded according to Bush et al. (1997). In accordance with the prevalence, the parasite species were defined as accidental (P% < 10), component (10 < P% < 20) and core (P% > 20) by Kennedy (1993). In the study, were reported the total number of species, the mean number of parasites and the Brillouin's diversity index (HB) (Magurran, 1988).

RESULTS AND DISCUSSIONS

During the spring of 2020, 30 specimens of *Chondrostoma nasus* were caught from the Danube River near the village of Kudelin.

The collected specimens were subjected to ecoparasitological investigation.

The common nase (*Chondrostoma nasus* L., 1758) belongs to the family Cyprinidae. The species is found in the Danube River and other rivers on the territory of Bulgaria.

The common nase uses aquatic plants for food (Karapetkova & Zhivkov, 2006).

Helminth community structure

For the spring of 2020, two species of parasites were identified - one species from class Nematoda (*Contracaecum* sp., larvae) and one species from class Cestoda (*Proteocephalus torulosus* (Batsch, 1786), juvenile) (Table 2).

Table 2. Diversity of parasite species of *Chondrostoma nasus* from the Danube River (Kudelin village) in 2019 and 2020

Parasite species	***Chondrostoma nasus*, Danube River, Kudelin**	
	Spring, 2019 (Zaharieva & Kirin, 2020b)	**Spring, 2020**
Allocreadium isoporum (Looss, 1894)	•	
Bothriocephalus acheilognathi (Yamaguti, 1934), immature	•	
Proteocephalus torulosus (Batsch, 1786), juvenile		•
Pomphorhynchus laevis (Müller, 1776)	•	
Raphidascaris acus (Bloch, 1779), larvae	•	
Contracaecum sp., larvae	•	•
Hysterothylacium sp., larvae	•	
Pseudocapillaria tomentosa (Dujardin, 1843)	•	

Zaharieva & Kirin (2020b) studied 49 specimens of *C. nasus* from the Danube River (Kudelin) in the spring of 2019 and reported seven parasite species.

The cestode *P. torulosus*, which was reported in 2020, was not identified in the 2019 study (Table 2). The reasons for the lower infection in 2020 compared to 2019 are probably related to the large differences in water levels of the two years and general to the different climatic conditions during these two periods.

Kirin et al. (2013) studied the parasite fauna of 16 species of fish from Lake Srebarna and the Danube River's lower current in Bulgaria.
One of the studied fish species was a common nase. The authors found that the studied specimens of *C. nasus* were not infected. Few authors examined parasites on *Chondrostoma nasus* from the Danube River basin.
P. torulosus was reported as a parasite on *Alburnus alburnus* (Linnaeus, 1758), *Leuciscus idus* (Linnaeus, 1758), *Rutilus rutilus* (Linnaeus, 1758) (Margaritov, 1959; Kakacheva et al. 1978), *Squalius cephalus* (Linnaeus, 1758) (Syn. *Leuciscus cephalus*; Cakić et al., 2004); from the Danube River for the territory of Bulgaria.
Djikanović et al. (2012) reported the cestode *Proteocephalus torulosus* (Batsch, 1786) in common nase from the Danube River, Serbia.
Marković & Novakov (2015) established the trematode *Posthodiplostomum cuticola* (Nordmann, 1832) on common nase from Međuvršje Reservoir located along the West Morava River, part of the Danube River basin in Serbia.

Component community
In the present study, the nematodes were represented by the largest number of specimens (a total of 490 specimens, of which the maximum number of parasite specimens (*Contracaecum* sp., larvae) established in one specimen common nase was 109 specimens). The nematode *Contracaecum* sp. larvae was a core parasite species (P% = 90.00), while the cestode *P. torulosus*, juvenile, was an accidental parasite species (P% = 3.33) in the parasite community of common nase. *Contracaecum* sp. had the highest mean intensity (MI) and the highest mean abundance (MA), respectively MI = 18.15 and MA = 16.33 (Table 3). Zaharieva & Kirin (2020b) identified seven parasite species of common nase from the Danube River (Kudelin) for the spring season of 2019 and reported the highest mean intensity (MI = 36.38) and the highest mean abundance (MA = 17.82) for the nematode *Contracaecum* sp., larvae, which was also a core species in the parasite community of common nase (P% = 48.98). The nematode *Raphidascaris acus*, larvae was also mentioned as a core species (P% = 44.90).

Table 3. Ecological terms of parasites and parasite communities of *Chondrostoma nasus* from the Danube River (Kudelin)

Parasite species	Kudelin N = 30					
	n	p	MI	MA	P%	Range
Proteocephalus torulosus (Batsch, 1786), juvenile	1	2	2.00	0.07	3.33	2
Contracaecum sp., larvae	27	490	18.15	16.33	90.00	1-109

N - number of examined fish, n - number of infected fish, p - number of fish parasites, MI - mean intensity, MA - mean abundance, P% - prevalence.

Infracommunity
Of the studied 30 specimens of *C. nasus*, three specimens of common nase (10%) were not infected, 26 specimens of common nase (86.67%) were infected with one parasite species, and one specimen of common nase (3.33%) was infected with two parasite species (Figure 2; Table 4).
The parasites number in the infracommunity of common nase from the Danube River (Kudelin) varied from 1 to 109 in one specimen of *C. nasus*. During the study, 492 parasite specimens were investigated. The Brillouin's diversity index is very low (HB = 0.024) due to the infection with only two species of parasites, one of which (*P. torulosus*) was represented by only two specimens (Table 4).

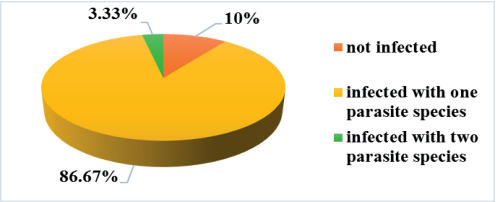

Figure 2. Infection of *C. nasus* from the Danube River (Kudelin)

Table 4. Infracommunity of *C. nasus* from the Danube River (Kudelin)

Number of specimens *Chondrostoma nasus*	Number of parasite species		
	0	1	2
	3	26	1
Total number of species (Mean number of species ± SD)	2 (0.96 ± 0.36)		
Total number of specimens (Mean number of specimens ± SD)	492 (9.48 ± 27.67)		
Brillouin's diversity index (HB)	0.024		

CONCLUSIONS

Thirty specimens of common nase (*Chondrostoma nasus* L., 1758) were studied for the presence of parasites. The fish were collected from the Bulgarian section of the Danube River near the village of Kudelin in the spring of 2020. During the ecoparasitological examination, two species of parasites were found - *Proteocephalus torulosus*, juvenile (class Cestoda) and *Contracaecum* sp., larvae (class Nematoda). *C. nasus* is a new fish host for *P. torulosus* in the studied area (Kudelin). The highest prevalence (P% = 90.00) was for the nematode *Contracaecum* sp. The number of larvae *Contracaecum* sp. found in one specimen of common nase ranged from 1 to 109. The Danube River near the village of Kudelin is a new habitat for *P. torulosus* as a parasite of common nase. *C. nasus* is a new host for *P. torulosus* in Bulgaria.

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NEW DATA ON CADMIUM (Cd) CONTENT IN *CHONDROSTOMA NASUS* (LINNAEUS, 1758), WATER AND SEDIMENTS FROM THE DANUBE RIVER, BULGARIA

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Abstract

In 2020, 30 specimens of common nase (Chondrostoma nasus Linnaeus, 1758) were caught. A total of 6 samples of water and sediments were collected from the Danube River, near the Kudelin village, north western Bulgaria. Tissues and organs of common nase, water and sediments from the Danube River ecosystem for cadmium (Cd) content were studied. In the samples of tissues and organs of common nase, the highest concentrations of Cd were found in the liver (C_{Cd} = 1.21 ± 0.39 mg.kg^{-1} wet weight). The concentrations of cadmium decrease in the order: liver > skin > muscles. The study presented the bioconcentration factor and the linear correlation coefficient of Spearman. The study compared the norms specified in national and international documents with obtained concentrations for cadmium in tissues and organs of common nase and water and sediments.

Key words*: fish, freshwater, heavy metals, tissues.*

INTRODUCTION

The Danube River passes over and connects ten countries' territory in Europe (Western, Central, and Eastern) (Juhásová et al., 2019). The Bulgarian section of the river includes 470 km from its lower course (Zarev et al., 2013). The lower current of the Danube River is subject to pollution of heavy metals due to different activities, including mining. The extraction and processing of ores in Eastern Europe countries (Serbia, Bulgaria, Romania) have strongly affected how developed these activities. Heavy metals are hazardous because they do not decompose in the environment but accumulate (Ilie et al., 2016). Few authors study the concentrations of heavy metals in fish, water and sediments from the Danube River in the territory of Bulgaria (Kirin et al., 2013; Kirin et al., 2014; Chunchukova et al., 2016; Chunchukova & Kirin, 2017; Chunchukova & Kuzmanova, 2017; Kirin & Chunchukova, 2017; Shukerova et al., 2017; Chunchukova et al., 2020; Zaharieva & Kirin, 2020a; Zaharieva & Zaharieva, 2020c; 2020d). The common nase investigations from the Bulgarian section of the Danube River are even less (Zaharieva & Kirin, 2020b; Zaharieva & Zaharieva, 2020a; 2020b).

The purpose of the present study is to provide new data on the cadmium (Cd) content in tissues and organs of common nase, and in water and sediments of the Danube River ecosystem, in a section located on the border of these three countries - Bulgaria, Serbia and Romania.

MATERIALS AND METHODS

In 2020, a study of 30 specimens of common nase (*Chondrostoma* nasus, Linnaeus, 1758) was performed. The common nase specimens were caught from the Danube River section immediately after the river enters the Bulgarian territory, near the Kudelin village.

Three samples of water and three sediments from the same area of the river were also collected. The Kudelin village (44°11′30″N, 22°40′5″E) is situated near the Danube River, Vidin Lowland, north western Bulgaria (Figure 1).

The fish were caught with fishing gear after a fishing permit was issued for scientific purposes. The collected fish specimens were identified in accordance with Karapetkova & Zhivkov (2006); Kottelat & Freyhof (2007). Each caught specimen of common nase was weighed and measured.

Figure 1. Danube River (Kudelin village), Vidin, north western Bulgaria (www.icpdr.org)

The metric data - TL (total length), MH (maximum height) and BW (body weight) were recorded. The mean values for TL, MH and BW, respectively 33.45 cm, 8.09 cm and 369.33 g, were calculated for 30 examined specimens.

Tissues and organs of common nase, water and sediments samples were subjected to chemical analyses to determine the cadmium (Cd) content. Liver, skin and muscles samples were prepared according to standard methods. Water and sediments samples from the Danube River were collected according to accepted standards (ISO 5667-6:2016; ISO 5667-12:2017).

The chemical analysis was performed on measuring equipment (ICP "OPTIMA 7000" Perkin-Elmer) in an accredited laboratory at the Institute of Biodiversity and Ecosystem Research (IBER), Bulgarian Academy of Sciences (BAS), Sofia.

The bioconcentration factor and the linear correlation coefficient of Spearman were calculated in the conducted study. The data were processed on MS Excel (Microsoft, 2010) and Statistica 10 (StatSoft Inc., 2011).

RESULTS AND DISCUSSIONS

The study's subject was the common nase – a freshwater fish from the family Cyprinidae. The species inhabits the Danube River and the rivers that flow into it, preferring areas with moderate currents. Fishers are interested in this species (Karapetkova & Zhivkov, 2006).

The study analysed the cadmium (Cd) content in samples of liver, skin and muscles of 30 specimens of common nase and samples of water and sediments from the Danube River near the Kudelin village. The results of the chemical analyses of the tissues and organs samples of common nase are presented in $mg.kg^{-1}$ wet weight and $mg.kg^{-1}$ dry weight (Table 1); and the results of the analyses of the water and sediments samples in $mg.l^{-1}$ and $mg.kg^{-1}$ dry weight, respectively (Table 2).

Table 1. Cadmium (Cd) in tissues and organs of *C. nasus* from the Danube River, near Kudelin village

Tissues and organs of *Chondrostoma nasus*		**Min.-Max.**	**Mean ± SD**
LIVER	$mg.kg^{-1}$ wet weight	0.62-1.61	1.21 ± 0.39
	$mg.kg^{-1}$ dry weight	1.69-4.48	3.18 ± 1.08
SKIN	$mg.kg^{-1}$ wet weight	0.04-0.19	0.10 ± 0.06
	$mg.kg^{-1}$ dry weight	0.08-0.47	0.26 ± 0.15
MUSCLES	$mg.kg^{-1}$ wet weight	0.02-0.07	0.04 ± 0.03
	$mg.kg^{-1}$ dry weight	0.08-0.28	0.16 ± 0.09

Table 2. Cadmium (Cd) in water and sediments from the Danube River, near Kudelin village

Danube River		Min.-Max.	Mean ± SD
WATER	$mg.l^{-1}$	0.001-0.011	0.008 ± 0.006
SEDIMENTS	$mg.kg^{-1}$ dry weight	0.15-4.27	1.55 ± 2.35

The highest cadmium (Cd) concentrations were reported in samples of liver (C_{Cd} = 1.21 ± 0.39 $mg.kg^{-1}$ wet weight; 3.18 ± 1.08 $mg.kg^{-1}$ dry weight) and the lowest – in samples of muscles (C_{Cd} = 0.04 ± 0.03 $mg.kg^{-1}$ wet weight; 0.16 ± 0.09 $mg.kg^{-1}$ dry weight). The cadmium concentrations in tissues and organs of common nase from the Danube River decreased in the order: liver > skin > muscles (Table 1). The following mean values of cadmium (Cd) in water and sediments from the Danube River were found: $C_{CdWater}$ = 0.008 ± 0.006 $mg.l^{-1}$ and $C_{CdSediments}$ = 1.55 ± 2.35 $mg.kg^{-1}$ dry weight (Table 2).

Cd's content in liver, skin and muscles samples of common nase was compared to the values specified in Ordinance No. 31 of 2004 on the maximum levels of contaminants in foodstuffs and by the Food and Agriculture Organization (FAO). Cd's norm in Ordinance No. 31 of 2004 is 0.05 mg/kg, and the maximum value for Cd given by the FAO is 0.2 mg/kg. It found that the Cd concentration in liver samples of common nase exceeded 24.2 times the norm specified in Ordinance No. 31 and 6.05 times the maximum value shown by the FAO. It was also found that the concentration of cadmium in skin samples exceeded the norm only in Ordinance No. 31 by twofold. Cd concentration in muscles samples of common nase was within accepted levels (Figure 2).

Figure 2. Exceedances of Cd in liver, skin and muscles of *C. nasus* from the Danube River, near Kudelin village ($mg.kg^{-1}$)

The concentrations of Cd in water samples from the Danube River were juxtaposed with the Ordinance values on environmental quality standards for priority substances and certain other pollutants of 2010 and Ordinance No. 18 of 2009 on the quality of water for irrigation of crops. The maximum permissible concentration (MPC) for Cd in Ordinance on environmental quality standards of 2010 is 0.0009 mg/l, and the norm for Cd in Ordinance No. 18 of 2009 is 0.01 mg/dm^3. The established mean values for Cd in water from the Danube River near the Kudelin village exceeded 8.89 times the MPC specified in the Ordinance on environmental quality standards of 2010. They did not exceed the norm in Ordinance No. 18 (Figure 3).

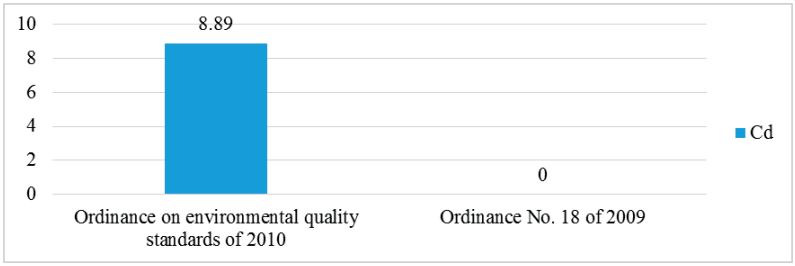

Figure 3. Exceedances of Cd in water from the Danube River, near Kudelin village (mg.l^{-1})

The cadmium concentrations in sediments samples from the Danube River were compared with the values of Ordinance No. 3 of 2008 on the norms for the permissible content of harmful substances in soils and with the Dutch Target Values. The MPC for cadmium in Ordinance No. 3 of 2008 is 2 mg/kg (at pH 6.0-7.4). The Dutch Target Values for cadmium are 0.8 mg/kg. The reported mean Cd concentrations in sediments from the Danube River near the village of Kudelin exceeded 1.94 times the Dutch Target Values and did not exceed the MPC in Ordinance No. 3 at pH 6.0-7.4 (Figure 4).

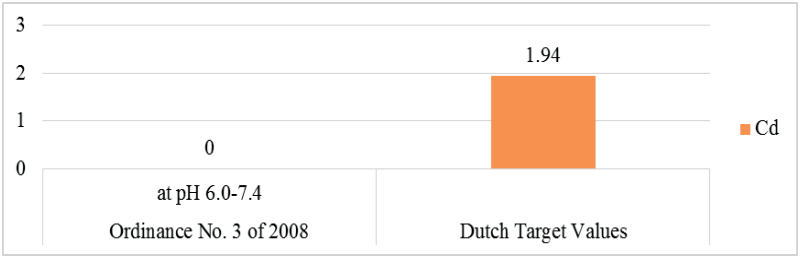

Figure 4. Exceedances of Cd in sediments from the Danube River, near Kudelin village (mg.kg^{-1})

The bioconcentration factor (BCF) in water ((BCF = [$C_{host\ tissues}$]/[C_{water}])) and sediments ((BCF = [$C_{host\ tissues}$]/[$C_{sediments}$])) was calculated. It is the highest in the liver samples and respectively the lowest in the muscle samples, both for water and sediments (Tables 3 and 4).

Table 3. Bioconcentration factor in water

***C. nasus*/Water**	**BCF_{Cd}**
C_{liver}/C_{water}	151.25
C_{skin}/C_{water}	12.50
C_{muscle}/C_{water}	5.00

Table 4. Bioconcentration factor in sediments

***C. nasus*/Sediments**	**BCF_{Cd}**
$C_{liver}/C_{sediments}$	2.05
$C_{skin}/C_{sediments}$	0.17
$C_{muscle}/C_{sediments}$	0.10

The linear correlation coefficient of Spearman (r_s = 0.94-1.0) shows very high correlations between the Cd content in the studied biological samples of common nase and those in the samples of water and sediments.

The obtained tendencies for the accumulation of cadmium in tissues and organs of common nase from the Danube River (Kudelin village) confirmed those obtained in previous studies with the fish species from the same area of the Danube River (Zaharieva & Kirin, 2020b; Zaharieva & Zaharieva, 2020b) - the highest concentrations of Cd in liver samples and the lowest - in muscles samples. According to the Zaharieva & Kirin (2020b) research with common nase from the Danube River (Kudelin biotope), the highest concentrations of Cd were found in liver (0.08 ± 0.04 mg.kg^{-1}), followed by skin (0.07 ± 0.04 mg.kg^{-1}) and muscles (0.01 ± 0.01 mg.kg^{-1}). Zaharieva & Zaharieva (2020b) provided data on Cd content in liver and muscles of common nase from the Danube River (Kudelin biotope), and they reported high Cd concentrations in liver samples (0.32 ± 0.25 mg.kg^{-1}) and low in muscles samples (0.07 ±

0.05 mg.kg^{-1}). In the present study, higher concentrations of Cd were found in the liver and skin samples than those found in the previous year's studies for the same section of the Danube River.

Research on Cd concentrations in tissues and organs of two other fish species from the Danube River (Vetren) was carried out by Shukerova et al. (2017).

The authors examined the liver, skin, and muscles of bleak (*Alburnus alburnus*) and vimba bream (*Vimba vimba*) for cadmium content and reported the highest Cd concentrations in the liver – 0.062 ± 0.025 mg.kg^{-1} (*A. alburnus*) and 1.062 ± 1.78 mg.kg^{-1} (*V. vimba*), followed by those in skin – 0.057 ± 0.026 mg.kg^{-1} (*A. alburnus*) and 0.623 ± 0.877 mg.kg^{-1} (*V. vimba*), and the lowest in muscles – 0.046 ± 0.027 mg.kg^{-1} (*A. alburnus*) and 0.214 ± 0.271 mg.kg^{-1} (*V. vimba*).

The concentrations of Cd established in the present study in tissues and organs of common nase from the Danube River (Kudelin) were lower than those reported by Shukerova et al. (2017) of *V. vimba* from the Danube River (Vetren), except those in the liver samples. They were higher than the Cd concentrations in tissues and organs of *A. alburnus* from the Danube River (Vetren), except those in the muscles.

CONCLUSIONS

In 2020, samples of fish, water and sediments were collected from the Danube River near the village of Kudelin.

There were examined tissues and organs (liver, skin, muscles) of common nase, water and sediments for cadmium (Cd) content.

The concentrations of Cd in the studied tissues and organs decreased in the order: liver (C_{Cd} = 1.21 ± 0.39 mg.kg^{-1} wet weight) > skin (C_{Cd} = 0.10 ± 0.06 mg.kg^{-1} wet weight) > muscles (C_{Cd} = 0.04 ± 0.03 mg.kg^{-1} wet weight).

Concentrations of Cd in muscle samples of common nase did not exceed the values specified in Ordinance No. 31 of the Bulgarian legislation and compared to the FAO's values.

The bioconcentration factor is the highest in the liver samples, from both water and sediments. All studied biological samples proved very high correlations between the Cd content in water and sediments.

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