

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

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PROBLEMS OF EARLY CULLING OF COWS IN BREEDING STOCKS FOR MILK PRODUCTION

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Review paper

Abstract: A common problem of dairy cattle in all production systems and production directions is their reduced production life and therefore their reduced lifetime span. Among the main reasons of dairy cattle premature culling the problems related to reproduction, udder and legs are prevailing. The order of these reasons according to their significance is not the same in all dairy cattle populations and depends on production direction, level of production and specific technology of cattle breeding. There are also other reasons of culling but these three groups are the main ones. Due to frequency of their incidence, as well as economic damage which they can cause in dairy cattle production, today they must be taken into account when defining breeding goals for certain breeds and populations of cattle.

Key words: functional traits, leg problems, reproductive problems, udder problems

Reproductive problems as a factor of early culling of cows

Reproductive problems (sterility) are a consequence of action of many exogenous and endogenous factors. The causes of sterility can be different and can be classified in different ways but the most often are: nutritive factors (errors in nutrition- insufficient and deficient nutrition over a longer period), the way of keeping (cow barn and tie stall), infections and diseases of sex organs, decreased function and insufficiency of ovaries and other reproductive organs (functional disorder of reproductive organs, neurohormonal disorders), congenital deficiencies and defects of reproductive organs, insemination errors, diseases of metabolism and interior organs diseases, hereditary factors (existence of lethal and semi-lethal genes), immunological sterility and other factors.

Among all factors that affect reproduction the effect of nutrition (in quantitative and qualitative sense) is the highest (60-75% of overall sterility), while all the other

factors together account for 25- 40% of sterility. This kind of sterility is called "hunger sterility".

An improved nutrition has an effect on the occurrence of oestrous in breeding females of all species of domestic animals. It is confirmed by the results of numerous authors obtained in previous research studies (*Morris et al., 1993*; as well as *McClure, 1961*; *Staples et al., 1990*; *Beam and Butler, 1998*; cited by *Vuković et al., 2011*). Fertility disorders can be a consequence of inadequate nutrition (particularly energy-deficient diet). There is an effect of negative energy balance and high milk yield postpartum on cow fertility status and the occurrence of ovarian cysts. Diet-originated glucose is necessary both for milk synthesis and for normal functioning of central nervous system (CNS), including hypothalamus and hypophysis where GnRH is being synthesised, i.e. FSH and LH. The requirements for milk synthesis, especially at the start of lactation, have a priority over other requirements, what induces silent reproductive functions as a consequence (*Lotthammer 1985*), cited by *Vuković et al. (2011)*.

Properly balanced bovine diets in certain production stages are very important prerequisites for attaining optimal fertility in cows at an annual level. However, nutrition of cows is not the only factor and therefore it is not a warrant for optimal fertility in cows. The occurrence of oestrous in cows, after uterine involution, depends to a great degree on cow body condition. Apart from nutrition, milk yield in the first few months postpartum affects to a great degree the state of cow condition, as well as the expressiveness and duration of negative energy balance.

Apart from "hunger sterility" there is also a "genital sterility" caused by pathomorphological changes in bovine reproductive organs. These changes can be congenital developmental anomalies, but also different acquired diseases of genital organs, most often induced by an infection. Genital sterility might account for 10-15% in overall sterility.

In dairy cattle breeding stocks the fertilization failure, early embryonic loss and resorption of embryo, prolonged or reduced oestrous cycle, delayed ovulation, acyclicity and other problems may often occur as a consequence of action of many exogenous and endogenous factors. Taking into account that these incidents are related to herds and occur occasionally they are also called "herd sterility".

Taking into consideration numerous factors that can affect fertility in cows, as well as the fact that it is impossible to fulfil all requirements that animals have in certain production stages, a decreased fertility in cows (fertility of breeding stock) may occur as a consequence at an annual level. With a view of increasing fertility in cows, particularly in dairy cattle herd, conducting of induction and synchronisation of oestrus is imposed as obligatory. For that purpose preparations of prostaglandine and their analogues are used in several different protocols (administration occurring once, administration occurring twice, repeated administration). The preparations based on GnRH and progestin in combination

with prostaglandines are used for synchronisation of preovulatory follicular development and induction of ovulation.

Vakanjac and Maletić (2013) state that the effect of prostaglandin depends on the stage of a cycle in which it is applied i.e. on the stage of development of corpus luteum. In heifers corpus luteum reacts to prostaglandin administration from the day 5 of the oestrous cycle (from the day 7 of oestrous cycle in cows) all up to day 17 when spontaneous luteolysis by endogenous prostaglandines originating from endometrium occurs. Due to these reasons it is not justifiable to apply hormone in the period up to day 5 and after day 17 of the cycle. The incidence of oestrous upon application of prostaglandine or its analogues depends on the stage of development of dominant follicle in the moment of hormone application. If the preparation is applied in the time of development of dominant follicle of the first follicular wave, the occurrence of ovulation can be expected in 2-3 days. If the preparation is applied in later stage when follicle has lost its dominancy the ovulation can be expected only after 4-5 days when dominant follicle which is going to ovulate is being separated from the second follicular wave. *De Jarnette et al. (2001)*, *Dogan et al. (2008)*, as well as *Perišić et al. (2016)* report the results of hormonal treatments on the incidence of oestrous, i.e. the application of various protocols of induction and synchronisation of oestrous in cows.

Udder problems as a reason of early culling of cows

Among the all udder problems the most common are different types of mastitis. In the occurrence of mastitis 3 bio-systems take place: a cow with its defensive mechanisms, environment with numerous possibilities of transmitting infection and causative agent of mastitis with its infection. All causative agents of mastitis can be divided in two groups: genetic factors (effect of breed or population within breeds) and non-genetic factors (way of cattle keeping, type of bed, type of diet and kind of nutrition, season, way of milking, milking machine, milking hygiene).

The effect of genetic factor on the incidence of mastitis is associated with procedure of improvement conducted in dairy cattle with the view of improving production traits in cows.

Over a few last decades the selection aimed at increasing the milk production was conducted what negatively reflected on the animal's resistance to diseases (mastitis). In that way a production life and lifetime of cows considerably decreased while replacement and repairing rates increased. High replacement and repairing rates significantly negatively affect and increase the cost of milk production. Production traits have a negative genetic correlation with functional traits such as health (general resistance, resistance to mastitis) and fertility in cows. For these reasons in breeding goals and programmes of improving cattle breeds in

some European countries besides improving of production traits the improvement of functional traits is obligatory as well. Economic parameters of milk production depend to a great degree on functional traits. The milking traits, which also include resistance to mastitis, belong to the group of functional traits and they increase profitability of production via decreasing the costs of production (*Groen et al., 1997, Rensing, 2005, cited by Bobić, 2014*).

In the group of milking traits there are several traits: uniformity of udder quarters (balanced udders), attachment, depth and height of udders, morphological appearance of the parts of udders, shape and dimensions of teats, shape and strength of sphincter, milk flow, udder index, and other. The shape and structure of udders determine predisposition of cow to mastitis. Besides the speed of milk flow the duration of individual stages of milking is very important as well. Thus *Bobić (2014)*, citing *Mijić et al. (2003)*, points out that selection of cows with shorter declining stage and longer plateau stage could make an effect on decrease of number of somatic cells in milk i.e. health state of udders could be affected. In cows with too fast flow of milk the risk of incidence of mastitis is increased along with the increase of the number of somatic cells.

At defining breeding goals for high yielding populations of Simmental breed of the direction milk – meat the functional traits are being emphasized. Therefore in Austria dual production has been defined as a breeding goal in a following ratio: 38% milk, 16% meat and 46% functional traits. It is similar in German Simmental in which functional traits account for over 40%. A particular significance is being put on fitness traits of fertility, course of calving, calves resistance and somatic cells. A special emphasis is on increasing the length of lifetime and production life as well as on realising an overall lifetime production of milk of minimum 30000 kg with realising lactation minimums which are defined in breeding goals (www.asr-rind.de) cited by *Perišić et al. (2014)*.

Nemeš (2016) citing *Rosenberger et al. (2004)* reports that raisers of Bavarian Simmental cattle recorded the decrease in longevity, higher percentage of still born and dead calves, more frequent incidence of hereditary disorders, direct relationship between produced yield of milk protein and health as main deficiencies of selection on higher production of milk.

The opinion of cattle raisers and researchers about cross-breeding of dairy cattle (*Fleischer et al., 2001; Weigel and Barlass, 2003; cited by Nemeš, 2016*) indicate that the research and practical experiences showed that unilateral selection which in the last decade was conducted exclusively towards high production of Holstein-Friesian breed, irretrievably affected metabolism, fertility and health of animals in the most unfavourable way while in cross-bred animals (mostly crosses of Holstein cows with Brown Swiss and Jersey bulls), improved health, fertility, longevity and profitability of production was observed. Many authors think that cross-breeding is a key for increasing the level of fertility and prolonging the life span of dairy cows. Antagonism between constant increase of milk production and

decrease (fall) of fertility is a growing problem in German Holstein as well, as reported by *Freyer et al. (2008)*, and cited by *Nemeš (2016)*. The same authors think that cross-breeding of dairy cattle can be an efficient way for improving functional traits and health of cows for milk production.

In the countries which have a great populations of dairy cattle it is possible to conduct cross-breeding of the parts of those populations. The results of conducted research on farms of dairy cattle in California are reported by *Heins et al. (2012a)* and *Heins et al. (2012b)*. By the analysis of data it was determined that all groups of crossbreds had significantly better traits of fertility and health compared to cows of Holstein during the first five lactations.

Table 1. The results of cross-breeding of Holstein with other breeds (*Heins et al., 2012a*)

Traits	Holstein	Holstein x Nordic breed	Holstein x Montbeliard	Holstein x Scandinavian red
Days to 1 st conception	70	66	63	66
Service period	148	128	122	136
Number of somatic cells (1000)	121	119	98	108
Milk yield (kg)	11417	9843	10774	10627
Yield of protein and milk fat (kg)	762	687	738	733
Survival until 2 nd calving (%)	75	88	89	85
Survival until 3 rd calving (%)	51	73	75	71
Survival until 4 th calving (%)	29	53	55	50
Duration of production life (day)	946	1263	1358	1306
Lifetime profit (\$)	4347	5467	6503	6272
Daily profit (\$)	4,17	3,89	4,39	4,32

Crossbred cows also had significantly longer lifetime span what had an effect on production profitability (*Heins et al., 2012b*). The effects of selection aimed at improving fertility traits cannot be attained in a short period of time. In addition, the situations in which it is not possible to conduct cross-breeding are frequent (small population of cattle of some breed). In such cases besides improving non-genetic factors which affect fertility (nutrition, way of keeping), there remains as an additional possibility, the application of hormones in the regulation of reproduction (*Perišić et al., 2016; Vakanjac and Maletić, 2013*). Information about the need of introducing functional traits in defining breeding goals for certain breeds can be found in the research papers by *Perišić et al. (2008); Perišić et al. (2009); Perišić et al. (2012)*.

Leg problems (lameness) as a factor of early culling of cows

Lameness is, besides mastitis and sterility, one of the most important economic and health problems in modern farm management of dairy cows and very

often a predisposing factor for the occurrence of the other two. *Relić et al. (2015)* concluded that in 88% lame cows some of the indicators of disturbed fertility (prolonged service period, higher index of insemination and/or prolonged interval from the first insemination postpartum up to a successful conception) are observed.

Lameness represents inability to use the limbs functionally along with the changes in gait which occur because of the pain and bad feeling in that part of the body (*Clarkson et al., 1996*). It occurs in all intensive production systems of dairy cows while a frequency of incidence and consequences thereof differ from farm to farm depending on the conditions of breeding. There are numerous causes of the occurrence of lameness, the claw disease being the most frequent one in cattle (*Toholj and Stevančević, 2015; Hristov et al., 2016*). Among the most common and the most important claw diseases are digital and interdigital dermatitis, interdigital phlegmon and laminitis, among which the first three are of infectious character and of similar aetiology (*Weber et al., 2013; Relić et al., 2015; Bojkovski et al., 2020*).

Claw disorders are usually present in more than half of the animals in a herd (in some herds in more than 70% cows) and lameness occurs in about 30% animals at an annual level (*Griffiths et al., 2018*). The economic losses due to lameness take place because of a consequent decrease of milk production, poor condition of the animal (decreased food intake and prolonged resting), problems in reproduction (less visible signs of oestrous and/or absent oestrous), increased working hours and treatment costs for affected cows, as well as a premature culling from breeding stock and from production in general. A direct reason of premature culling happens in the case when claw disorder has developed into incurable, chronic course and when the animal health is permanently impaired and it is no longer capable for production. An indirect reason is seen in the case when due to claw and leg disorders there occurred some other disease what led to a decision that the animal be removed from production and sent to economic slaughter.

A decrease in milk production due to claw disease may be even up to 30% (*Toholj and Stevančević, 2015*). The overgrown dermis of toes, even without visible lameness, can affect the decrease of milk production from 0.5 to 1 liter of milk daily per cow. In breeding stocks in which claw disease occurs in 5 to 10 per cent of animals the decrease of daily quantity of milk can be up to 4 to 5 liters of milk per cow (*Relić and Radenković-Damnjanović, 2009; Stojić et al., 2012*).

The occurrence of lameness shows that animal feels a strong pain what is an indicator of its impaired welfare. If a considerable number of animals in the herd has an expressed lameness it can be a sign that general standards of welfare are not fulfilled in the herd (*Whay et al., 2003*).

Risk factors for incidence of lameness in cows

Factors which represent the risk for the occurrence of claw disease can be classified into two groups. The first group includes the factors whose effect it is not

possible to avoid: the age of an animal, season, pregnancy and stage of lactation, parity, previous diseases and genetic predisposition to claw disorder. The number of interventions due to pathological changes on claws usually increases with increased age of animal and increase of the number of lactations in order while the season and parity are in a positive correlation with the incidence of some of claw diseases (Mülling *et al.*, 2006; Weber *et al.*, 2013; Relić *et al.*, 2015).

The second group of factors on which it is possible to make an effect includes the conditions of housing, hygiene of cows, application of biosafety measures, social relationship in the herd, diet composition and skill at performing the corrections of claws. An increased risk for incidence of claw infectious diseases is observed in animals that have no chance of using pastures and whose movements are restrained, as well as in the farm facilities in which the cattle population is large (Somers *et al.*, 2005; Barker *et al.*, 2009).

According to Mülling *et al.* (2006) the state of claws in cows and occurrence of lameness are mostly affected by quality, hygiene and comfort of the surfaces for lying and walking. These surfaces must not be slippery (for example, wet, smooth concrete covered by liquid manure and faeces, wet and dirty straw) or damaged in any way and covered by small stones which can stuck in the claw sole. Twisting and swinging of lower part of limbs on hard and abrasive floor (for example, at sudden changes in body positions) can lead to damage of live parts within the capsule of dermis. In addition, anatomic structure of the bones of hind limbs in cattle (initial asymmetry of metatarsal bones) can cause that on a hard floor outer claw (external toe) bears greater burden. The consequence thereof is a more intensive growth of dermis on outer toe than on the internal one what affects further increase of pressure. Negative consequences of a hard floor can be avoided by an expert and regular shortening of claw dermis.

Composition of a ration for cows is also very important for the incidence of claw disease and lameness. Rumen microbiota needs six weeks to adapt to sudden change in the composition of a diet and during that time present microelements and other nutritive materials will not be absorbed at an optimal level. Zinc, biotin, iodine, selenium, copper, manganese and cobalt are particularly important in prevention of claw disease.

The share of certain components in a diet (more than 50% of dry matter) can affect biochemical processes in rumen and induce impossibility of synthesis of sufficient quantity of biotin which by its activity affects preventing rumen acidosis due to accumulated lactates. A lactic acid, high yield of carbohydrates and small quantity of fibres in diet, energy rich diets and nutrition by maize silage are reported as significant for the incidence of acidosis and therefore for the incidence of lameness as well (Mülling *et al.*, 2006).

Diet which induces rumen acidosis very often has laminitis for a consequence. The reason why these two states may be associated is a fall of rumen pH what results in a death of a certain number of microorganisms and in releasing

of the content from their cells. It is thought that the greatest role in the incidence of laminitis have vasoactive matters (histamine) released from decomposed bacterial cells which enter the bloodstream and lead to damage of corium of claw but also a great importance is given to bacterial endotoxins, lactic acid and other biologically active substances (*Radojičić et al., 2009*).

An excessive quantity of protein in a diet means that by its degradation there occurs a greater quantity of aminoacids and ammonia as well (*Grubić and Adamović, 2003*), i.e. creation of some toxic products which can act as allergens. The greatest risk for incidence of claw infectious diseases is purchase of new animals. For that reason, before their including into the herd and immediately after their arrival, it is necessary to have records about a health status of every animal and herd which the animals come from and which should prove that an animal be free from digital dermatitis and other infectious claw diseases. Newly purchased animals should be isolated from other animals at least 2-3 weeks during which period the housing must suit the needs of dairy cows regarding comfort, hygiene and quality of air, with minimal action of stressors. Suspicious cows should be isolated and treated immediately upon their arrival while for all animals it is recommended thorough claw cleaning and trimming followed by preventive foot bathing over a few consecutive days upon arrival on the farm.

Preventing the contact with other ungulates, hygiene of litter and bed, barn paths, yards, disinfection of equipment for claw trimming, then cleansing, trimming and disinfection of foot, as well as the isolation of affected animals and their timely and adequate treatment represent biosafety measures in control of claw disease. Their aim is to prevent the occurrence of a disease causative agent on the farm and its spreading inside it (*Mülling et al., 2006; Relić and Radenković-Damnjanović, 2009*). Non-regular and selective conducting of these measures represents the risk not only for the incidence of claw disease in the herd but also of its spreading inside it.

Conclusion

A decreased production life in dairy cattle is a common phenomenon in breeding stocks of dairy and combined production traits (direction milk-meat) nowadays. Main reasons of premature culling of cows are: reproductive problems, problems of udders and claw and leg disorders.

Functional traits (regular fertility, general resistance and resistance to mastitis, good fundament - legs, claws), are regularly included into breeding goals and programmes of high yielding cattle breeds. The ways of improving aforementioned traits are: selection in pure breed but also an application of ameliorative cross breeding. Improvement of functional traits by application of these methods of improvement is a slow process if the effects are regarded per generations. Particularly the effects of selection aimed at improving fertility traits

cannot be regarded in a short period. With of view of faster improvement of fertility some countries with large populations of dairy cattle resort to the application of classical two-breed crossbreeding or to the application of rotational crossbreeding. Along with the changes in hereditary basis a negative effect of all non-genetic factors should be eliminated, i.e. to fulfil all requirements (nutrition, way of keeping, milking hygiene and prevention of mastitis, regular trimming of claws, etc.) that animals have according to certain production stages.

The goal of these activities is to increase the longevity in dairy cattle what directly affects the decrease of replacement and repairing rates in breeding stock and the increase of the economy of production.

Problemi ranog izlučenja krava u zapaćtima za proizvodnju mleka

Predrag Perišić, Renata Relić, Cvijan Mekić, Stefan Stepić

Rezime

U svim proizvodnim sistemima i proizvodnim usmerenjima skraćen je proizvodni vek krava. Posebno je to izraženo kod specijalizovanih mlečnih rasa, kao i rasa kombinovanog smera mleko:meso. Glavni razlozi prevremenih izlučenja krava iz proizvodnje su problemi u vezi sa reprodukcijom, vimenom i nogama. Redosled ovih razloga po znaćaju nije isti u svim populacijama krava, već zavisi od proizvodne usmerenosti, nivoa proizvodnje i konkretne tehnologije gajenja krava. Postoje i drugi razlozi izlučenja krava, ali ove tri grupe su glavne. Zbog učestalosti njihove pojave, kao i ekonomskih šteta koje uzrokuju u govedarskoj proizvodnji, funkcionalne osobine (redovna plodnost, opšta otpornost i otpornost ka mastitisu, dobar fundament - noge, papci), redovno su uključene u odgajivaćke ciljeve i programe visoko proizvodnih rasa goveda. Naćini poboljšanja navedenih osobina su: selekcijom u čistoj rasi, ali i primenom meliorativnog ukršćanja. Poboljšanje funkcionalnih osobina primenom ovih metoda oplemenjivanja je spor proces, ako se efekti sagledavaju po generacijama. Posebno se efekti selekcije u cilju poboljšanja osobina plodnosti, ne mogu sagledati u kratkom roku. U cilju bržeg poboljšanja plodnosti, pojedine zemlje koje imaju velike populacije mlečnih rasa goveda, pribegavaju primeni klasićnog dvorasnog ukršćanja ili primeni rotacijskog ukršćanja. Uporedo sa izmenom nasledne osnove, treba eliminisati negativni uticaj svih negenetskih činilaca, odnosno ispoštovati sve zahteve (ishrana, naćin držanja, higijena muže i prevencija mastitisa, redovna korekcija papaka i dr.), koje životinje imaju u skladu sa proizvodnim fazama. Cilj svih aktivnosti je povećanje

durovečnosti krava, čime se direktno utiče na smanjenje remontne stope u zapatu i povećanje ekonomičnosti proizvodnje.

Ključne reči: funkcionalne osobine, problemi reprodukcije, problemi vimena, problemi nogu

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INFLUENCE OF REARING CONDITIONS ON REPRODUCTION, GROWTH, MILK YIELD AND QUALITY OF MEAT AND MILK OF SHEEP AND GOATS

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Review paper

Abstract: The most important stressors in our country are unfavourable climatic and nutritional conditions. Extreme deviations in climatic and nutritional conditions are immediate stressors for the body of sheep and goats. Appetite in sheep and goats decreases if they are exposed to extremely high ambient temperatures, while cold increases the body's energy needs and leads to the mobilization of fat from body fat and the consequent oxidation of fat and the formation of non-esterified fatty acids. Thyroid activity is reduced when these animals are exposed to high temperatures, and increased when they are exposed to cold. In an environment with high temperatures, blood glucose and cholesterol concentrations decrease due to unsuccessful maintenance of homeostasis. Sheep and goats are naturally kept in a group, which basically protects them from predators. They successfully register the action of new stimuli, especially noise and sudden movements of nearby predators. Early weaning breaks the bond between mother and offspring and adversely affects the development of normal behavioural relationships in lambs and kids, because artificial feeding systems do not fully meet the needs of oral activity, so sucking of the umbilical and scrotal region often occurs. Investigations of the influence of various technological procedures on the reactivity of the adrenal cortex indicate that the separation of the animal from the herd and immobilization are the most significant stimuli. Electrical stunning of sheep at the slaughterhouse causes significant stimulation of the sympathetic system with a sudden release of adrenaline and noradrenaline. In conditions of intensive production, the most significant stressors and physiological conditions that lead to stress are: parturition, birth, puerperal period, weaning, oestrus, high pregnancy, various surgical procedures, veterinary and zootechnical procedures, extreme variations of micro- and macroclimatic conditions, variations in quantity and food quality, etc.

Key words: rearing conditions, reproduction, production, sheep, goats

Introduction

In our climatic conditions, sheep and goats are raised on pastures for a longer period of the year, where the most significant stressors are unfavourable climatic and nutritional conditions (*Hristov and Bešlin, 1991*). Climate variations directly affect these species, influencing their biological reactions (*Hristov et al., 2002*). Detailed studies of certain haematological and biochemical changes related to pregnancy and lactation in sheep and goats in different climatic conditions, different rearing and housing conditions, as well as different feeding conditions have been performed. Periods requiring an abundant supply of nutrients, such as immediately before and after birth in highly productive breeds of sheep and goats under conditions of insufficient and/or unbalanced diet, may include mobilization of body fat and protein, leading to fatty liver and clinical ketosis (known as pregnancy toxæmia) and results in decreased productivity and in some cases even death (*Helman et al., 1995*). Similar metabolic changes can occur in low-yielding breeds of sheep and goats if these animal species are malnourished and/or fed unbalanced rations (*Hristov and Bešlin, 1991*).

Influence of stressors on reproduction, growth, milk yield and meat and milk quality

The connection between stress and animal welfare is a well-known issue in scientific observations (*Hristov et al., 2007*). Climate change is one of the most significant stressors affecting the welfare and production of sheep and goats (*Hristov et al., 2007a*). The impact of climate change is manifested through numerous losses in livestock production. It should be borne in mind that analyze of the conditions of production is an important issue, since each breed has different characteristics of adaptability. Changes in biological functions when exposed to heat stress include physiological, hormonal, haematological and biochemical reactions, which make sheep and goats resilient and enable them to survive in an unfavourable environment (*Darcan et al., 2007*). The extreme variations in climatic and nutritional conditions are the direct stress to the organism of sheep and goats (*Hristov and Bešlin, 1991*).

Appetite in sheep and goats, for example, decreases if they are exposed to extremely high ambient temperatures (*Sarangi, 2018*). Cold, on the other hand, increases the body's energy needs and leads to the mobilization of fat from body fat in sheep and goats and the consequent oxidation of fat, which leads to the formation of non-esterified fatty acids (NEFA – Non Esterified Fatty Acids) as a

constituent of blood plasma. NEFA level in blood plasma of sheep or goat is reliable indicator of nutritive status and reactivity to environmental stressors (Gupta *et al.*, 2013; Milošević-Stanković *et al.*, 2020).

The intensity of the detrimental effects of high environmental temperatures on sheep and goats depends on the efficiency of their thermoregulatory mechanisms (Caroprese *et al.*, 2009; Shaji *et al.*, 2017). Thyroid activity is reduced when these animals are exposed to high temperatures, and increased when they are exposed to cold (Habibu *et al.*, 2016; El-Tarabany *et al.*, 2017). Well-adapted animals react quickly to changes in the environment so they can make the necessary physiological adjustments. Research shows that in the environment with high temperatures, the concentrations of glucose and cholesterol in the blood decrease, which points out the unsuccessful maintenance of homeostasis.

Heat stress has a greater impact on total cholesterol concentration, which may be due to increased use of fatty acids for energy production and decreased glucose concentration in animals exposed to heat stress (Gupta *et al.*, 2013; Das *et al.*, 2016). Similarly, researchers point out protein level increase when these animals are exposed to heat stress (Nazifi *et al.*, 2003; Gupta *et al.*, 2013). During physical exertion a faster release of oxygen is present as well as an increase of oxygen consumption and thus an increase of the concentration of haemoglobin. Reduced food intake in animals under long-term heat stress reduces erythrocyte production and haemoglobin levels, resulting in a reduction in the number of red blood cells in the bloodstream (Alam *et al.*, 2011; Gupta *et al.*, 2013).

Lambs and kids born by caesarean section or exposed to cold have an increased concentration of NEFA in the blood plasma (Clarke *et al.*, 1994; Hristov *et al.*, 2012). Other adverse factors such as, for example, complete lack of food or malnutrition combined with multiple pregnancy also lead to a significant increase in the concentration of NEFA in the blood plasma of sheep and goats, which is closely related to the intensity of stressors (Eşki *et al.*, 2015; Di Giacomo *et al.*, 2016; Milošević Stanković *et al.*, 2020). In sheep and goats exposed to cold as well as humid climate conditions, the adrenal cortex is stimulated to increase hormone secretion while the number of eosinophils in the blood is significantly reduced. The metabolic reaction to repeated exposures of sheep and goats to cold in a short duration is progressively reduced. Progressive changes in climate conditions are likely to lead to relatively wide ranges in haematological values in sheep and goats in different geographic areas (Ghosh *et al.*, 2014; Snyman *et al.*, 2017).

Increased cortisol levels in sheep and goats occur after acute heat stress (Marai *et al.*, 2007; Seijan *et al.*, 2008); intensive chronic stress can result in periods of high concentrations of cortisol, followed by a decrease in individual health condition through immuno-suppression and atrophy of the body's defence tissues. In addition, cortisol reduces the energy available to the immune system, which increases the susceptibility of animals to infectious diseases.

Today, the most intensive production in sheep and goat breeding has contributed to the production of lambs and kids throughout the year. Basically, the existing systems in use in the housing and rearing of sheep and goats during the year and the artificial induction of oestrus have contributed to the reduction of exposure of sheep and goats to daylight (*Alcalde et al., 2017*).

Highly productive breeds of sheep and goats have been subjected to rigorous selection and crossbreeding with tested rams and goats to improve meat quality. The lambs and kids are weaned and fed with artificial milk using rubber teats from the second day of age. A study of the behaviour of groups of lambs and kids reveals that housing systems where the social bond between mother and offspring is broken, adversely affect the development of normal behavioural relationships (*Sevi et al., 2009*). As it was found for calves (*Johnsen et al., 2016*), artificial feeding systems do not fully meet the needs of oral activity of lambs and kids, since it was noticed that those lambs and kids develop the habit of sucking the umbilical and scrotal regions by individual lambs and kids in the group (*Stephens and Baldwin, 1971; Napolitano et al., 2008*). Previous attempts to improve the diet in order to achieve the most optimal in accordance with the biological needs of sheep and goats have not been completely successful in preventing the occurrence or reduction of abnormal oral activity of lambs and kids (*Miranda-de la Lama and Mattiello, 2010*).

Surgical transplantation of the adrenal gland to the neck where the blood supply is easy enabled the use of sheep as a model for studying adrenocortical function in mammals. The relatively simple surgical procedure made it possible for the uterus to connect with the adrenal cortex of the foetus and consequently easily stimulate with ACTH and induce a reaction in the foetus and newborn lambs (*McDonald et al., 1958*). Studies of the dynamics of adrenal gland reactions to experimental ACTH stimulation in adult sheep showed that the maximum secretion of cortisol is achieved within 10 to 20 minutes. When the ACTH infusion is continuous, maximum cortisol secretion is maintained for the next 30 to 60 minutes, after which normal secretion is slowly established (*Beaven et al., 1964; Salemi et al., 2000*).

Sheep and goats are naturally kept in a group, which basically protects them from predators. They have a very developed observation about the action of new stimuli, especially noise and sudden movements of nearby predators (*Rutter, 2002*). Sudden loud noise, such as a loud gunshot, is a stressor and causes a significant initial reaction in sheep movement. Depending on the duration and intensity of the sound, the herd moves quickly in different durations and at different distances (*Hargreaves and Hutson, 1990; Grandin, 2011*).

Aberrations from stationary level limits of many constituents in blood plasma have also been found in sheep and goats exposed to qualitatively new stimuli. An increase in thyroid hormone release rate was found in sheep within 15 to 30 minutes, when they were exposed to a series of fireworks explosions or barking dogs. The increase in the concentration of these hormones usually lasts for about 2

hours (Falconer and Hetzel, 1964; Bobek et al., 1986). Plasma NEFA concentrations also increase in sheep and goats when exposed to qualitatively new stimuli, such as vein puncture (Paterson, 1963). This increase usually does not last longer than 2 hours (Slee and Halliday, 1968), and the increase rate becomes progressively lower with repeated seizures in order to take blood samples (Reid and Hinks, 1962; Westgren et al., 1987). Similarly, the consequent progressive decrease was caused by venous puncture when taking blood to determine the concentration of corticosteroids in the blood plasma (Bassett and Hinks, 1969).

Circadian variations in plasma cortisol concentrations in sheep and goats are absent for the first 2 to 3 days after the introduction of noise and sudden movements of nearby predators. During the stay in the new ambient conditions, the diurnal rhythm of cortisol concentration in the blood plasma is reestablished. However, a significantly higher concentration of cortisol in blood plasma is found in unadapted individuals compared to the concentration in fully adapted sheep and goats. It is considered that in sheep and goats require a period of 7 to 28 days for complete adaptation to the new conditions of keeping and accommodation (McNatty and Young, 1973).

In the study of various technological procedures and their effects on the reactivity of the adrenal cortex, it was found that the separation of sheep and goats from the herd and immobilization are the most influential stimuli. Prolonged shear time from 5 to 15 minutes causes an increase in plasma cortisol concentration by 50% (Kilgour and de Langen, 1970).

It has been proven that an increase in the concentration of cortisol in the blood plasma of sheep can be induced by an intravenous infusion of adrenaline. The increasing the concentration of cortisol was dependent on the applied dose of adrenaline, as well as higher in sheep that were housed in the stall. Studies have shown that clinical signs of disorders and disturbances in sheep are partly related to the release of adrenaline. The researchers point out that significant dynamic adrenocortical activity is found when a group of sheep is exposed to disturbance and suggest that the adrenal gland response depends on the intensity of the stressor (Thurley and McNatty, 1973).

The use of inexperienced dogs to chase the flock in sheep causes a greater and longer-lasting response of the organism than the use of trained dogs. Grouping of sheep by humans causes a less stressful reaction of the sheep organism (Beausoleil et al., 2005). Surprisingly, sheep kept together with lambs, although more often in a distressed state, show less reaction to grouping with experienced dogs compared to the same treatment in the same sheep before parturition.

Sheep housed in stables immediately before transport are less susceptible to stress or increased corticosteroid concentrations. However, sheep caught and loaded into a trailer directly on pasture show a greater reaction (Miranda-de la Lama et al., 2012; Pascual-Alonso et al., 2017).

Electrical stunning of sheep at the slaughterhouse causes significant stimulation of the sympathetic system with a sudden release of adrenaline and noradrenaline into the circulation, 14 to 20 times higher than normal values. Concentrations of cortisol, adrenaline and noradrenaline in blood plasma are significantly higher in sheep in large slaughterhouses compared to the values found in the same species in small non-specialized slaughterhouses. This is thought to be due to heavier catching, higher noise and greater disturbance of sheep in large slaughterhouses (*Linares et al., 2008; Sabow et al., 2016*).

Mean concentrations of adrenal hormones in the blood serum of sheep gradually increase with an increase in the duration of transport to 2 hours. When extending the duration of transport to 8 hours, a lower rate of increase in the concentration of these hormones in the blood serum of sheep was observed (*Shannon et al., 1976; Zarogiannis et al., 2006; Kadim et al., 2007*).

Meat breeds of sheep and goats are inevitably exposed to various procedures before and during loading, during transport, during unloading, as well as food deprivation before slaughter. Procedures with sheep and goats before, during and after transport can affect animal welfare and production profitability. Expressed stress before slaughter negatively affects the quality of meat in animals. Weight loss during transport and storage in pre-slaughter depots are of particular importance because in small ruminants the digestive tract represents a larger proportion of live weight than in cattle and pigs (*Ekiz and Yalcintan, 2013; Hashem et al., 2013*).

The researchers used several physiological indicators to assess stress levels in farm animals. Cortisol concentration was used as a reliable indicator of short-term physical stress (*Kannan et al., 2003*). Leptin, a protein hormone synthesized by adipose tissue, has been reported to inhibit the activity of the hypothalamic-pituitary-adrenal axis in response to stress. (*Macedo et al., 2019*). Creatine kinase (CK), an enzyme in skeletal muscle, is released into the blood plasma in response to muscle damage under stress (*Nazifi et al., 2003; Chulayo and Muchenje, 2013*). Stress in sheep and goats due to lack of food causes the degradation of proteins and increase the level of urea in the blood (*McDougal et al., 1991; Wheelock et al., 2010*). The effects of various stressors on leukocyte profiles in farm animals were determined. Physiological responses to pre-slaughter stress have been studied in all species of farm animals, with limited data on goats (*Ekiz and Yalcintan, 2013; Sabow et al., 2016*).

Immediately before and after birth, highly productive breeds of sheep and goats need large amounts of nutrients. If then there is insufficient and/or unbalanced diet, body fat and protein are mobilized, which leads to fatty liver and clinical ketosis (known as pregnancy toxemia), reduced productivity, and in some cases the death of these species (*Brozos et al., 2011; Milošević-Stanković et al., 2020*). Similar metabolic changes can occur in poorly productive breeds of sheep and goats if they are not fed and/or fed an unbalanced diet. Concentrations of free

fatty acids, triglycerides, cholesterol, urea and creatinine in the blood serum are higher in sheep and goats with the occurrence of pregnancy ketosis and multiple pregnancies compared to non-pregnant sheep and goats (*Donoghue and Kronfeld, 1990; Brozos et al., 2011*). Calcium intake decreases with reduced food intake during ketosis, which leads to hypocalcaemia. Serum calcium levels were found to be significantly reduced in sheep and goats with hypocalcaemia and pregnancy toxemia. Also, reduced food intake during grazing is a possible cause of hypocalcaemia (*Kessler, 1991*).

Conclusion

Based on a detailed review of the literature data related to the influence of sheep and goat rearing conditions as a stressor on reproduction, growth, milk yield and quality of meat and milk, the following can be concluded:

- Extreme deviations in climatic and nutritional conditions are immediate stressors for sheep and goats;
- Appetite in sheep and goats is reduced if they are exposed to extremely high ambient temperatures. Cold, on the other hand, increases the body's energy needs and leads to the mobilization of fat from body reserve in sheep and goats and the consequent oxidation of fat, which causes the formation of non-esterified fatty acids;
- Thyroid activity is reduced when these animals are exposed to high temperatures and increased when they are exposed to cold. In an environment with high temperatures, blood glucose and cholesterol concentrations decrease and homeostasis fails;
- Sheep and goats have very good registration of the action of new stimuli, especially noise and sudden movements of nearby predators;
- Breaking the social bond between mother and offspring by early weaning adversely affects the development of normal behavioural relationships in lambs and kids;
- Separation of sheep and goats from the herd and immobilization are the most influential stress stimuli;
- Electrical stunning of sheep and goats at the slaughterhouse causes significant stimulation of the sympathetic system with a sudden release of adrenaline and noradrenaline into the circulation.

In the conditions of modern intensive sheep and goat production, the most significant stressors, regardless of origin, and physiological conditions in which these species are more susceptible to stress are: parturition, birth, puerperal period, weaning, oestrus, high pregnancy, various surgical procedures, veterinary and zootechnical procedures, extreme variations of micro- and macroclimatic conditions, variations in the quantity and quality of food, etc.

Uticaj uslova gajenja ovaca i koza na reprodukciju, prirast, mlečnost i kvalitet mesa i mleka

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Rezime

Najvažniji stresori kod nas su nepovoljni klimatski i nutritivni uslovi. Ekstremna odstupanja u klimatskim i nutritivnim uslovima predstavljaju neposredne stresore za organizam ovaca i koza. Apetit kod ovaca i koza se smanjuje ako su one izložene izrazito visokim ambijentalnim temperaturama, dok hladnoća povećava energetske potrebe organizma i dovodi do mobilizacije masti iz masnog tkiva organizma i posledične oksidacije masti i stvaranja neesterifikovanih masnih kiselina. Aktivnost štitne žlezde je smanjena kada su ove životinje izložene visokim temperaturama, a povećana kada su izložene hladnoći. U ambijentu sa visokim temperaturama smanjuju se koncentracije glukoze i holesterola u krvi usled neuspešnog održavanja homeostaze. Ovcе i koze se prirodno drže u grupi čime se u osnovi štite od predatora. One uspešno registruju delovanje novih stimulusa, naročito buke i iznenadnih pokreta predatora u blizini. Rano odbijanje prekida vezu majke i mladunčeta i nepovoljno utiče na razvoj normalnih bihevioralnih odnosa kod mladunčadi, jer veštački sistemi ishrane ne zadovoljavaju u potpunosti potrebe oralne aktivnosti, pa se često javlja sisanje pupčane i skrotalne regije. Ispitivanja uticaja različitih tehnoloških postupaka na reaktivnost kore nadbubrežne žlezde ukazuju da je izdvajanje jedinke iz stada i imobilizacija predstavljaju najznačajnije stimulse. Električno omamljivanje ovaca na klanici izaziva znatnu stimulaciju simpatičkog sistema sa naglim oslobađanjem adrenalina i noradrenalina. U uslovima intenzivne proizvodnje najznačajniji stresori i fiziološka stanja koja dovode do stresa su: porodaj, rođenje, puerperalni period, odbijanje mladunčadi, estrus, visoki graviditet, razni hirurški postupci, veterinarske i zootehničke mere, ekstremna variranja mikro- i makroklimatskih uslova, variranja u količini i kvalitetu hrane i dr.

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PHENOTYPIC CHARACTERISTICS OF LINEAR TRAITS OF UDDER AND ANGULARITY IN HOLSTEIN-FRIESIAN COWS AND THEIR CORRELATION WITH MILK YIELD TRAITS

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Abstract: Data set including 10860 primiparous Holstein-Friesian breed cows first calved in the period from 2011 to 2015, was used in determining phenotypic variability and correlation between the traits of milk yield and linearly estimated traits of udder and angularity. The average values obtained for type traits (angularity, fore udder attachment, front teats placement, teats length, udder depth and rear udder height) were 6.47; 5.74; 4.96; 5.20; 5.99 and 6.25, respectively. The values obtained for phenotypic correlations between linear type traits and traits of milk yield ranged from -0.042 (udder depth and milk yield) to 0.335 (fore udder attachment and protein yield). Positive phenotypic correlation (0.293) was recorded also between fore udder attachment and milk yield which is deemed the most important trait of milk yield while the lowest correlation between milk yields was determined in relation to udder depth (-0.033). The results obtained indicate a possibility of applying direct and indirect multiple traits selection which should be conducted within a national progeny-testing programme on Holstein-Friesian bulls by using the method of selection indexes.

Key words: Linear type traits, phenotypic correlations, milk yield traits

Introduction

Modern trends in dairy industry require a permanent work on improving genotypes and phenotypes of cattle. Recently, more and more attention has been

paid to body conformation, body development and type traits of cattle. It has been determined that certain malfunctions in type traits, particularly udder traits, can lead to lower production and bad health state of an animal and therefore to the early culling of the cows from the breeding stock. Visual assessment and recognising the characteristics of cow's udders are preliminary indicators of milk yield, longevity and reproductive capacity of animal. A linear type estimation as an objective and unbiased method for estimating an animal body development has been used and it represents the basis of modern classification systems as well as the foundation of all systems for describing dairy cows (*Janković, 2017*).

Selection of cows directed exclusively to the traits of milk yield can decrease the values of other traits which relate to a type, conformation, durability and longevity. These traits have a great economic importance, therefore in order to increase productive life of an animal it is essential to direct selection also to the traits of type which are phenotypically and genetically connected with milk yield traits. The application of selection on multiple traits can decrease unwanted cullings and increase lifetime profitability per animal (*Stanojević et al., 2018*).

Studying the correlation between the traits of milk yield and linear type traits and therefore the traits of udder and angularity *Živanović, 2002; Pantelić et al., 2012; Tapki and Güzey, 2013; Bohlouli et al., 2015; Janković, 2017*, determined that their mutual interdependence was relatively weak but also that a dairy character-angularity showed constant positive correlations with milk yield. *Berry et al. (2005)* determined also that all phenotypic correlations between type traits and milk yield traits were weak and positive except for an udder depth and teats length.

The objective of this study was to determine phenotypic variability and correlation between the traits of milk yield and the traits of udder and angularity in primiparous cows, primarily due to the significance that these traits have on final milk production and necessity that these traits be included in the national selection programme of Holstein-Friesian breed bulls and cows.

Material and Method

A trial conducted included 10860 primiparous cows of Holstein-Friesian breed that were first calved in the territory of Vojvodina Province, Republic of Serbia, in the period from 2011 to 2015. Primiparous cows were being examined by 22 evaluators who have finished a specialist training according to the Instructions for the evaluation of linear type traits and body development in Holstein-Friesian breed cows (*Janković, 2012*). Each dairy farm had on average 10 cows and the cows first calved in the age of 27 months. At the time of estimation the average age of primiparous cows was 30 months while average number of days

realized in lactation on the day of assessment in primiparous cows was 95 with variability of 15 to 210 days.

The available traits were: milk yield (MY), fat content (FC), fat yield (FY), protein content (PC) protein yield (PY), and 6 type traits: angularity (ANG), fore udder attachment (FUA), front teats placement (FTP), teats length (TL), udder depth (UD) and rear udder height (RUH).

Values of standard statistical parameters (arithmetic mean, standard deviation (SD), variation coefficients (CV), variation interval (Min-Max)) for phenotypic expressivity of studied traits were calculated by means of standard statistical procedures using a statistical package (*SAS Institute, 2013*).

Data used for the estimation of phenotypic correlation coefficients of milk yield and type traits were encoded in a PEST software package (*Groeneveld et al., 1990*), while the estimation of phenotypic variances and covariances was done by means of VCE v6 software package within a programme package (*Groeneveld et al., 2010*) with application of multiple traits model. The values of phenotypic correlations were calculated by means of two mixed models. For type traits a following mixed model was used:

$$Y_{ijklmno} = \mu + F_i + GG_j + YxS_k + AFC_l + O_m + Y_n + YO_o + \text{animal} + e_{ijklmno}$$

(model Eq. 1)

Where:

$Y_{ijklmno}$ – is a phenotype expressiveness of tested trait,

μ - population general average

F_i - fixed effect of the size of farm (6 classes, according to the number of first calvings, I (1-5); II (6-10); III (11-15); IV (16-50); V (51-100); VI (>100));

GG_j - fixed effect of genetic group, (interaction of bull's year of birth (1980-2011) and country of bull's origin (12), 79 genetic groups in total);

$YxSk$ – fixed effect of interaction of the year and calving season (5 years, every year being divided into 4 seasons: winter, spring, summer, autumn);

AFC_l – fixed effect of the age at first calving, (animals's age expressed in months and allocated into 5 classes: I (19-23); II (24-26); III (27-30); IV (31-33); V (34-44));

O_m – fixed effect of the evaluator;

Y_n – fixed effect of the year of evaluation, (4 years, from 2012-2015 during which the animals were evaluated);

YO_o – fixed effect of the age at evaluation, (animal's age expressed in months and allocated into 5 classes: I (20-24); II (25-29); III (30-34); IV (35-39); V (40-45));

animal – random effect of an individual for whom the kinship matrix has been created,

$e_{ijklmno}$ – random error.

For calculating phenotypic correlations of milk yield traits a following model was used:

$$Y_{ijkl} = \mu + F_i + GG_j + Y_{xSk} + AFCl + \text{animal} + e_{ijkl} \quad (\text{model Eq. 2})$$

Where:

Y_{ijkl} - phenotypic expression of the investigated trait; e_{ijkl} - random error; μ , F_i , GG_j , Y_{xSk} , $AFCl$, animal – model variables are defined in the previous model (model Eq. 1).

Results and Discussion

Table 1 shows average values, standard deviations, variation coefficients and range of phenotypic variability of studied traits as well as ideal score for udder traits and angularity in primiparous Holstein-Friesian breed cows.

A pronounced milk production yield presupposes a cow with well developed and broad chest with open ribs and strongly expressed angularity. Such a cow has a harmonious frame which suggests resistance and high milk production yields.

For the angularity in primiparous Holstein Friesian cows a mean score of 6.47 was obtained. A considerably lower mean values of 5.60 and 5.50 for angularity were obtained by *Nemcova et al. (2011)* and *Zavadilova et al. (2009)* in Holstein cows in Czech Republic.

Table 1. Phenotypic variability of linear type traits and traits of milk yield (n=10860)

Trait	Abbreviation	Ideal score	Mean	SD	Cv (%)	min	max
Angularity (points)	ANG	9	6.47	1.40	21.64	1	9
Fore udder attachment (points)	FUA	9	5.74	1.46	25.44	1	9
Front Teats Placement (points)	FTP	5	4.96	1.14	22.98	1	9
Teats length (points)	TL	5	5.20	1.12	21.54	1	9
Udder depth (points)	UD	5	5.99	1.22	20.37	1	9
Rear udder height (points)	RUH	9	6.25	1.33	21.28	1	9
Milk (kg)	MY		6672	1740	26.07	1811	14395
Con. Fat (%)	FC		3.81	0.45	11.81	2.04	5.96
Fat (kg)	FY		252.83	67.66	26.76	59.00	612.00
Con. Protein (%)	PC		3.21	0.21	6.54	2.02	5.09
Protein (kg)	PY		213.86	56.94	26.62	51.00	472.00

Tapki and Guzey (2013) also obtained low mean score of 5.18 for angularity in Turkish primiparous Holstein cows. A lower mean score of 5.60, for angularity in Holstein cows in Australia, was obtained by *Haile-Mariam et al. (2014)*, while *Kern et al. (2014)* obtained similar mean score of 6.35 for the trait of angularity in population of Holstein cows in Brazil. Mean score for angularity which is closest to one calculated in this research was determined in Holstein cows in Brazil being 6.44 (*Campos et al., 2012*), while the mean values of over 6.0 for this trait were obtained by *Otwinowska-Mindur et al. (2016)* in Polish Holstein-Friesian population (6.12), then by *Bohlouli et al. (2015)* in Holstein cows in Iran (6.24), by *Janković et al. (2016)* in primiparous Holstein-Friesian cows in Vojvodina province (6.33) and by *Almeida et al. (2017)* in Holstein cows in Brazil (6.30). In relation to a mean value reported herein a higher value of 6.67 for angularity was obtained by *Dadpasand et al. (2012)* in Holstein population in Iran while *Van der Laak et al. (2016)* obtained a significantly lower value of 4.83 in tested sample of Holstein-Friesian cows in Holland.

An absolute variability of angularity expressed in standard deviations ranged from 1.05 in the research of *Tapki and Guzey (2013)* to 1.57 in the research of *Bohlouli et al. (2015)*. A calculated standard deviation of 1.40 for angularity in primiparous Holstein-Friesian cows in Vojvodina province is closest to a standard deviation of 1.36 reported in the research of *Van der Laak et al. (2016)*, while a relative variability of angularity expressed by variation coefficient was 21.64 % what is similar to variation coefficient of 22.23% obtained by *Zavadilova and Štipkova (2012)*.

Although a mean value of 6.47 obtained for angularity in primiparous Holstein-Friesian cows in Vojvodina province is closer to a mean value (5) than to an ideal score (9) for Holstein Friesian breed, it was higher than majority of mean values calculated in the above mentioned studies. The importance of dairy character and its improvement in dairy cattle conformation is reflected also in the fact, that besides the effect on milk production (*Brotherstone, 1994; Bohlouli et al., 2015*) and productive lifetime of dairy cows (*Weigel et al., 1998*), there is also a correlation between angularity and fertility traits (*Pryce et al., 2000; Makgahlela et al., 2009; Almeida et al., 2017*). The research by *Berry et al. (2005)*, showed that milk production affects also a functional longevity of cows in commercial herds and that higher yielding cows exhibit better ability to remain longer in productive herds.

A particular importance in dairy cows conformation is given to udder traits which, according to the *WHFF (2016)* recommendations make 40% of total evaluation of type traits. *Nemcova et al. (2011)* obtained similar mean values of evaluation in Holstein population in Czech Republic (from 4.90 for front teat position to 5.80 for udder depth), while *Campos et al. (2012)*, in Holstein population in Brazil, obtained higher mean scores for almost all udder traits (from 5.22 for front teat position to 6.36 for teat length), except for udder depth (4.85) and rear udder height (5.77). *Tapki and Guzey (2013)*, in their research on Turkish

The values obtained for correlations ranged from -0.042 (between udder depth and milk yield) to 0.335 (between fore udder attachment and protein yield). Phenotypic correlations between milk yield as the most important trait of milk production capacity in our national programme and studied type traits such as angularity, fore udder attachment, front teats placement, teats length, udder depth and rear udder height) were 0.310; 0.321; 0.101; 0.241; -0.042 and 0.022, respectively.

Similarly to the results obtained in this paper a number of studies shows that there is a positive and negative phenotypic correlation between milk yield traits and linear type traits in dairy cows. According to *Pantelić et al. (2012)* phenotypic correlations between milk yield and udder traits ranged in the interval from -0.11 (rear udder height) to +0.1 (front teats placement).

The phenotypic correlations between type traits and milk yield according to *Tapki and Güzey (2013)* ranged from -0.31 (udder depth and milk yield) to 0.29 (angularity and milk yield); -0.23 (udder depth and fat yield) to 0.26 (angularity and fat yield) for fat yield and -0.29 (udder depth and protein yield) to 0.25 (angularity and protein yield) for protein yield.

Brotherstone (1994) determined that all phenotypic correlations between type traits and milk yield traits were low, while moderate correlations were obtained between milk yield, milk fat and protein and angularity (0.43), as well as between milk yield and udder depth (-0.44). Also, *Bohlouli et al. (2015)* in their study on correlations between type traits and milk yield determined positive correlations in all type traits which ranged in the interval from very weak (0.02) for front teats placement to weak (0.26) for angularity. All mentioned studies indicate that by application of selection on type traits and by an appropriate improvement of the structure of dairy cow's udder a simultaneous effect can be obtained both on the increase of milk yield and of milk content.

The values of phenotypic correlations obtained in this paper are mainly lower compared to mentioned studies. These differences can be a consequence of different models applied for their estimation as well as of different systems of linear estimation of dairy cows, but also of the differences in the size of population, size of studied sample, number of evaluators and their competence as well as of the intensity of previously applied selection on studied traits.

Conclusion

The values obtained for phenotypic correlations between type traits and milk yield traits showed that higher milk yields, milk fat and protein were obtained by cows that have more pronounced angularity as well as by those that have a good connection of fore and rear udder. These results also indicate a possibility of improving the traits of milk yield and type traits by application of selection on

multiple traits primarily by using the method of selection indices within national selection and breeding programmes. Milk yield traits and type traits should be included in optimal relationship in national selection programme in line with the aim of improvement in order to obtain a maximal selection effect.

In the future the success of bull and cow testing on the traits of interest shall be significantly improved by means of genomic selection because it will be known in advance whether the potential breeding animals inherited favourable gene patterns of their parents before they start to be exploited in the breeding stock.

Fenotipske karakteristike linearnih osobina vimena i uglatosti holštajn frizijskih krava i njihova povezanost sa osobinama mlečnosti

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Rezime

Za utvrđivanje fenotipske varijabilnosti i povezanosti između osobina mlečnosti i linearno procenjenih osobina vimena i uglatosti korišćena je evidencija o 10860 prvotelki holštajn frizijske rase, koje su se prvi put telile u periodu od 2011. do 2015. godine. Prosečne vrednosti analiziranih osobina tipa: (uglatost, veza prednjeg vimena, položaj prednjih sisa, dužina sisa, dubina vimena i visina zadnjeg vimena) iznosile su: 6,47; 5,74; 4,96; 5,20; 5,99 i 6,25 respektivno. Fenotipske korelacije između ispitivanih linearnih osobina tipa i osobina mlečnosti imale su vrednosti od -0,042 (dubina vimena i prinos mleka) do 0,335 (veza prednjeg vimena i prinos proteina). Pozitivna fenotipska korelacija (0,293) zabeležena je i između veze prednjeg vimena i prinosa mleka kao najvažnije osobine mlečnosti, dok je najniža korelacija između prinosa mleka utvrđena u odnosu na dubinu vimena (-0,033). Dobijeni rezultati ukazuju na mogućnost primene direktne i indirektno selekcije na više osobina koju je neophodno sprovoditi u okviru nacionalnog programa progenog testiranja bikova holštajn frizijke rase koristeći metod selekcijskih indeksa.

Cljučne reči: linearne osobine tipa, fenotipske korelacije, osobine mlečnosti

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PRODUCTION PERFORMANCES OF COWS OF DIFFERENT ORIGIN AND HOUSING METHOD

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Abstract: In order to examine the variability of production performance traits (lactation duration, milk yield for the whole lactation, milk yield in standard lactation, milk fat content, milk fat yield in standard lactation, protein content and yield in standard lactation), 954 cows, domestic and imported Simmental populations, were included in the study, with a total of 3641 completed lactations. Cows were located in the area of Toplica district, reared on individual farming households (tied system) and on the farm with intensive farming (free system). Based on the rearing method and origin, animals were divided into four groups: Group 1 (domestic animals reared by individual agricultural producers on individual farming households); Group 2 (imported animals reared by individual agricultural producers on individual farming households); Group 3 (domestic animals reared on the farm) and Group 4 (imported animals reared on the farm). The fourth group of studied cows showed the highest yield and protein content, while the cows of the third group had the longest lactation and the highest milk fat content. All production performance traits varied highly significantly ($p \leq 0.001$) under the influence of the combined factor of housing/rearing method and origin.

Keywords: breed, milk, milk fat, origin, Simmental farm

Introduction

Milk production has biological and production significance. Together with reproduction, it is the material basis for the maintenance of the species. In terms of production, in addition to being the highest quality human food, it also provides the basis for meat production through rearing calves. Milk yield is the most important feature of milk performance and is determined for the whole lactation, as well as for the standard 305 day lactation. Milk production in standard lactations, i.e. 305 day lactation, enables the mutual comparison of lactations of different duration.

Different genotypes of Simmental cows (domestic cows and Simmental cows imported from Germany and Slovenia), their production and reproductive traits, are examined by *Perišić (1998)*, in the region of upper flow of the Kolubara River. The study shows the average duration of the first three lactations of 307.18 days, in all animals included in the study, and the average milk yield for the duration of lactation of 4311.1 kg. The lowest milk yield is recorded in cows of Domestic spotted breed with production in the first three lactations of 3738.8 kg, 4033.4 kg and 4384.3 kg, respectively and the highest yield by cows of the German Simmental breed - 4120.4 kg, 4669.2 kg and 5153.2 kg. The study shows the average milk fat content for the whole lactation of 3.83%, for all animals included in the study, an average milk fat yield of 165.06 kg and 4191.17 kg of 4% FCM.

In the study of the major milk performance traits of F₁ daughters of the same Simmental bulls in Germany and Serbia, *Kučević et al. (2005)* have established significantly greater absolute difference in the quantity of milk produced of 1057 kg in the F₁ generation in Germany, the difference at the level of significance ($p \leq 0.05$). A statistically significant difference ($p \leq 0.01$) has been determined for milk fat and milk fat content, with daughters tested in Germany achieving higher production.

By comparing the three-year average of the best Simmental herd in our country and the average established for imported first calving heifers from Austria during 2004-2005, *Medić et al. (2006)* have determined the milk yield of 4472 kg for 305 days, for domestic animals, which is lower compared to imported animals by significant 1171 kg of milk. Austrian animals also had a significantly higher average milk fat content of + 0.49% and a total milk fat content of +73 kg. These differences of mean values are highly significant.

Pantelić (2006) states that the average duration of lactation in first calving Simmental heifers in Serbia is 311.72 days, with a minimum duration of 241 and a maximum of 586 days.

In the study of the production potential of first calving Simmental heifers calving in the period 2007-2010 in the individual sector in Serbia, *Nikšić et al. (2011)* have recorded the average milk yield of 4348 kg, 3.93% milk fat content and milk fat yield of 171.1 kg. With the exception of 2009, the milk yield of first calving heifers has been steadily increasing on average by about 100 kg of milk per head per year.

In the analysis of the phenotypic variability of Simmental bull dams, *Pantelić et al. (2013)* have determined the milk yield of 5754.49 kg, with 3.98% and 230.24 kg of milk fat, i.e. 5755.47 kg of 4% FCM.

In the study of the effect of the import of breeding animals of the Simmental breed from Germany on the implementation of the breeding program on the territory of the city of Kragujevac, *Kostić (2014)* has obtained the following production results in his research: the imported animals in all three lactations have achieved higher milk yield (449.08 kg in I, 568.52 kg in II and 488.73 kg in III),

milk fat (19.87 kg, 22.66 kg and 18.52 kg) and 4% fat corrected milk (477.64 kg; 567.26 kg and 473.36 kg). Domestic cows have recorded lower milk fat content in the first lactation by 0.05%, but the same trait in the second and third lactation has shown higher levels by 0.01% in each lactation.

The average production of all cows under control in Austria in 2012 (*Genetic Austria*) was 7073 kg of milk, 4.15% milk fat (293.53 kg), and 3.43% protein (242.60 kg), while the average production of first calving heifers was 6419 kg, with 4.13% milk fat (265.1 kg) and 3.40% protein (218.25 kg).

Materials and Methods

Basic data on production traits, as well as data on the origin of all the studied cows, were collected in collaboration with the “Lazar” Blace farm, which housed part of the cattle included in this study. For the animals that were reared on the households/farms of individual agricultural producers, data on the above performance traits were collected in cooperation with the breeding organizations, which are responsible for the implementation of the breeding program in the area of Toplica district. This study identified differences in the production traits of imported cows reared on the “Lazar” Blace farm and by individual agricultural producers, then differences in the milk performance traits between the imported and cattle of domestic origin on the farm, but also with individual agricultural producers of Simmental breed cows in the same area.

The total number of animals and their completed lactations is divided into four groups, with each group having approximately the same percentage of cows of different lactations by order, as follows:

Group 1: Domestic animals reared by individual agricultural producers on individual farming households (n = 1526);

Group 2: Imported animals reared by individual agricultural producers on individual farming households (n = 234);

Group 3: Domestic animals reared on the farm (n = 1100);

Group 4: Imported animals reared on the farm (n = 781).

When examining the impact of the unified factor of housing/rearing and origin, the model with fixed unified factor of housing/rearing and origin (NP) was used:

$$Y_{ij} = \mu + NP_i + e_{ij}$$

Y_{ij} : trait studied,

μ : population average for a given trait,

NP_i : fixed unified effect of housing/rearing and origin (i = 1,2,3,4)

e_{ij} : random error

The analyzed factors are combined/merged due to their interaction, because to the greatest extent their impact is reflected through the interaction of these two factors.

Subsequent to the variance analysis and determining the basic parameters of descriptive statistics by using the test of least significant differences (LSD), the differences between the groups were determined individually for all observed traits.

SPSS Statistics software for Windows, Version 23.0 was used for the statistical data processing and application of the said model.

Results and Discussion

In this study, the following milk performance traits were analyzed: lactation duration, whole and standard lactation milk yield, milk fat content and yield, and protein content and yield.

Table 1 shows the lactation duration of the observed cows divided into four groups. The shortest duration of lactation was recorded in cows of domestic origin reared by individual agricultural producers, 304.12 days. The imported cows reared by individual agricultural producers had an average lactation duration of 313.67 days, while slightly longer lactation time was recorded in cows of domestic origin reared on the farm, 323.96 days, and slightly longer in imported animals, 314.13 days. The results obtained are higher than the average lactation duration reported by *Perišić (1998)* and *Pantelić (2006)*, except for the first group of cows, where the duration of lactation is shorter compared to values obtained by these two authors. The combined housing/rearing and origin factor showed statistically very highly significant ($p \leq 0.001$) effect on the observed four groups of cows for the trait tested. If the milk yield values for the whole and standard lactation by groups are observed, as shown in Table 1, the lowest values were recorded in cows of domestic origin housed/reared on individual agricultural households/farms, slightly higher values were recorded in the second and third group of cows, and the highest in imported cows reared on the farm (Group 4). Milk yield values for the whole and standard lactation varied statistically very highly ($p \leq 0.001$) under the unified factor of housing/rearing method and origin of the animal. The obtained values for milk yield in all four groups were significantly higher than the values for first calving heifers in Serbia, as reported by *Nikšić et al. (2011)*. Groups of imported animals (3 and 4) achieved higher production than animals originating from Germany in the research of *Perišić (1998)* and bull dams in Serbia, cited in their research by *Pantelić (2013)*.

Table 1. Mean values and variability of milk yield traits by groups of cows

Trait	Group	Number of lactations	\bar{X}	SD	SE	95% confidence interval		Min.	Max.
						LB	UB		
Duration of lactation (days)	1	1526	304.12	39.096	1.001	302.15	306.08	200	563
	2	234	313.67	34.845	2.278	309.18	318.15	236	434
	3	1100	323.96	46.084	1.389	321.23	326.68	243	514
	4	781	314.13	19.382	0.694	312.77	315.49	256	412
Total		3641	312.87	38.851	0.644	311.61	314.13	200	563
		F=58.647***			p=0.000				
Milk yield whole lactation (kg)	1	1526	4817.57	1123.811	28.768	4761.14	4874.00	1877	13150
	2	234	5028.50	873.375	57.094	4916.01	5140.99	2610	7438
	3	1100	6181.03	1160.670	34.996	6112.37	6249.70	3050	13367
	4	781	6592.19	659.728	23.607	6545.85	6638.53	4273	9135
Total		3641	5623.71	1291.620	21.405	5581.74	5665.67	1877	13367
		F=663.926***			p=0.000				
Milk yield standard lactation (kg)	1	1526	4802.84	963.159	24.656	4754.48	4851.20	1289	9434
	2	234	4907.26	823.760	53.851	4801.17	5013.36	2609	7015
	3	1100	5872.75	928.905	28.008	5817.79	5927.70	3031	9643
	4	781	6437.63	590.128	21.116	6396.17	6479.08	4434	9000
Total		3641	5483.45	1106.311	18.334	5447.50	5519.40	1289	9643
		F=721.625***			p=0.000				
Milk fat content standard lactation (%)	1	1526	3.89	0.109	0.003	3.88	3.89	3.32	4.81
	2	234	3.92	0.079	0.005	3.91	3.93	3.69	4.17
	3	1100	3.94	0.131	0.004	3.93	3.94	3.53	4.91
	4	781	3.92	0.092	0.003	3.92	3.93	3.48	4.28
Total		3641	3.91	0.113	0.002	3.91	3.92	3.32	4.91
		F=42.177***			p=0.000				
Milk fat yield standard lactation (kg)	1	1526	187.22	39.968	1.023	185.21	189.22	47.95	403.56
	2	234	192.46	33.526	2.192	188.14	196.78	106.04	276.10
	3	1100	231.11	37.102	1.119	228.92	233.31	120.05	411.16
	4	781	252.56	22.846	0.817	250.96	254.17	172.91	360.97
Total		3641	214.83	44.789	0.742	213.38	216.29	47.95	411.16
		F=704.633***			p=0.000				
Milk protein content standard lactation (%)	1	1526	3.15	0.087	0.002	3.15	3.16	2.61	3.43
	2	234	3.15	0.074	0.005	3.14	3.16	2.87	3.36
	3	1100	3.16	0.088	0.003	3.16	3.17	2.95	3.93
	4	781	3.24	0.096	0.003	3.23	3.25	2.83	3.43
Total		3641	3.17	0.095	0.002	3.17	3.18	2.61	3.93
		F=176.346***			p=0.000				

Milk protein yield standard lactation (kg)	1	1526	151.44	30.685	0.786	149.90	152.99	40.86	297.31
	2	234	154.70	26.320	1.721	151.31	158.09	85.45	221.98
	3	1100	185.81	29.875	0.901	184.04	187.58	99.43	305.04
	4	781	208.57	20.779	0.744	207.11	210.03	144.51	298.27
Total		3641	174.29	36.559	0.606	173.10	175.48	40.86	305.04
		F=811.078***			p=0.000				

N=number; \bar{x} =average; SD=standard deviation; SE=standard mean error; LB= lower limit; UB=upper limit; F=statistical value; p=significance ***- $p \leq 0.001$; ** - $p \leq 0.01$; * - $p \leq 0.05$; nz - $p > 0.05$

Observing by groups (Table 1), the highest milk fat content was recorded in cows of domestic origin reared on the farm - 3.94%, 3.92% of milk fat content was recorded in imported animals reared on the farm and by individual agricultural producers, while the lowest content of milk fat was recorded in domestic cows reared by individual agricultural producers (3.89%). The values obtained are in the concordance with those obtained by *Nikšić et al. (2011)*, slightly lower than the milk fat content of bull dams in Serbia reported by *Pantelić et al. (2013)*, and by 0.10% higher in relation to the values obtained by *Perišić (1998)*. The influence of the factors of the housing/rearing method and the origin of the animals had statistically very high ($p \leq 0.001$) impact on the milk fat content of the four observed groups.

Table 1 also see shows the realized milk fat yields by groups based on origin and method of housing/rearing. The highest yields were obtained from imported cows on the farm (252.56 kg), followed by cows of domestic origin also on the farm (231.11 kg), imported cows reared by individual agricultural producers originating (192.46 kg), and the lowest yields were achieved by domestic cows reared by individual producers (187.22 kg). All four groups of cows achieved significantly higher milk fat production than those cited by *Perišić (1998)* and *Nikšić et al. (2011)*, while the milk fat production of bull dams cited by *Pantelić et al. (2013)* is the same as the third group of cows. The influence of these factors on the variability of milk fat yield of the observed population by groups was statistically very high ($p \leq 0.001$).

Looking at the population by groups, it can be concluded that the animals reared by individual agricultural producers had the same protein content (3.15%), regardless of their origin. Slightly higher values for protein content were obtained from farm-raised animals (3.16%), while a significantly higher percentage was obtained from farm-raised imported animals (3.24%). Protein content values obtained are significantly lower than the results reported in Austria for the entire examined population of cows and first calving heifers in their 2012 annual report. The effect of these two combined factors on the observed trait of the four groups of cows was statistically very highly significant ($p \leq 0.001$).

Table 1 also lists the milk protein yield values in the observed population divided into four groups. The lowest yield was achieved by cows of domestic origin reared by individual producers, 151.44 kg, which is 3.26 kg less than the yield of cows originating from import reared under the same conditions. The farm reared domestic animals had a protein yield of 185.81 kg, while under the same conditions the imported animals realized a yield of 208.57 kg. As for the protein content, the protein quantity was significantly lower than the results reported in Austria for the whole examined population of cows and first calving heifers in their 2012 annual report. Protein yield varied statistically very high significantly ($p \leq 0.001$) under the influence of the unified factor of rearing method and origin.

Table 2. Differences of averages for all examined traits by groups of cows (LSD test)

Duration of lactation	2	3	4	Milk yield whole lactation	2	3	4
1	-9.551***	-19.840***	-10.017***	1	-210.931**	-1363.467***	-1774.622***
2		-10.289***	-0.465 ^{nz}	2		-1152.536***	-1563.691***
3			9.824***	3			-411.155***
Milk yield standard lactation	2	3	4	Milk fat content	2	3	4
1	-104.423 ^{nz}	-1069.906***	-1634.784***	1	-0.0288***	-0.0469***	-0.0354***
2		-965.483***	-1530.361***	2		-0.0181*	-0.0066 ^{nz}
3			-564.878***	3			0.1154*
Milk fat yield	2	3	4	Milk protein content	2	3	4
1	-5.2448*	-43.8904***	-65.3441***	1	0.0015 ^{nz}	-0.0107*	-0.0854***
2		-38.6492***	-60.0993***	2		-0.0122 ^{nz}	-0.0869***
3			-21.4500***	3			0.0747***
Milk protein yield	2	3	4				
1	-3.2514 ^{nz}	-34.3674***	-57.1273***				
2		-31.1160***	-53.8759***				
3			-22.7600***				

Based on the test of the least significant difference (LSD, Table 2) it can be stated that the difference between the groups was statistically significant at different levels of significance ($p \leq 0.001$ *** to $p \leq 0.005$ *), in all examined traits, except between groups 1 and 4 for the trait lactation duration, 1 and 2 for the trait milk production in standard lactation, 2 and 4 for the milk fat content, 1 and 2 and 2 and 3 for the protein content, and 1 and 2 for the trait of protein yield, where no statistically significant differences were established ($p > 0.05$ nz).

Conclusions

Farm-reared cows (domestic and imported) achieved by 1614.34 kg higher yield than cows reared on individual agricultural households (domestic and imported), while imported animals (farm-reared and reared on individual agricultural households) produced by 1261.50 kg more milk compared to animals of domestic origin (farm-reared and reared on individual agricultural households).

All observed production performance traits varied very significantly ($p \leq 0.001$) under the influence of the combined factor of the rearing method and the origin of animals.

Based on the results obtained for production performance traits, it can be concluded that the yield of milk, milk fat and protein in the observed population was above the average of the whole population of cows of the Simmental breed in the Republic of Serbia.

From the results obtained by comparing cows by origin and method of rearing, it can be concluded that domestic cows can have significantly higher production if they are provided with favorable rearing conditions present on the studied farm. It can also be concluded that imported animals contribute significantly to the improvement of production on individual agricultural households, but at the same time that their potential is not utilized to the maximum of their potential in such rearing conditions.

Proizvodne performanse krava različitog porekla i metoda držanja

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Rezime

U cilju ispitivanja varijabilnosti proizvodnih osobina (trajanje laktacije, prinos mleka u celoj laktaciji, prinos mleka u standardnoj laktaciji, sadržaj mlečne masti, prinos mlečne masti u standardnoj laktaciji, sadržaj proteina i njegov prinos u standardnoj laktaciji) domaće i uvežene populacije krava simentalske rase, u ovom istraživanju obuhvaćeno je 954 krava, sa ukupno 3641 zaključenom laktacijom. Sve krave su se nalazile na području Topličkog okruga, kod individualnih poljoprivrednih proizvođača (vezani sistem) i na farmi sa intenzivnim načinom gajenja (slobodni sistem). Na osnovu načina držanja (vezani i slobodni sistem) i porekla (damaća grla, grla iz uvoza) grla su bila podeljena u četiri grupe: grupa 1 (grla domaće porekla, gajena kod individualnih proizvođača); grupa 2 (grla

poreklom iz uvoza, gajena kod individualnih proizvođača); grupa 3 (grla domaćeg porekla, gajena na farmi) i grupa 4 (grla poreklom iz uvoza, gajena na farmi). Četvrta grupa posmatranih krava ostvarila je najveće prinose i sadržaj proteina, dok je treća grupa imala najdužu laktaciju i najveći sadržaj mlečne masti. Sve proizvodne osobine vrlo visoko značajno ($p \leq 0.001$) varirale pod uticajem objedinjenog faktora načina držanja i porekla.

Ključne reči: rasa, mleko, mlečna mast, poreklo, farma krava simentalске rase

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OESTRUS SYNCHRONIZATION EFFICIENCY IN EWES AND RAM MATURITY EFFECT ON FERTILITY DURING SUMMER SEASON

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Abstract: The purpose of this study was to investigate oestrus synchronization efficiency and ram maturity effect on fertility of ewes of MIS sheep population during summer season. Research was performed on the experimental sheep farm of the Institute for Animal Husbandry in Belgrade, Serbia. The study included 70 crossbred ewes of MIS sheep population (Pirot Pramenka, Merinolandschaf and Ile de France breed) and 3 Ile de France rams (2 young, sexually unexperienced rams and 1 mature ram). Oestrus was synchronized in all 70 ewes using progestagen impregnated vaginal sponges (30 mg fluorogestone acetate) in combination with 500 IU PMSG. Ewes were mated with rams 48 h later. Obtained fertility and productivity results were as follows: lambing rate 57.14% (40/70), gestation length 147.11, litter size 1.82, stillborn rate 15.07%, birth weight of lambs 4.29 kg and body weight of lambs at 30 days of age 13.07 kg on average. Based on the results obtained in this study it was concluded that presented oestrus synchronization and mating protocol yielded lower than expected lambing rate. The effect of ram maturity on lambing rate was significant ($P < 0.05$), as ewes mated to mature ram had higher lambing rate compared to those mated to two young unexperienced rams. Mature ram also had bigger litter size and higher stillborn rate, as well as lower birth weight of lambs.

Key words: ram, maturity, fertility, oestrus synchronization, sheep

Introduction

The use of exogenous hormones for oestrus synchronization is a common practice in intensive sheep farming. Most commonly used procedure for oestrus induction/synchronization is application of progestagens via vaginal sponges in combination with PMSG or FSH, especially outside the breeding season. Progestagens are widely used to synchronize oestrus in sheep and usually result in greater than 90% of ewes in heat in a 24-hour period and conception rate of 70–

80% (Evans et al., 2001). However, fertility results achieved after hormone induced/synchronized oestrus vary depending on a number of factors, such as season, dose of PMSG used, fertilization method, body condition and physiology status of ewes. These are mostly female factors, but male factors, such as sperm fertility and libido, are also important. However, it is difficult to determine exactly which factors, female or male, contribute to fertility results and in what amount, but both must be taken into consideration.

MIS sheep population is a meat type breed of strong constitution, good carcass conformation and good meat quality properties. It was obtained by using a complex combination crossing according to a precisely defined genetic procedure, using Pirot Pramenka, Merinolandschaf and Ile de France breeds (Petrović, 2006). Ewes of MIS sheep population reach puberty at age of 6-8 months, which classifies them as the group of early maturing populations. In regard to fertility, MIS sheep population is very good if well managed and the use of progestagens in addition to PMSG hormone appears to be effective in the induction/synchronization of oestrus and in increasing of mean litter size outside the breeding season (Maksimović et al., 2015). However, there is not enough information in the literature on synchronization efficiency and fertility in ewes of MIS sheep population.

A significant percentage of domestic yearling rams are sexually inhibited when first exposed to oestrus ewes (Price et al., 1999b). Sexually inexperienced rams or ram lambs can often exhibit orientation problems with respect to mounting behavior. Among a group of oestrus ewes, rams can also show some preference for specific females while ignoring other ewes and this accounts for some differences in the lambing distribution (Fitzgerald and Morgan, 2007).

The role of experience on sexual performance suggests that early exposure to females will increase the probability of rams becoming sexually active at an earlier age (Perkins and Roselli, 2007). According to Perkins et al. (1992), the exposure of rams to oestrous ewes can increase the levels of LH and consequently the testosterone secretion in the ram, which in turn has been suggested to positively influence the production of pheromones (Haynes and Haresign, 1987). The close contact with ewes in oestrous also increases the libido of rams (Rodriguez Iglesias et al., 1991).

The purpose of this study was to investigate oestrus synchronization efficiency using progestagen+PMSG protocol and ram maturity effect on fertility in the ewes of MIS sheep population during summer season.

Material and Methods

Location and animals

Research was performed on the experimental sheep farm of the Institute for Animal Husbandry in Belgrade, Serbia at the end of July and the beginning of

August 2020. The study included 70 crossbred ewes of MIS sheep population (Pirou pramenka x Merinolandschaf x Ile de France) and 3 Ile de France rams. Ewes were 2 to 6 years of age, two young previously unexperienced rams (marked as ram 2 and ram 3) were both 1.5 year old and one mature ram (marked as ram 1) was 3 years old. Previous to mating, as well as after mating both rams and ewes were kept outdoors at pasture, but separately.

Experimental design

All 70 ewes were synchronized into oestrus using progestagen impregnated vaginal sponges (30 mg fluorogestone acetate, FGA, Syncro-Part, Ceva sante animale, France) in combination with pregnant mares serum gonadotropin (PMSG). Sponges remained *in situ* for 12 days. On the day of sponge removal, ewes received an intramuscular injection of 500 IU PMSG (Folligon[®], MSD Animal health). Rams were introduced to ewes 48 hours later and were kept with them for mating for about 8-10 hours/day. Ram to ewe ratio for mating was 1:3/day. Mounting activities of rams were not recorded, neither visually or with harnesses (crayons). Ram breeding soundness was considered by inspection and examination of reproductive organs.

The measured traits

The following reproductive parameters were measured: lambing rate (number of ewes lambed/number of ewes mated), stillborn rate (number of stillborn lambs/number of lambs born), gestation length, litter size (number of lambs born/number of ewes lambed), birth weight of lambs, birth weight of lambs at 30 days of age.

Statistical analysis

Statistical analysis of the experimental data was performed using the statistical package Statistica for Windows 7 (Stat. Soft. Inc.). The equality of variances of analyzed treatments was tested using the Leven's test. Lambing rates and stillborn rates were tested by Chi square analysis. Effect of ram on litter size and birth weight of lambs was tested using ANOVA and the mean comparison between rams was done by Fisher's LSD test. Analyses were performed for the significance level of 5% and 1% and the results were presented as mean \pm SEM.

Results and Discussion

Table 1 shows fertility traits (lambing rate, gestation length, litter size, stillborn rate) of ewes from synchronized oestrus and body weights of lambs at birth and 30 days of age.

Table 1. Fertility results of ewes and body weights of lambs (Mean±SEM)

Lambing rate, %	Gestation length, days	Litter size (all lambs)	Litter size (live born only)	Stillborn rate, %	Birth weight of lambs, kg	Body weight of lambs at 30 days of age, kg
57.14 (40/70)	147.11±0.14	1.82	1.55	15.07	4.29±0.13	13.07±0.46

Of the total of 70 ewes in the trial, 40 of them lambed after the synchronization of oestrus and joining with rams, representing lambing rate of 57.14%. Average gestation length was 147.11±0.14 days. Litter size was 1.82 lambs/ewe born (including stillborn lambs) and 1.55 lambs/ewe born including only live born lambs. Stillborn rate was 15.07%. Birth weight of lambs was 4.29±0.13 kg and body weight of lambs at 30 days of age was 13.07±0.46 kg on average. When compared to previous study of *Maksimović et al. (2015)* investigating the fertility of MIS sheep population after progestagen+PMSG synchronized oestrus outside breeding season, present study resulted in lower lambing rate, lower litter size, higher body weight of lambs at birth and at 30 days of age, as well as lower stillborn rate. *Maksimović et al. (2017)* also report higher lambing rate of 66.66% in MIS sheep population, although after repeated prostaglandin treatment for oestrus induction outside breeding season. *Mekić et al. (2014)* report lambing rates of 48.47% on average in Ile de France ewes after progestagen+PMSG oestrus synchronization protocol outside breeding season. *Evans et al. (2001)* state that the use of progestagen for oestrus synchronization typically results in greater than 90% of ewes in heat in a 24-hour period and conception rate of 70–80%. However, fertility results achieved after hormone induced/synchronized estrus vary depending on a number of factors, such as season, dose of PMSG used, fertilization method, body condition and physiology status of ewes. Also, male factors, such as sperm fertility and libido, are also important.

Table 2 shows effect of ram on lambing rate, litter size, stillborn rate and birth weight of lambs.

Table 2. Effect of ram on fertility results and birth weight of lambs (Mean±SEM)

Parameters	Lambing rate, %	Litter size	Stillborn rate, %	Birth weight of lambs, kg
Ram 1	75 ^a	2.22 ^a ±0.25	25 ^a	3.81 ^A ±0.15
Ram 2	47.83 ^b	1.91 ^{ab} ±0.28	4.76 ^b	4.25 ^A ±0.2
Ram 3	47.83 ^b	1.18 ^b ±0.12	7.69 ^b	5.52 ^B ±0.22

^{a, b} - Column means with different superscript letters differ significantly at P<0.05

^{A, B} - Column means with different superscript letters differ significantly at P<0.01

As presented in Table 2, the effect of ram on all studied parameters was significant; 75% of ewes conceived and lambed with older, mature ram, compared to 47.83% of ewes that lambed after being mated to each of two young previously unexperienced rams ($P < 0.05$). Mature ram also had bigger litter size and higher stillborn rate, as well as lower birth weight of lambs. Higher rates of stillbirths are common in more numerous litters, as well as lower birth weights are (Berger, 1997; Maksimović *et al.*, 2015). With the use of exogenous hormones there is a greater chance for intense ovarian activity and ovulation of greater number of oocytes that will potentially be able to become fertilized. Highly prolific sheep breeds normally have higher mortality rates of lambs at birth compared to less fertile breeds, because of the more numerous litters (Berger, 1997).

It is common for young rams to give lower fertility results when first introduced to breeding. Price *et al.* (1994) point out that intermittent exposure of ram lambs to estrous ewes early in life can improve their sexual performance as yearlings. In later study, Price *et al.* (1999a) state that direct contact with females was necessary for enhanced sexual performance in young rams. Authors have found that 59% of the rams (age 7-8 months) reared without contact with females failed to become active during their first sexual performance test. In contrast, the same study showed that only 15% of the rams reared with direct contact with females were inactive during their first sexual performance test. Also, 38% of the rams that had fence-line contact with ewes was sexually inactive during their first test. High percentage of domestic yearling rams are sexually inhibited when first exposed to ewes in estrous, however, most of these rams will eventually mate with ewes after repeated or prolonged exposure to females (Katz *et al.*, 1988; Price *et al.*, 1999b). In the study of Price *et al.* (1999b), only 56% of tested rams successfully mated with estrous ewes on first exposure. Stellflug and Lewis (2007) conclude that early exposure of 7–8 months old ram lambs to oestrus ewes improves sexual performance in serving capacity tests at 16–19 months of age in most rams. However, some authors did not find clear link between maturity and previous experience of rams to fertility results in ewes. Kenyon *et al.* (2007) found that two-tooth rams previously unused and mature rams previously used for mating are a better option than unused ram hoggets to maximize the pregnancy rate of ewe hoggets, but that it was unclear why lower conception rates were observed in ewe hoggets joined with mature rams which had been used previously. Authors suggested that as a result, the advantage of using mature rams may have been underestimated.

With the practice of hand mating within restricted time it is ideal to have one on one ewe to ram ratio. When given a choice to choose between more females, rams tend to mate with same ewe/s more than once and not pay attention to others (Tilbrook *et al.*, 1987; Fitzgerald and Morgan, 2007), or they chase after all of them at once and become exhausted, which is common in first time breeders, or get intimidated by females, also common in young rams. Therefore, one ram to

three ewes ratio for limited time period mating that was used in this study could have contributed to less fertilized ewes and lower lambing rate.

However, it is difficult to determine exactly which factors, female or male, contributed to fertility results and in what amount, but both must be taken into consideration.

Conclusion

Based on the results obtained in this study it can be concluded that presented oestrus synchronization protocol of using progestagen+PMSG during summer months subsequently followed by mating of ewes through single natural service, yielded lower than expected lambing rate. Ram maturity had significant effect on lambing rate as ewes mated to mature ram had higher lambing rate compared to those mated to two young unexperienced rams. Mature ram also had bigger litter size and higher stillborn rate, as well as lower birth weight of lambs. Ram to ewe ratio of 1:3 may have also contributed to lower conception and lambing rate. Perhaps 1:1 to 1:2 ram to ewe ratio would be better when young rams are used for limited time breeding. Fertility results are influenced by both management and animals and therefore further studies are needed in order to determine what contributes the most. Also, modification of oestrus synchronization protocol and usage of different hormone levels to ensure better oestrus response for MIS sheep population can be considered. Use of teaser rams to detect ewes in heat and prolonged mating time could ensure higher conception rates. Also, concept of ram maturity and previous experience effect on mating abilities and fertility results should be further investigated.

Efekat sinhronizacije estrusa i uticaj zrelosti ovna na plodnost ovaca tokom letnje sezone

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Rezime

Cilj istraživanja bio je da se ispita efikasnost sinhronizacije estrusa i uticaj zrelosti ovna na plodnost ovaca MIS populacije tokom letnje sezone. Ispitivanja su sprovedena na eksperimentalnoj farmi Instituta za stočarstvo u Beogradu, Srbija. U ogled je bilo uključeno 70 plotkinja MIS populacije (dobijene složenim kombinacijskim ukrštanjem pirotske pramenke, virttemberg i il de frans rase ovaca)

i tri il de frans ovna, od kojih su dva mlada priplodnjaka prvi put uvedena u priplod i jedan zreo ovan sa prethodnim iskustvom u reprodukciji. Estrus je sinhronizovan kod svih 70 ovaca upotrebom progestagenskih vaginalnih sunđerica (30 mg FGA) u trajanju od 12 dana i u kombinaciji sa 500 i.j. SŽK. Plotkinje su pripuštene za parenje sa ovnovima nakon 48 h od aplikacije SŽK. Ostvareni su sledeći rezultati: stopa jagnjenja 57,14% (40/70), dužina bremenitosti 147,11, indeks jagnjenja 1,82, stopa mrtvorodenja 15,07%, masa jagnjadi na rođenju 4,29 kg i masa jagnjadi u uzrastu od 30 dana 13,07 kg, prosečno. Uticaj zrelosti ovna na stopu jagnjenja bio je statistički značajan ($P < 0,05$) pri čemu su se plotkinje koje su se parile sa zrelim ovnom sa prethodnim iskustvom u reprodukciji ojašnjile u većem broju u poređenju sa plotkinjama koje su parene sa mladim, reproduktivno neiskusnim ovnovima. Zreo ovan je takođe imao i veći broj rođene jagnjadi, veću stopu mrtvorodenja, kao i manje porođajne mase jagnjadi. Rezultati plodnosti se nalaze istovremeno pod uticajem samih životinja kao i menadžmenta u proizvodnji, a dodatna istraživanja potrebna su da bi se utvrdilo u kolikoj meri koji faktori doprinose. Fokus bi trebalo staviti na eventualnu modifikaciju protokola sinhronizacije estrusa da bi se osigurala efikasnija pojava estrusa. Upotreba ovnova probaća za detekciju ovaca u estrusu, kao i produžetak vremena trajanja pripusta ili dvokratni pripust mogli bi osigurati bolju stopu koncepcije. Takođe, trebalo bi dodatno ispitati koncept uticaja zrelosti i prethodnog reproduktivnog iskustva ovna na sposobnost parenja i rezultate plodnosti.

Ključne reči: ovnovi, zrelost, plodnost, sinhronizacija estrusa

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STUDY OF THE RELATIONSHIP BETWEEN THE AGE OF THE RAMS AND THE QUALITY OF THEIR EJACULATES OBTAINED OUTSIDE THE BREEDING SEASON

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Original scientific paper

Abstract:The present study aims to research the effect of age on the quality of ejaculates of rams from Synthetic population of Bulgarian dairy breed. Rams divided into two age groups were used for the experiment: G1 (2.5 years) versus G2 (6 years). Ejaculates were obtained by the method of artificial vagina and diluted with medium 6A-G. The tested ejaculates were obtained outside the breeding season. A Sperm Class Analyzer (SCA Microptic, Barcelona, Spain) was used to study sperm motility. The biochemical activity of the enzymes lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT) was determined using an automatic Mindrai BA88 spectrophotometer. The activity of the enzymes was studied in sperm plasma, in aqueous and tritonic extract. Significant differences between the groups were found in total sperm motility ($P \leq 0.05$) and LDH enzyme activity in sperm plasma ($P \leq 0.01$). Regarding the efficiency of the LDH enzyme in aqueous and tritone extract, no significant differences between the groups was found. No significant differences were found between groups for GGT activity, while age had significant impact on LDH enzyme in sperm plasma.

Keywords: ram, spermatozoa, motility, LDH, GGT

Introduction

In rams, fluctuations in reproductive capacity during different seasons of the year are evaluated (*Boland et al., 1985*). The choice of fertility in sheep can be made by selecting correlated traits in young rams such as age, testicular size, testicular length and others (*Land and Carr, 1975*). In general, the sexual development of rams appears to be more closely related to body growth than to their age (*Dyrmundsson and Lees, 1972*). The qualitative indicators of ejaculate are highest in autumn and winter and low in spring and summer, the same pattern is

observed in quantitative indicators such as volume and concentration of sperm (Hafez, 1952). Sexual activity in rams changes with seasonality, which is influenced by changes in day light periods, age and social hierarchy (Mickelsen et al., 1982). They have shown that both sexual behaviour and sperm quality vary with age and breed (Aisen, 2004; Pelayo, 2019), although other authors believe that libido may be influenced more by season than by age (Aisen and Venturino, 2004). There are scientific reports of a reduction in the quantity and quality of sperm production and sperm fertility in rams during the non-breeding season (Makawi et al., 2007; Azawi and Ismaeel, 2012).

Sperm analysis involves a series of tests that evaluate various factors or functions of the sperm. Evaluation of sperm motility is one of the most widely studied parameters (Gallego et al., 2018), as well as the activity of key enzymes for the reproductive system such as lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT) (Stefanov et al., 2013). The activity of certain enzymes varies in seasonally polycyclic animals (sheep, goats, horses) and shows reproductive seasonality (Gündoğan, 2005). The enzyme lactate dehydrogenase (LDH) has been shown to be important for various metabolic processes that provide energy for viability, mobility and sperm fertility (Duan and Goldberg, 2003). The function of the enzyme gamma-glutamyl transferase (GGT) is not well defined. According to some authors, the enzyme is a specific marker of sperm, plays an important role in redox balance and performs a protective function during the transport and storage of sperm in the epididymis (Hinton et al., 1991). Therefore, the evaluation of biochemical components and enzymes in semen plasma can be recommended as biological markers of sperm quality, as their values determine sperm function, integrity and damage (Tvrda et al., 2013; Tejaswi et al., 2016).

The aim of the present study was to investigate the effect of age on the quality of ejaculates from rams of the Synthetic Bulgarian Dairy population.

Material and Methods

Animals

The experiment was conducted with four clinically healthy rams of the breed Synthetic population of Bulgarian dairy, divided into two age groups: Group 1 (2.5 years - young) vs Group 2 (6 years - mature). A total of 16 ejaculates from each group obtained on 8 consecutive days were examined. The studied ejaculates were obtained outside the breed's breeding season. Rams were kept under the same breeding conditions and nutrition.

Sperm production and analysis

Sperm collection is performed by the method of an artificial vagina by an experienced operator. All obtained ejaculates were subjected to an initial macroscopic evaluation and those outside the standard requirements were discarded. Immediately after that, each ejaculate was diluted in a ratio of 1:12 with medium 6A, prepared at the Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov" - BAS. The obtained ejaculates were analyzed by computer sperm analyzer (SCA, Microptic SL, Barcelona, Spain) to determine the total motility of sperm (TM,%), progressively motile sperm (PM,%), sperm with non-progressive movement (NPM). and immotile sperm (Immotile,%).

Biochemical analysis

The extracellular activity of the enzymes lactate dehydrogenases isoenzyme C4 (LDH-C4, U / L) and gamma-glutamyl transferase (GGT, U / L) was determined in the sperm plasma. Aqueous and newt extracts were prepared to determine the intracellular activity of both enzymes. After dilution of the ejaculate, the seminal plasma was removed by centrifugation at 3500 rpm at 37 ° C for 15 minutes. The resulting sperm plasma from each tube was carefully aspirated with a micropipette into sterile Eppendorf tubes and the extracellular activity of the enzymes was determined. Distilled water (1ml) was added to the resulting precipitate and frozen at -20 ° C. After 24 h, the samples were thawed and sonicated (150 W MSE ultrasonic disintegrator) by ultrasound three times for 10 s. The tubes were then centrifuged at 12,000 rpm for 15 min. In this way, an aqueous extract is obtained, in which the sperm cells are destroyed and the intracellular enzymes are extracted. Triton extract was also obtained to completely destroy the sperm cells. To obtain it, the resulting precipitate was resuspended with 1% Triton X-100 and centrifuged under the same conditions. In this way, the intracellular activity of the enzymes in the sperm was determined. The activity of the enzymes LDH-C4 and GGT was determined by a semi-automatic spectrophotometer for clinical chemistry BA-88 (Mindray, Medical Germany GmbH, Bensheim, Germany) using a set of reagents manufactured by Via Campania - Italy.

Statistical analysis

The analysis of all results was performed with a specialized statistical analysis package IBM SPSS Statistics 23 (SPSS Inc., Chicago, USA). Comparison of sperm characteristics was performed by ANOVA statistical tests. Significance of group differences was assessed with One Sample T-Test. Results are presented as mean \pm standard error (SE).

Results

Results of sperm motility analysis in both groups of rams are presented in Table 1.

Table 1. Sperm motility

Group	N	TM, % Mean \pm SE	PM, % Mean \pm SE	NPM, % Mean \pm SE	Immotile, % Mean \pm SE
G1	16	99.60 \pm 0.11*	75.82 \pm 2.59 ^{ns}	23.78 \pm 2.50 ^{ns}	0.39 \pm 0.11*
G2	16	98.08 \pm 0.53*	72.05 \pm 4.19 ^{ns}	26.03 \pm 3.89 ^{ns}	1.91 \pm 0.53*

Note: * Significant differences at $P \leq 0.05$; ^{ns} – non significant

The effect of age on sperm motility for semen obtained outside the breeding season of the breed was found to be significant only for the total motility ($P \leq 0.05$). Regarding progressively motile (PM) and non-progressively motile (NPM) sperm, no significant differences between groups were found.

The extracellular and intracellular activity of the enzyme lactate dehydrogenase (LDH-C4) is presented in Table 2. The activity of the enzyme in sperm plasma in young rams (G1) is almost twice lower than in mature rams (G2) ($P \leq 0.01$). Regarding the activity of the enzyme in aqueous and newt x100 extract, the values obtained in both groups are similar.

Table 2. Enzymatic activity of LDH-C4

Group	N	LDH-C4(U/L)		
		Sperm plasma Mean \pm SE	Water extract Mean \pm SE	Triton extract Mean \pm SE
G1	16	6.75 \pm 0.85**	6.75 \pm 1.65 ^{ns}	8.25 \pm 1.25 ^{ns}
G2	16	11.25 \pm 1.03**	6.50 \pm 2.32 ^{ns}	8.00 \pm 1.41 ^{ns}

Note: ** Significant difference at $P \leq 0.01$; ^{ns} – non significant

The extracellular and intracellular activity of the enzyme gamma-glutamyl transferase (GGT) is presented in Table 3.

Table 3. Enzymatic activity of GGT in sperm plasma, water and Triton extract within age groups

Group	N	GGT (U/L)		
		Sperm plasma Mean \pm SE	Water extract Mean \pm SE	Triton extract Mean \pm SE
G1	16	41.50 \pm 5.23 ^{ns}	4.25 \pm 0.47 ^{ns}	4.75 \pm 0.47 ^{ns}
G2	16	42.75 \pm 4.55 ^{ns}	3.75 \pm 0.47 ^{ns}	4.50 \pm 0.28 ^{ns}

Note: ^{ns} – non significant

The effect of ram's age was not significant on the activity of the enzyme in sperm plasma or any of studied extracts.

Discussion

The present study showed a high percentage of motile sperm in both young rams (G1) and mature rams (G2). Our results for total sperm motility are higher than those obtained by *Abadjieva et al. (2014)* (97-97.5%), who also studied sperm motility in rams outside their breeding season. High total sperm motility in rams outside the breeding season was also found by *Farshad et al. (2012)*. They have examined seasonal changes in serum testosterone concentration of LDH and characteristics of sperm in other ruminants. In their research, however, total sperm motility (85.90%) was lower than our results, but the percentage of progressively motile sperm was slightly higher (78.69%).

We also found a significant difference between G1 and G2, similar to *Ntemka et al. (2019)*, which also reveal significant differences between age groups of the rams. According to *Stefanov et al. (2013)* the activity of the androgen-dependent enzymes LDH and GGT are influenced by seasonal change. These changes also affect sperm function. The increased percentage of vital and morphologically normal sperm corresponds to increased LDH activity in the sperm. This enzyme has been found to play a significant metabolic role in sperm capacitation and fertility (*Zamiri and Khodaei, 2005*).

Despite the obtained high percentage of motile sperm, in our study we found extremely low extracellular and intracellular activity of the enzymes LDH and GGT. Low LDH activity is one of the main causes of low fertility in ram sperm (*Brooks, 2001*). According to *Duan and Goldberg (2003)*, complete inhibition of LDH activity blocks capacitance. Despite the low activity of LDH, we found that age had some effect on enzyme activity. Different from our results were results obtained by *Atroshcenko et al. (2019)*, who examined the age factor in stallions and didn't found significant differences between the groups.

In their research, *Zakrzewska et al. (2002)* obtained GGT enzyme values several times higher than ours (430.9 U/L) during the breeding season. The epidermal origin of GGT suggests the involvement of this enzyme in sperm maturation (*Kohdaira et al., 1986*). The metabolism of germ cells and the secretory glands are an important expression of GGT. This could explain why the enzyme appears as a sperm marker. In our studies, we did not find significant differences between age groups in terms of GGT activity. Like us, *Viudes-de-Castro et al. (2015)* didn't found significant correlation between the levels of GGT and sperm motility.

From the results obtained for the activity of the two enzymes during the different seasons, we believe that the activity of LDH and GGT is influenced by the breeding season of the animals and the seasons, and less by age.

Conclusions

- The age of rams affects sperm motility in ejaculates obtained outside the breeding season.
- Significant differences between age groups in LDH-C4 activity were found only in the extracellular activity of the enzyme in sperm plasma.
- No significant differences were found between groups for GGT activity.

Studija povezanosti starosti ovnova i kvaliteta njihovih ejakulata dobijenih van sezone parenja

Madlena Andreeva, Rossen Stefanov

Rezime

Cilj ove studije bio je da se istraži uticaj starosti na kvalitet ejakulata ovnova iz sintetičke populacije bugarske mlečne rase. Za eksperiment su korišćeni ovnovi podeljeni u dve starosne grupe: G1 (2,5 godine) i G2 (6 godina). Ejakulati su dobijeni metodom veštačke vagine i razblaženi korišćenjem medijuma 6A-G. Testirani ejakulati dobijeni su van sezone parenja. Analizator klase sperme (Sperm Class Analyzer, SCA Microptic, Barselona, Španija) korišćen je za proučavanje pokretljivosti sperme. Biohemijska aktivnost enzima laktat dehidrogenaze (LDH) i gama-glutamil transferaze (GGT) određena je pomoću automatskog spektrofotometra Mindrai BA88. Aktivnost enzima proučavana je u plazmi sperme, u vodenom i tritonskom ekstraktu. Pronađene su značajne razlike između grupa u ukupnoj pokretljivosti sperme ($P \leq 0,05$) i aktivnosti enzima LDH u plazmi sperme ($P \leq 0,01$). Što se tiče efikasnosti LDH enzima u vodenom ekstraktu i ekstraktu tritona, nisu pronadene značajne razlike između grupa. Nisu pronadene značajne razlike između grupa za aktivnost GGT, dok je starost imala značajan uticaj na LDH enzim u plazmi sperme.

Ključne reči: ovan, spermatozoidi, pokretljivost, LDH, GGT

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GENOTYPE AND BREEDER FLOCK AGE IMPACT ON BROILER PERFORMANCE IN SUBOPTIMAL CONDITIONS

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Original scientific paper

Abstract: A comparative study of production traits was performed between COBB 500 broilers from a 52-week-old breeder flock and a ROSS 308 from two breeder flocks of different ages (52 weeks and molted flock 79-week-old) in equal, relatively suboptimal ambiental (temperature oscillations) and nutritional conditions (market feed of average quality). The experiment was performed on a total of 720 day-old chicks of both sexes in 4 experimental groups (Cobb; Ross 52; Ross 79; Ross mix - a mixture of Ross broilers from two breeder ages), with 6 replications per group and 30 chickens in each replication. Mortality, body weight (days 0, 7, 21 and 42), weight gain, feed conversion and production index (EPEF) were monitored in 42 days of fattening. It was determined that heavier day-old chickens ($p < 0.01$) with better gain in the first week of production, were obtained from the older breeder flock. Except in the first week, Cobb generally had the highest ($p < 0.05$) body weights and gains in the experiment, aside from in mid and the end of the test, where did not differ significantly from the body weight of the Ross 79 and Ross mix group of broilers, respectively. Results also indicate that genetics (used hybrid) may have a greater influence on the final body weight and daily gain in suboptimal test conditions than the breeder age. Cobb 500 broilers also had significantly higher mortality under test conditions ($p < 0.05$). Feed conversion and the achieved EPEF production index did not vary significantly in the test in chickens of different genotypes or parents. In general, suboptimal conditions in our research constrained realisation of the genetic production potential, with a suppressive effect on both hybrids, higher on the growth in Ross 308, and on the resistance measured by mortality in Cobb 500 hybrids.

Key words: broiler strain, breeder age, suboptimal conditions, production

Introduction

The two most popular breeds of fast-growing chickens in Serbia (and probably worldwide) are Cobb 500 and Ross 308, for a reason, because behind these two brands are genetic companies with almost hundred-year tradition and investment in improving the genetics of their products. According to the manufacturer, the current genetic potential allows in optimal conditions of six-week fattening of both sexes to reach an average body weight of 2952 g, with a feed conversion of 1.61 (*Cobb-Vantress, 2018b*), or 2918 g in Ross 308 hybrids, also with consumption of 1.61 kg of feed per kilogram of gain (*Aviagen, 2019b*). The success of the farm depends on the value of these two parameters, together with low mortality, because the price of feed and live weight of broilers depending on the market, on which the primary producer has no influence but must adjust to.

With long genetic selection focus on the growth rate, meat yield, and feed efficiency, modern fast-growing broilers approaching close to the biological limit and become very vulnerable, especially their immune, musculoskeletal, and cardiovascular systems (*Tixier-Boichard, 2020; Hartcher and Lum, 2020*), resulting in the need for very sophisticated nutrition and care, and often the so-called yield gap on farms. The achievement of the genetic potential of broilers on the farms themselves is limited by a number of factors, among which the quality of delivered day-old chicks and feed as well as the environment and health status of the farm have the greatest impact. Hence, in practice, every farm, and even every facility and flock within the farm, can have different production results, because during fattening, many things can deviate from optimal conditions for a longer or shorter time, more or less. Despite the constant yearly improvement of the genetic potential of these hybrids, the average final weight of chickens delivered to slaughterhouses in Serbia in the previous three years was the same and amounted to 2.4 kg (*Statistical Office of the Republic of Serbia, 2020*). In the US, chickens have been delivered on average after 47 days of fattening in the previous three years, with weights growing steadily (from 2.81 kg in 2017 to 2.87 kg in 2019), but also with an increase in farm mortality (from 4.5 to 5%) in the observed three-year period (*National Chicken Council, 2020*).

The production technologies of these two hybrids differ somewhat, especially in terms of feeding programs, which producers often neglect when using standard feeding strategy for broiler production. Also, adapting to market and constantly optimizing broiler production often requires suboptimal solutions to make feed cheaper. On the other hand, research has found that, due to the application of different selection goals, Ross 308 and Cobb 500 hybrids respond differently to the suboptimal diet (*Sterling et al., 2006; Gous, 2007; Tallentire et al. 2016*).

A raising problem is maintaining optimal ambient conditions on farms, due to increasingly extreme weather conditions caused by climate change, as well as

increasing the final broiler weights of broilers, which require lower optimal ambient temperatures while emitting more heat and gases. New, more efficient environmental control technologies require significant investments, so it is not uncommon for farms with older technologies in use and poorer conditions in the facility, which, despite poorer results, negatively assess the feasibility of new investments and loans (Gillespie et al., 2017; El-Tahawy et al., 2017).

Therefore, it is necessary to check both the resistance and the ability to adapt the latest genotypes of hybrid broilers by measuring productivity in suboptimal breeding and feeding conditions. It is usually up to the broiler producer to choose or test which genotype best suits his production conditions and available feed, especially since in recent literature, comparative testing of these two hybrids are relatively rare, especially independent research carried out in conditions that are not completely optimal for any of the tested hybrids. Also, different strain, sex, stocking density, environmental and health conditions, feeding levels, and programs, among others, interact and define the response of birds, and consequently make difficult the comparison and interpretation of results in different tests. In addition to *in vivo* tests, software programs for modelling broiler growth and optimization of nutrition and production are increasingly in use, which mainly take into account and can predict the impact of the above (input) parameters of fattening *in silico*. For them, the success of prediction depends on the availability and accuracy of the input data, as well as the sophistication of the model itself (Gous, 2007). However, they also do not take into account or interpolate the differences between the latest genotypes of hybrids, resulting from the application of different selection targets and selection pressures in different genetics companies, which is why biological tests are still irreplaceable.

On farms, the problem can be not only the choice of a suitable hybrid for fattening, but also the availability of a sufficient number of chicks from the same breeding flock or from parents of similar age, so mixing chickens is often inevitable. That is why we wanted to compare chickens of the same genotype from breeding flocks of different ages, reared separately and together.

The aim of the experiment is to evaluate the production performance of broiler chickens of different hybrids (Cobb 500 and Ross 308) and the same hybrid (Ross 308) from two breeding flocks of different ages in equal, relatively suboptimal production conditions.

Materials and Methods

Experimental design and used birds

Comparative testing of production traits was performed on broilers of COBB 500 (from a 52-week-old breeding flock) and ROSS 308 proveniences from two breeding flocks of different ages (52nd and 79th week of age, with the older flock molted and in the second laying cycle). All chickens were procured from the

same local producer, incubated in the same machine by the same procedure, hatched and delivered to the experimental farm on the same day.

The trial was performed on a total of 720 one-day-old broiler chicks of both sexes in 4 experimental groups (**Cobb** - Cobb 500 broilers from the breeding flock aged 52 weeks; **Ross 52** - Ross 308 broilers from the 52-week-old parent flock; **Ross 79** - chickens of Ross 308 hibrid from 79-week-old breeding flock; **Ross mix** - a mixture of an equal number of Ross chicks from breeding flocks of different ages), with 6 replications per group and 30 chickens in each replication (180 chickens of both sexes in each group).

At the beginning of the trial, an individual initial visual inspection and weighing of the chickens were performed. All used birds in the trial were vital and in good condition. During the trial, the usual legally prescribed and technologically recommended preventive health care measures were performed.

Testing was performed at the Institute of Animal Husbandry, Belgrade - Zemun, Serbia, at the Experimental Broiler Farm in the period August/September 2020. The experiment and experimental procedures were evaluated and approved by the Ethics Committee of the Institute of Animal Husbandry Belgrade - Zemun.

Housing, rearing and feeding conditions

The chickens were kept in a floor system, in one room with group boxes for 30 broilers (stocking density 10 birds/m²), on a litter of chopped straw. Food and water were given to the broilers ad libitum from one automatic bell drinker and two standard round feeders per box.

The lighting program during the first 10 days and the last three days of fattening was 23 hours of light (L) and 1 hour of darkness (D), and from the 11th to the 38th day in 24 hours 16L/4D/2L/2D.

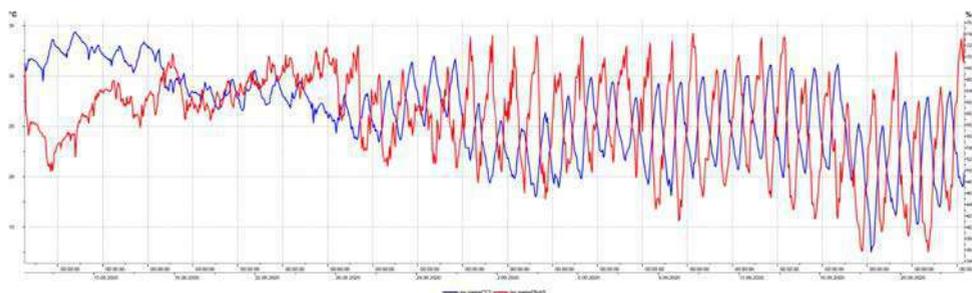
Ventilation and temperature in the facility are regulated by automatic switching on of heating (set temperature) and ventilation (by setting a timer, without the possibility of cooling the inlet air) according to the recommendations of the manufacturer of the used hybrids. Measurement of temperature and humidity data (Testo 174H, Testo SE & Co. KGaA, Germany) at the level of chickens was performed every hour. The ambient gases (Dräger X-am 7000, Drägerwerk AG & Co. KGaA, Germany), and air flow (Testo 410-2, Testo SE & Co. KGaA, Germany) were measured once daily in 5 different points in the facility, at the chickens level.

The average measured values of the indicators of ambient conditions during the experiment are shown in Table 1 and Graph 1.

Table 1. Weekly averages of ambient temperature (T), relative humidity (RH), air flow and concentration of individual ambient gases during the broiler test

Trial week	T Mean±SD (°C)	T Min/Max (°C)	RH Mean±SD (%)	RH Min/Max (%)	Air steam (m/s)	CO ₂ (%)	NH ₃ (ppm)
I	32±2	27/34	61±4	50/71	0-0.1	0.04	3
II	28±2	24/30	65±4	53/72	0.1-0.5	0.06	4
III	26±3	20/32	59±6	46/74	0.3-1.0	0.09	4
IV	24±3	18/30	59±8	41/74	0.3-1.0	0.1	5
V	25±3	20/31	57±9	41/74	0.3-1.0	0.3	10
VI	21±4	13/28	52±10	36/75	1.0	0.2	7

SD-standard deviation; Min/Max-minimal and maximal recorded values

**Graph 1. Temperature ranges (blue curve, °C) and air humidity (red curve, % rH) during the 42-day broiler test (one-hour measurement interval)**

Broiler nutrition

Broiler feed used up to the 35th day of fattening was purchased on the market. Starter and grower pelleted broiler complete feed mixtures were used, and the chosen commercial feed is an average quality (standard) option in the offer for broiler producers from the feed industry on the local market (feed factories mostly offer three price/quality options: economical, standard and premium program). From 36-42 days, the finisher complete mash broiler feed based on maize and soybean meal produced at the Institute of Animal Husbandry was used.

All used feed was chemically and microbiologically tested by accredited methods in the laboratory of the Institute of Animal Husbandry. The summarized results of the analysis of the basic chemical composition and microbiological correctness of all complete feed mixtures used in the test are shown in Table 2.

Table 2. Laboratory-determined characteristics of complete feed mixtures used in the test

Investigated parameters	Starter (0-15 days)	Grower (16-35 days)	Finisher (36-42 days)
Form of feed	Crumbles	Pellets	Mash
Moisture, %	8.69	8.03	10.28
Crude protein, %	22.21	19.45	18.71
Crude fat, %	5.04	5.44	5.72
Crude fibre, %	5.40	5.08	4.62
Ash, %	6.80	6.77	6.02
Calcium, %	0.94	0.96	0.82
Tot. phosphorus, %	0.90	0.81	0.67
Sodium, %	0.12	0.18	0.13
Starch, %	35.09	41.18	42.75
Soluble sugars, %	7.76	7.65	5.88
NFE*	51.86	55.23	54.65
ME, MJ/kg**	12.9	13.6	13.7
Mikrobiol. correct	Yes	Yes	Yes

* Nitrogen-free extractives (NFE) determined by calculation

** Metabolic energy (ME) estimated according to the Carpenter and Clegg equation (Leeson and Summers, 2005)

Measurement of broiler production parameters

The health condition of broilers was monitored and determined daily. All deaths in the trial were immediately recorded and measured, and mortality was calculated at the box level.

The individual body weight of each chick in the trial was measured when the chickens were housed, at the end of the first week, at the mid and at the end of fattening period (days 0, 7, 21 and 42). For the first two measurements, a calibrated scale CHYO MK 2000B (Chyo Balance Corp., Japan) with a measurement accuracy of 0.01g was used, and for the other measurements, a specialized scale for measuring poultry BAT 1 (Veit Electronics, Czech Republic) with a measurement accuracy of 1g was used. Simultaneously with the measurements of body weight, the consumption of feed per box was determined for the time interval between the two measurements.

Based on the recorded deaths, measured individual body weights of chickens and feed consumption per box, individual gain of chickens was calculated, as well as mortality and feed conversion (kg of feed/kg gain) at the box level for different observed periods of fattening.

For the duration of the experiment (42 days), the European Production Efficiency Factor (EPEF) was calculated, the usual cumulative indicator of broiler fattening success based on the achieved average body weight, vitality, feed conversion and fattening duration according to the equation: EPEF = (Liveweight,

kg x Livability, % / Age of depletion, days x Feed Conversion Ratio, kg feed/kg gain) x 100.

Statistical analysis

Body weight and weight gain were determined and analysed in each trial chicken, while box was used as the experimental unit for all other production parameters. The obtained results were processed with the statistical software package STATISTICA, version 8, (StatSoft Inc., 2009). The degree of statistical significance of differences in production parameters between groups was determined using the Tukey test. Significant were considered values with $p < 0.05$.

Results of measurements are shown in the tables as the mean values (Mean) of a certain number of repetitions (n), as well as its standard deviation (SD) and standard error (SE).

Results and Discussion

Ambient conditions and nutrition

Ambient conditions measured at the broiler level (Table 1, Graph 1) were relatively optimal in the first three weeks of fattening, but suboptimal conditions were often recorded during the last three weeks, especially in terms of temperature (oscillations influenced by the outside temperature) and elevated gas concentration. Our test also confirmed the justification of the great attention paid to the problem of environmental control in genetic selection (*Tixier-Boichard, 2020*), as well as in the latest recommendations of both hybrid manufacturers (*Aviagen, 2018; Cobb-Vantress, 2018a*), especially given that extreme hot summers and cold winters are becoming more frequent globally, so their impact should be minimized as much as possible.

The results of feed analyses (Table 2) correspond to the declared, legally prescribed and expected basic chemical composition and quality, indicating that the feed used could potentially meet the average needs of the tested hybrids in terms of nutritional composition. It should be noted that, according to the manufacturer's declaration on the product, in used food there were no other additives (e.g. prebiotics, probiotics, phytoadditives) except the basic ones (synthetic lysine and methionine, phytase, coccidiostat).

In relation to the recommendations for tested hybrids, the food quality and nutrition program was not optimal for any of the hybrids. In general, the Cobb 500 requires a slightly lower crude protein and energy content, and four complete mixtures with a different inclusion schedule over 42 days of fattening (*Cobb-Vantress, 2018b*), while Ross recommends a slightly higher protein content (and lower energy) in the feed, and four mixtures in the nutrition program (*Aviagen,*

2019b). Also, for both hybrids, pelleted food is recommended until the end of fattening.

Body mass, weight gain and feed conversion ratio

Body weights of broilers in the trial determined during measurements at a certain age are shown in Tables 4 and 5. The achieved daily gains in the monitored periods of fattening are shown in Table 6.

The weight of hatched chickens was statistically significantly ($p < 0.01$) lower in Ross 308 hybrids originating from the younger breeder flock (52 weeks), both compared to chickens from half a year older parents of the same hybrid, and compared to parents of the same age hybrid Cobb 500. Until the end of 42 days of fattening, these chickens remained nominally the lightest in the trial, and statistically significantly differed in body weight and daily gain from Cobb hybrids when measured at mid ($p < 0.01$) and end of the trial ($p < 0.05$), despite the fact that at the end of the first week of fattening they had uniform masses and gains.

In regard to the weight and quality of day-old chicks, it is known that with age in laying hens increases the size and weight of eggs and that larger one-day-old chicks can be expected from the breeding eggs of older parent flocks, which was confirmed in our research. *Nangsuay et al. (2016)* examined the influence of broiler parents age and broiler strain on hatched chickens, noting that primarily due to more feed for the embryo (especially yolks) and more oxygen due to better shell conductance in incubated eggs, differences in chicken development and quality can be expected. They have established that older parents also have more yolks in eggs of the same weight than younger ones, and that the Ross 308 is generally characterized by better conductivity of the shell of incubated eggs than the Cobb 500, which is not affected by the age of the flock. *Tona et al. (2010)* have monitored the parameters of embryonic development in the incubator and juvenile development in the first week after hatching of Cobb 500 and Ross 308 chickens originating from parents of the same age (52 weeks), concluding that Cobb and Ross embryonic chickens have different development trajectories, hence different patterns of juvenile growth, and that Cobb chickens reached higher weights at the end of the first week of growth, which was not confirmed in our study of the current genotypes of these hybrids.

Table 4. Average body weight (g) of chickens at housing and at the end of the first week of fattening

Group	0 day				7th day			
	n	Mean	SD	SE	n	Mean	SD	SE
Cobb	180	44.21 ^A	3.98	0.297	178	165.9 ^{ab}	23.59	1.768
Ross 52	180	42.23 ^B	3.32	0.247	177	162.9 ^b	21.42	1.609
Ross 79	180	43.35 ^A	3.45	0.257	175	169.5 ^a	21.56	1.649
Ross mix	180	43.15 ^{AB}	3.80	0.283	179	169.1 ^a	19.48	1.456
p value	p<0.01				p<0.05			

a-b - values between groups marked with different letters are significantly different ($p < 0.05$)
 A-B - values between groups marked with different letters are significantly different ($p < 0.01$)

Table 5. Average body weight (g) of chickens after three and after six weeks of fattening

Group	21st day				42nd day			
	n	Mean	SD	SE	N	Mean	SD	SE
Cobb	169	867.3 ^A	145.2	11.55	164	2644 ^a	380.3	31.26
Ross 52	175	798.2 ^B	132.9	10.16	173	2512 ^b	384.1	29.89
Ross 79	173	826.9 ^{AB}	119.9	9.19	172	2523 ^b	339.7	26.53
Ross mix	175	806.1 ^B	119.3	9.15	172	2584 ^{ab}	354.9	27.63
p value	p<0.01				p<0.05			

a-b - values between groups marked with different letters are significantly different ($p < 0.05$)
 A-B - values between groups marked with different letters are significantly different ($p < 0.01$)

Table 6. Average daily gain of broilers in the trial, g/chicken/day

Group	0-7 days				0-21 days				0-42 days			
	n	Mean	SD	SE	n	Mean	SD	SE	n	Mean	SD	SE
Cobb	178	17.41 ^{ab}	2.89	0.22	169	39.24 ^a	6.78	0.54	164	61.93 ^a	8.99	0.74
Ross 52	177	17.26 ^b	2.64	0.20	175	36.01 ^b	6.19	0.47	173	58.81 ^b	9.09	0.71
Ross 79	175	18.04 ^a	2.72	0.21	173	37.32 ^b	5.6	0.43	172	59.05 ^b	8.04	0.63
Ross mix	179	17.99 ^a	2.34	0.17	175	36.35 ^b	5.55	0.42	172	60.51 ^{ab}	8.39	0.65
p value	p<0.05				p<0.05				p<0.05			

a-b - values between groups marked with different letters are significantly different ($p < 0.05$)

Recent data on monitoring and analysis of differences in quality and weight of one-day-old Cobb 500 and Ross 308 chickens from parents aged 32, 42 and 52 weeks in an incubator in Poland (Nowak *et al.*, 2019) may be particularly relevant for our study, as they indicate relatively equal incubation results, quality and weights of hatched chickens in both genotypes, as well as to significantly higher weights of chickens of older parents. Also, El-Tahawy *et al.* (2017) following the results of 130 broiler farms in Egypt raised by these two hybrids, also have found no significant difference in the weights of day-old chicks. On the other hand, it must be emphasized that the weight of eggs and consequently the weight of hatched chickens can be influenced not only by genetics and age but also by broiler breeder flock nutrition, which is not included in the experimental design and cannot be excluded as the cause of different weights, especially Cobb and Ross day-old chicks from parents of the same age.

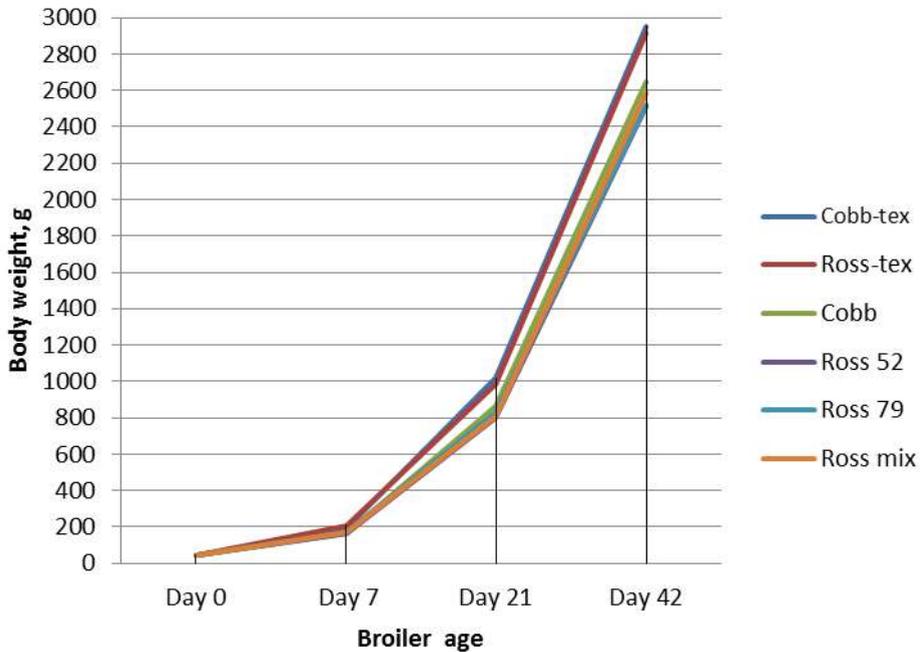
Except in the first week of fattening, Cobb 500 chickens generally reached the highest body weights and gains in the trial, although those at mid-fattening did not differ statistically significantly from the body weight of Ross 308 chickens of half a year older parents, and at the end of fattening from the mix of chickens from older and younger Ross parents. Achieved body weight and achieved daily gain of Ross 308 chickens originating from younger and older flocks did not differ statistically significantly in the mid and end of fattening, regardless of whether they

were reared separately or mixed, despite statistically significant differences in the initial ($p < 0.01$) and weight after the first week of fattening ($p < 0.05$).

As in the trial, chickens of different genotypes were kept in the same ambient conditions and fed the same feed, the differences in growth can be attributed primarily to the genetics of commercial hybrids, and consequently to a different reaction to suboptimal conditions. Combining previous research on the response of these two hybrids to marginal protein deficiencies in food, *Gous (2007)* points out that the Ross hybrid responds to such food with a lower growth due to lower food consumption compared to the Cobb hybrid and suggests that reason may be due to Ross breeding to growth improvement and the conversion efficiency of high-protein foods. The changes in food use in differently selected broiler genotypes depending on the differences in selection programs are indicated in the extensive study by *Tallentire et al. (2017)*. It should be noted that, since the food used in the test according to the protein content was conditionally more in line with the recommended needs of the Cobb 500 hybrid (*Cobb-Vantress, 2018b*) than the Ross 308 hybrid (*Aviagen, 2019a*) whose recommendations are 1-2% higher in terms of protein content in food, this may have favoured Cobb's response to dietary conditions in our trial. Similar observations, that Ross 308 broilers may have higher lysine needs and exhibit better production performance in appropriate dietary conditions, were also made by *Sterling et al. (2006)*.

By monitoring initial body weight at the beginning and end of the first week of fattening, it can be confirmed that heavier one-day-old chicks are obtained from older parent flocks, they grow better in the first week of fattening, in which the quality of the obtained chickens is crucial. However, from the results at the end of the test, it can be seen that the final body weight and daily gain in suboptimal fattening conditions are more influenced by genetics (used hybrid) than the age of the parent flock, despite higher initial weights of chickens from older parents. On the other hand, it is possible that in optimal conditions, the final test results would have been different and the influence of genetics on the final weights and achieved gains would have been lower, and the age of the parents higher. This is also indicated by *Leeson and Summers (2005)*, who reared broilers from parents aged 28, 38, 48 and 58 weeks in the same facility and standard conditions and noticed that with the age of the parents, the final and broiler carcass weights increased, especially in females. It should be noted that in our research, broilers from young and from middle-aged, the so-called prime breeders (*Yerpes et al., 2020*), were not used, but from parents in the final phase of the first cycle of laying (52 weeks) and broilers from parents in the second cycle of laying after moulting (79th week), i.e. relatively old and "very" old parents regarding production life, which, judging by the available literature, was rarely compared.

Body weights achieved in suboptimal test conditions were compared with the latest technological norms of hybrid producers in Graph 2.



Graph 2. Current technological goals for body weight in intensive fattening of hybrids Cobb 500 (Cobb-tex) and Ross 308 (Ross-tex), and achieved body weights in the test of broiler Cobb 500 (Cobb), broiler Ross 308 from the parent flock of old 52 weeks (Ross 52), a flock of 79 weeks (Ross 79) and a mix of Ross broilers (Ross mix.)

In terms of the manifestation of the genetic potential of hybrids expressed by the technological goal (reaching a certain growth and body weight for a certain time of fattening on optimal conditions), in suboptimal conditions of our test both hybrids did not reach the target mass at seven days, and by the end of 42 weeks the weights were about 300 g (Cobb) to 400 g (Ross 52) lower than the current technological norms of the tested hybrids. At the same time, the maximum difference in the achieved final weights in the test among the experimental groups was significantly lower (Cobb weighs 132 g more than Ross 52 broilers). Similar to our results, *Pascalau et al. (2017)* in their evaluation of the production characteristics of these two hybrids in the same farm conditions of nutrition and care in Romania, have obtained higher final body weights in 42 days of fattening in Cobb hybrids, but generally significantly lower than the technological target (2298g for Cobb 500 and 2219 g for Ross 308), emphasizing the importance of fodder composition and optimal breeding conditions, all these varying from country to country. *El-Tahawy et al. (2017)* in a study of the productivity and

economy of 130 farms in Egypt that rear these two hybrids, also have found better final weights and gains in Cobb 500 hybrids in fattening for up to 35 days and in conditions characteristic of this country.

Achieved feed conversion ratios in different periods of fattening are shown in Table 7.

Table 7. Average feed conversion ratio (FCR) in broilers in the trial, kg of feed/kg gain

Group	0-7 days				0-21 days				0-42 days			
	n	Mean	SD	SE	n	Mean	SD	SE	n	Mean	SD	SE
Cobb	6	0.952	0.12	0.05	6	1.334	0.05	0.02	6	1.676	0.12	0.05
Ross 52	6	1.019	0.11	0.04	6	1.391	0.11	0.04	6	1.657	0.08	0.03
Ross 79	6	0.978	0.05	0.02	6	1.387	0.09	0.04	6	1.665	0.09	0.04
Ross mix	6	1.056	0.17	0.07	6	1.367	0.06	0.03	6	1.624	0.07	0.03
p value	p=0.476				p=0.623				p=0.789			

The obtained results indicate that the conversion of food in the experiment did not differ between the examined hybrids or in chickens obtained from parent flocks of different age. Similar to our results, *Hristakieva et al. (2014)*, in the test including these two hybrids under the same conditions, report similar feed conversion ratio, as well as heavier day-old chick and better gain of Cobb 500 hybrids, without specifying more precise ambient and feeding conditions during the test. And the authors who monitored the productivity of these two hybrids on farms in different countries (*Pascalau et al. 2017; El-Tahawy et al., 2017*) did not find significant differences in FCR between hybrids.

Mortality

The cumulative mortality of the tested broilers at the end of the experiment is shown in Table 8.

Table 8. Mortality of broilers during 42 days of fattening (% of housed chickens)

Group	n	Mortality		
		Mean	SD	SE
Cobb	6	8.89 ^a	4.67	1.91
Ross 52	6	4.17 ^b	2.74	1.12
Ross 79	6	4.44 ^b	3.75	1.53
Ross mix	6	4.47 ^b	2.74	1.12
p value:		p<0.05		

a-b - values between groups marked with different letters are significantly different ($p < 0.05$)

In our trial, the Cobb 500 hybrid had a statistically significantly ($p < 0.05$) higher mortality compared to Ross 308 (Table 3). This may be due to the greater sensitivity of this hybrid and/or the influence of the breeder flock (genetics, health status, nutrition, ambient conditions of the farm) on the quality and performance of

broilers, but not on the incubation as all chicks in the trial came from the same incubator. On the other hand, the Ross 308 hybrid exhibited relatively higher resistance to test conditions, which did not differ significantly in chickens originating from two different parent flocks, whether reared separately or mixed. It should be noted that the increased mortality and generally high percentages are partly due to the non-culling during the first week of fattening (which is a common practice on farms), but also to the determination on a small sample (at the level of repetition - 30 housed chickens).

Our results are contrary to the results of the authors who did not determine differences in mortality of these two hybrids, monitoring a small number of individuals in the studies (*Hristakieva et al., 2014; Pascalau et al., 2017*). *El-Tahawy et al. (2017)* also find no significant difference in mortality between these two hybrids on farms in general (about 2%), but indicate significantly higher mortality on small farms (up to 10,000 broilers), where Cobb 500 has a mortality of 6.15% and Ross 308 4.06%, which is explained by the relatively poorer farm conditions. On the other hand, analysing the factors that influenced the cumulative mortality in the first week of broiler fattening (including culled birds) in 2267 flocks in 253 fattening farms on 104 farms of one poultry company in Spain from 2015-2018, *Yerpes et al. (2020)* conclude that, among others, the genotype (breed) used and the age of the parents have a significant impact. The authors state that during the first week, chickens of Ross genotype died more (1.85%) than the Cobb genotype (1.72%), without specifying specific hybrids, and that broilers originating from older parents had higher mortality.

Broiler fattening production index

The calculated European Production Efficiency Factor (EPEF), the aggregate performance indicator of broiler fattening for the duration of the trial, is shown in Table 9.

Table 9. Achieved European Production Efficiency Factor (EPEF) during 42 days of testing

Group	n	EPEF		
		Mean	SD	SE
Cobb	6	345.3	48.8	19.9
Ross 52	6	347.2	29.6	12.1
Ross 79	6	346.5	36.2	14.8
Ross mix	6	364.2	27.9	11.4
p value:		p=0.782		

The achieved EPEF, the cumulative indicator of the final result and the success of fattening, were very similar among the trial groups, since the differences in body weight and growth rate on the one hand and sensitivity expressed by mortality on the other hand between the used hybrids countermand each other. This

leads to the conclusion that neither the different hybrids nor the age of the breeder flock affected the final result of fattening, as much as the conditions in which the chickens were reared and fed in this research, otherwise not so rare on broiler farms.

Conclusion

From the results of the comparative broiler test, it can be concluded that genetics (used hybrid) had a greater influence on the resistance expressed by mortality, final body weight and achieved daily gain in suboptimal fattening conditions than the age of the parent flock. At the same time, the greatest influence were the (suboptimal) conditions in our research (primarily nutrition) on the achievement of technological goals, in which Ross 308 showed less growth and greater resistance than Cobb 500 hybrids. On the other hand, the feed conversion ratio and the achieved production index of fattening in suboptimal conditions did not significantly depend on either the hybrid or the age of the parents. The assumption that in optimal conditions the manifested influence of genetics would be lower, and the age of the parents higher, needs to be further examined.

Komparativno ispitivanje proizvodnih osobina dva hibrida brojlera poreklom od roditelja različite starosti u suboptimalnim uslovima

Miloš Lukić, Zdenka Škrbić, Veselin Petričević, Nikola Delić, Nataša Tolimir, Vladimir Dasković, Simeon Rakonjac

Rezime

Komparativno ispitivanje proizvodnih osobina izvedeno je između brojlera COBB 500 od roditeljskog jata starog 52 nedelje i ROSS 308 od roditelja različitih starosti (52. nedelje i mitarenog jata u 79. nedelji starosti) u jednakim, relativno suboptimalnim uslovima gajenja (temperaturne oscilacije) i ishrane (tržišna hrana prosečnog kvaliteta). Ogljed je izveden na ukupno 720 jednodnevnih pilića oba pola u 4 ogledne grupe (Cobb; Ross 52; Ross 79; Ross mix – mešavina Ross pilića od jata roditelja različite starosti), sa 6 ponavljanja po grupi i 30 pilića u svakom ponavljanju. Praćen je mortalitet, telesne mase (0, 7, 21. i 42. dana), prirast, konverzija hrane i proizvodni indeks (EPEF). Utvrđeno je da se od starijeg roditeljskog jata dobijaju teži jednodnevni pilići ($p < 0.01$), koji bolje prirastaju u prvoj nedelji tova. Rezultata ogleda ukazuju i da na završnu telesnu masu i

ostvareni dnevni prirast u suboptimalnim uslovima testa veći uticaj ima genetika (korišćeni hibrid) od starosti roditeljskog jata. Cobb je generalno tokom testa imao najveće ($p < 0.05$) telesne mase i priraste u ogledu, koje se na polovini tova nisu statistički značajno razlikovale od Ross 79 grupe pilića, odnosno na kraju tova od Ross mix grupe. Sa druge strane, Cobb 500 brojleri su imali značano veći mortalitet u uslovima testa ($p < 0.05$). Konverzija hrane i ostvareni EPEF indeks tova nisu u testu značajnije varirali kod pilića različitih po genotipu, niti po starosti roditelja. Najveći uticaj je upravo (suboptimalnih) uslova u našem istraživanju na dostizanje genetičkog proizvodnog potencijala, sa supresivnim dejstvom na oba hibrida, većim na porast kod Ross 308, odnosno na otpornost merenu mortalitetom kod Cobb 500 hibrida.

Ključne reči: hibrid broilera, starost roditelja, suboptimalni uslovi, proizvodnja

Acknowledgment

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ATTITUDES OF SERBIAN PRODUCERS AND CONSUMERS OF TABLE EGGS ON THE BAN ON CONVENTIONAL BATTERIES AND THE TRANSITION TO ENRICHED CAGES AND ALTERNATIVE PRODUCTION SYSTEMS

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Abstract: The aim of the survey was to determine how the egg production sector is complying with animal welfare legislation, which requires a ban on conventional cages and the transition to permitted systems for rearing laying hens - enriched cages and alternative systems, as well as the attitudes of egg producers and consumers on the impact of permitted systems of egg production/purchase and hen welfare. The survey included producers (50), with 1000 and more hens, where in the sample, the percentage share of producers was according to the capacity - the size of the farm, i.e. similar to the share in the total egg production in Serbia. A consumer survey (261) established their views on the impact of the welfare of layers and the rearing system when buying eggs. According to the test results, it can be stated that: when switching to permitted rearing systems, enriched cages were the choice of all surveyed producers (100%) and that in the transition period from 2014 to autumn 2020, 16.3% of respondents switched from conventional cages to enriched ones (2.04% in full and 14.29% in part). According to the results obtained, 80.49% of them are planning to switch from a conventional cage system to another rearing system, of which 60.98% said they would do so if they had the financial means, while about 19% of producers believe they will be forced to leave production. All producers expect that the transition to enriched cages will lead to a reduction in their production, and 83.33% of them believe that it will be from 20 to 40%. Surveyed producers (87.18%) expect that the transition from conventional cages will increase production costs, and 65.11% believe that this will not affect

the welfare of layers. A survey of consumer attitudes found that when buying eggs, the production system was very important for 19.91% of consumers, and for 19.41% of consumers it was not important at all, while the welfare of layers was very important for 26.84% and not at all important for 10.32% of consumers. The conclusion of the research is that in Serbia the transition to permitted rearing systems is slow, that harmonization with legal regulations is a great challenge and that changes are expected in the coming period that may have far-reaching consequences for the sector of table egg production.

Key words: laying hens, welfare, rearing systems, consumer, eggs, survey, Serbia

Introduction

In recent decades, the egg production sector in Serbia has recorded a decline, i.e. a decrease in the number of laying hens, which according to data from 2019 is less by 11.11% compared to the 2008-2017 average, which can be related to the inability of producers to meet the new high requirements of regulations in Serbia, which are harmonized with EU regulations (Krnjaić, 2019). Adaptation to European standards (*Directive 1999/74/EC*) in Serbia is related to the Law on Animal Welfare which entered into force in Serbia in 2009 (*Official Gazette of the Republic of Serbia No. 41/2009*) and in 2010 the Rulebook on rearing conditions that must be met by breeders was adopted (hereinafter the Rulebook), which was amended in 2014 (*Official Gazette of the Republic of Serbia No. 6/10; Official Gazette of the Republic of Serbia No. 57/2014-27*). The legislation requires abandoning the conventional battery system and switching to permitted systems - enriched cages and alternative production systems, which requires high costs and large changes, and can be considered a danger to the egg production sector in Serbia. Research indicates that the implementation of legislation in Europe has been influenced by producers, traders, consumers, lawmakers, the media and general pressure by the public (Appleby, 2003; Frewer, 2005). Observed by country, waiting strategies and producer requirements for delay, in some countries, proved to be a poorer choice in relation to acceptance and faster adaptation to new conditions (as cited in Rodić et al., 2014). Taking into account how the process took place in EU countries and considering the persistence of decision makers, Rodić et al. (2014) indicate that it is not realistic that the rules for Serbia could be different.

In Serbia, the first deadline for the transition to permitted systems for rearing laying hens was in 2012, but it turned out that the given transitional period of two years was insufficient for the period of adaptation of producers, which led to amendments to the Rulebook, i.e. extension to 31st of December, 2020. The results of the survey of the attitudes of producers of table eggs in Serbia on the regulations

for ensuring the welfare of laying hens presented by *Rodić et al. (2014)* indicate that producers are not aware of the seriousness of the problem, that the belief dominates among producers that welfare regulations in the EU are motivated by preventing imports from countries outside the EU, and in our country by copying European practices. Also, some producers expect that the ban will be implemented in practice only when we become a member of the EU. Six years from the extension of the deadline, it turned out that the extended transition period was not enough, which can be related to the previously stated attitudes of producers, however this situation is a consequence of difficult economic conditions in the egg production sector in the transition period, small and limited incentives at the national level, inability to use funds under the IPARD II program until 2019, as well as particularly difficult conditions in 2020 caused by the Covid 19 pandemic. Given the situation that a very small number of producers managed to use the transitional period to harmonize production with the Rulebook, in 2020, producers sent a request to the authorities for a new extension (personal contact of the author).

Within this issue, part of the survey related to consumer attitudes about domestic animal welfare, and it was found that there were differences between countries - in northern Europe, greater importance is attached to welfare than in the south and the newly acceded EU member states, which may be due to different levels of knowledge about rearing and different willingness to allocate more funds for products obtained according to the principles of welfare (*European Commission, 2005*). In Europe, consumers in 2019 go a step further in demand and in September 2019, a European citizens' initiative called "End the Cage Age", which called for the abolition of cage systems for laying hens and for which over a million signatures were collected, was given for public debate in the European Parliament (*European Parliament, 2019*). In Serbia, there is little research on consumer attitudes (*Rodić et al., 2010; Rodić et al., 2012; Pavlovski et al., 2011*), which indicate a tendency to increase awareness of welfare over the last decade, and *Tolimir et al. (2019)* indicate that consumers need to be educated in this area.

The aim of this paper is to determine the representation of different systems for rearing of laying hens, i.e. how many producers have switched from conventional cages to permitted rearing systems - enriched cages and alternative systems, as well as to determine producers' views on the impact of permitted systems on production and welfare of laying hens. The paper also aims to examine the attitudes of consumers about the impact of production systems and welfare conditions for laying hens on their decisions when buying eggs.

Material and Methods

In accordance with the aim of the research, a basic method was used in the paper - a survey method - producers of table eggs (in telephone communication) and consumers (by filling out a written questionnaire). Data on producers were obtained using the databases of the Republic Bureau of Statistics, based on the 2012 census (on the number of farms with a capacity of 1000 to 5,000), and the structure of farms with over 5,000 hens was taken from the document *Sectorial analysis of egg production and processing in Serbia (2019)*, (Krnjaić, 2019) in which the data of the Veterinary Directorate of the Ministry of Agriculture of the Republic of Serbia from 2019 were used. The structure of the sample in relation to the total number of farms is given in Table 1.

Table 1. Structure of the sample

Farm size	1000-5000	5000-10000	10000-25000	25000-50000	Over 50000	Total
Number of farms*	339	89	30	17	17	492
Share of respondents in the total sample (%)**	5.6	11.2	26.6	35.3	41.2	10.2

*Number of farms according to the data of the Veterinary Directorate of the Ministry of Agriculture of the Republic of Serbia in 2019 (Krnjaić, 2019)

**Share of the surveyed producers within the each group registered farms, split by size

Producers of table eggs from the entire territory of the Republic of Serbia were surveyed during the fall of 2020, using data (contacts) from the records of the Serbian Chamber of Commerce. The sample, share (%) of surveyed producers was formed on the basis of their capacity, according to the size of the farm, i.e. the share in the total egg production in Serbia. The total number of surveyed producers (50) was 10.2% of the total number of farms with 1000 and more layers. The total number of layers on these farms was 1,311,700, which is about 15% of the total number of laying hens in Serbia (8,426,673). The survey covered 31 farms with 5,000 and more laying hens, with 1,264,500 layers, which is about 40% of the total number of laying hens located on all farms with a capacity of over 5,000 in Serbia (3,217,510), according to the data by Krnjaić (2019).

The survey used a structured survey questionnaire of closed type - questions that are with the offered answers, and related to:

a) Switching to permitted rearing systems - questions 1) Have you left the conventional production system (Answers: Yes and No), 2) Do you plan to switch from the conventional to another production system (Answers: Yes, Yes if I have financial means and No), 3) Which production system do you plan to switch to? (answers: Enriched cage system, Floor system, Aviary system, Free range system and Organic production);

b) The assessment of the impact of the transition on permitted rearing systems - questions: 1) By how many % do you expect the number of layers in your production to decrease? (Answers; by 0-20%, 20-40% and 40 and more %), 2) In your opinion, will the transition to a new rearing system have impact on the increase in production costs (answers: Yes and No) and the quality of eggs answers: Yes and No);

c) the welfare of laying hens - question 1) In your opinion, will the transition to enriched cages have impact on the welfare of chickens? (Yes or No);

d) to questions about the awareness of producers about consumer initiatives in Europe - question 1) Are you familiar with the initiative in Europe to abolish all cage systems? (Yes and No).

Survey research of 261 consumers of table eggs from the Belgrade region, as the largest in Serbia was conducted. The structured questionnaire consisted of closed-ended questions, based on the principle of a nine-point Likert scale - where 1 was marked as not important to me at all, and 9 - very important to me, applied to the questions "How important is the welfare of laying hens when buying eggs?" and "How important is the layer rearing system to you when buying eggs?", and where 1 was marked as not at all and 9 - I completely agree in regard to the question, "The price of eggs from the new systems is higher (%)".

Standard data analysis methods in Microsoft Excel program was used in data processing.

Results and Discussion

Table 2. Attitudes of producers of table eggs about the transition to permitted rearing systems

Survey questions							
Have you abandoned the conventional production system?			Are you planning to switch from conventional to another production system?			Which production system are you planning to switch to?	
Yes	No	Partially	Yes	Yes, if I can	No, I will abandon the production	Enriched cage system	All other systems*
%							
2.04	83.67	14.29	19.51	60.98	19.51	100	0

* All other production systems (floor system, aviary, free rearing systems – free range, organic production)

Table 2 shows the results related to monitoring of the implementation of welfare legislation, i.e. the extent to which manufacturers have abandoned the system with conventional batteries during the transition period, their intention to switch/abandon with the production and to which system of those that are allowed

will they switch - enriched cages or some of the alternative systems: floor system, aviary, free-range and organic production.

According to the obtained results, it can be stated that in Serbia, by the fall of 2020, only 16.3% of producers switched to permitted systems, of which 2% in full and 14.3% in part. All surveyed producers had a conventional cage system, which is in line with a study conducted in 2019, which stated that in Serbia the conventional cage rearing system was dominant and that it was estimated that only about 15% of this equipment has been replaced (*Krnjajić, 2019*). The obtained results indicate that the process of transition to permitted systems is slow, although a transition period is close to expiration. In Europe, compliance with legislation is the obligation of all countries, with some members deciding to go beyond EU standards by introducing stricter national or regional laws (*Van Horne and Bondt, 2017*), but some countries, such as Belgium, with transition deadline 2012, was slow in accepting alternative layer farming systems (*Tuytens et al., 2011*). The same author states that Belgian producers, in 2010, in examining their plans to switch to one of the alternative systems showed the following commitments: 55.3% opted for the aviary system, 39.4% for the enriched and colony cages which are the type of enriched cages and 5.3% for non-cage systems. However, according to the results of this research, all producers in Serbia choose enriched cages (100%), and the authors got the impression during the survey that they would not switch from the classic cage system, if they were not forced by law. The obtained results can be related to the research of *Stadig et al. (2016)*, according to which only 8.2% of the surveyed egg producers would switch from the battery system to one of the alternative systems for farming laying hens if it was not stipulated by law. A part of the surveyed producers (19.5%) believe that they will be forced to abandon production, mainly due to high initial costs for the purchase of equipment, which was also the case in European countries. *Tuytens et al. (2011)* indicates that the problem was more pronounced in older farmers. In Serbia, according to the obtained results, the age structure of the surveyed producers is as follows: 50% is aged 56 to 65, 26.09% 46 to 55, 15.22% 36 to 45 and at least in the youngest category 8.7%, with about 50% of producers stating that they have a successor in their business.

In the sector of egg production in the coming period, major changes are to be expected, with a far-reaching impact on primary production, which will be reflected in the market. Producers are aware that the transition to permitted systems will lead to a reduction in production. A reduction should also be taken into account due to the inability of a certain number of producers to adapt and abandon the production. For these reasons, the self-sufficiency of production may be called into question, that is, there may be dangers of deficits in Serbia. The shortage of eggs on the market is a threat to consumers in Serbia, who said that when buying eggs, the choice of all respondents (100%) was for eggs from domestic production, compared to imported eggs.

Consumer attitudes about the impact of enriched cage systems on production are shown in Table 3 and relate to expectations regarding the reduction in the number of laying hens on the farm, as well as the impact on production costs and egg quality.

Table 3. Assessment of the impact of the transition to permitted farming systems on the production of table eggs

Survey questions						
By what % do you expect that the number of layers in your production will decrease?			In your opinion, will the transition to the new farming system affect:			
			Increase of production costs		Egg quality	
0-20%	20-40%	40 and over	Yes	No	No	Yes
%						
2.78	83.33	13.89	87.18	12.82	73.18	26.82

The analysis of the obtained test results shows that the largest number of producers (83.33%) believe that the transition to a system with enriched cages will result in a reduction of production by 20 to 40% compared to their current production in conventional cages. They also believe that it will have a negative impact on the increase of costs, to more difficult maintenance of hygienic conditions and greater possibility of injuring layers.

The increase in production costs in systems with enriched cages, compared to conventional ones, according to the research of *Van Horne and Bondt (2017)* are higher by 6%, and according to *Rodić et al (2010)* the increase in costs is up to 15%. The results can also be related to the research of *Stadig et al. (2016)* which shows that producers in Europe rated battery systems as better than alternatives, in terms of layer health, profitability, production results, operating costs, type and amount of work, egg prices, but in their opinion, animal welfare is better in alternative then in battery systems. According to the data from this research, producers who were of the opinion that the change in the system would affect the quality of eggs, mostly stated an increase in breakage and lower purity of eggs, i.e. poorer physical properties of egg quality. In the available literature, research indicates differences in egg quality depending on the production system, but mainly referring to comparisons of enriched cages and alternative systems, in which eggs from enriched cages have a higher grade for purity (*Denli et al., 2016*), and according to research by *Englmaierová et al. (2014)*, from the point of view of egg quality, suitable systems for transition are enriched cages and aviary in relation to the floor system, stating that the lowest values for the total number of bacteria are recorded in eggs from conventional production and from systems with enriched cages.

Table 4 shows the results of consumer attitudes about the welfare of laying hens in permitted systems - enriched cages, as well as indicators of awareness of producers in Serbia on consumer initiatives in Europe on the ban of all cage systems for farming laying hens.

Table 4. Attitudes of producers about the welfare of laying hens in permitted farming systems

Survey questions			
In your opinion, will switching to enriched cages affect the welfare of layers?		Are you aware of the initiative in Europe to ban all cage systems?	
Yes	No	Yes	No
%			
34.89	65.11	61.70	38.30

In Serbia, 65.11% of producers are of the opinion that the transition to enriched cages will not affect the welfare of layers. Some scientists are not convinced that the welfare of laying hens is better in alternative systems than battery systems (*Duncan, 2001; Savory, 2004*), but the fact is that both systems have advantages and disadvantages. Hens in non-cage systems have a greater ability to exhibit their natural behaviour, but also an increased risk of injury, parasites, disease, and predators compared to cage systems (*Laing, 1988; Häne et al., 2000*). Information from egg producers, according to *Tyttens et al. (2011)*, are very important, because they can be used to determine whether legally imposed changes in the breeding environment really result in improved welfare of laying hens in practice. The same author points out that the attitudes of producers who have already implemented legislation and have some of the allowed systems in production are especially important, since research indicates that their opinions about a system are better than those producers who do not have that experience.

A survey of producers' awareness of the initiative to ban all cage systems in Europe showed that 61.7% of producers had this knowledge, while the awareness of producers of smaller farm capacity was lower.

Producers in Serbia have expressed concern about the transition to farming in enriched cages, while initiatives are being launched in Europe to eliminate them (*European Parliament, 2019*).

Table 5 presents the results related to the attitude of consumers of table eggs towards farming systems and the welfare of layers, in terms of their impact when buying eggs.

Table 5. Attitudes of consumers of table eggs in Belgrade towards farming systems and welfare of laying hens

When buying eggs, evaluate the importance of the following for you									
Respondents' answers *	1	2	3	4	5	6	7	8	9
System for production of eggs (%)	19.41	6.21	6.27	9.16	11.32	11.41	10.10	6.21	19.91
Welfare of layers (%)	10.32	6.47	7.32	4.86	14.30	10.88	9.30	9.71	26.84

* Not important at all - 1; It is very important to me - 9

According to the results of the research, when buying eggs, the farming system is very important for about 19% of consumers and not important at all also for 19%, while the welfare of laying hens is very important for a larger number of consumers (26.84%) and not important at all for only 10.32% consumer. The study by *Tolimir et al. (2019)*, which included a survey of the attitudes of consumers of table eggs on farming systems and the welfare of laying hens, indicates that there are no major differences between the regions in Serbia. Also, comparing the data with research in previous periods in Serbia (*Pavlovski et al., 2011*) relating to the acceptability of the battery system shows a decrease in the share of consumers for whom the battery system is acceptable for farming of laying hens and egg production, from 70.6% to 35, 6%, in the period from 1981 to 2001. *Stadig et al. (2016)* indicates a significant influence of consumers in the selection of farming systems by the producers, while in Serbia this influence is much less significant.

The results of consumer attitudes regarding the price of eggs from permitted systems are shown in Table 6 and show the willingness of consumers to allocate more money for eggs from “new farming systems”.

Table 6. Attitudes of consumers in Belgrade about the price of eggs from non-battery systems

Respondents' answers*	1	2	3	4	5	6	7	8	9
The price of eggs from new systems should be higher (%)	10.39	2.60	4.76	8.23	18.18	7.79	14.29	9.09	24.68

*I do not agree at all -1; I totally agree - 9

According to the obtained results, 10.39% of consumers do not agree that eggs from the new systems should have a higher price, while 24.68% of consumers completely agree. Also, 17.6% of surveyed consumers believe that they are always ready to pay a higher price for eggs from permitted systems, while 18.25% of respondents believe that they never have such an option (data not presented in table). Research by *Pavlovski et al. (2011)* indicates that the share of consumers willing to pay a higher price for eggs from non-battery systems has increased from

46 to 71.5%. However, producers surveyed in this survey, when asked: "How much are consumers willing to pay more for eggs from non-battery systems, expressed scepticism on this issue and stated that for Serbian consumers the most important when buying eggs is the lowest possible price, except for a very small number consumers, mainly in the area of the city of Belgrade, and that in addition to the price, the freshness and size of the eggs are very important to them, while they are not sufficiently informed about the farming systems. Research by some authors suggests the need for consumers to be better informed and educated about the importance of domestic animal welfare (*Autio et al., 2017*), which would include farming systems. Also, in the coming period, more attention should be paid to the research of the attitudes of consumers in Serbia, whose choice of eggs from a certain system can influence producers when choosing a system to transition to. Certain differences between producers and consumers in terms of consumer willingness to pay more for eggs from alternative systems impose the need that in the coming period attention should be focused to communication between them, as well as to consumer education.

Conclusion

Based on the research, conducted with the aim of monitoring the implementation of the Law on Animal Welfare and the related Rulebook, which require the transition from conventional to permitted systems - enriched cages and alternative systems, which significantly changes the egg production sector in Serbia, it can be concluded that the process is slow and that in the transitional period (2014-2020) about 85% of producers failed to harmonize their production with regulations. Manufacturers who have not converted production feel uncertain and hope for a new extension of the transition period, with all surveyed producers (100%) stating that they would transition to enriched cage systems. They have a negative opinion about the transition and believe that the transition will increase production costs and make it more complicated, and according to the opinion, about 65% of producers it will not have an impact on the welfare of laying hens. Further research should focus on examining the attitudes of producers who have switched to a production system with enriched cages, in order to more realistically assess the impact of this system on the production of table eggs in Serbia, which may be important for producers who have yet to convert production. Also, certain differences between producers and consumers regarding the willingness of consumers to pay more for eggs from alternative systems impose the need that in the coming period attention is focused to communication between them, as well as to consumer education.

Stavovi proizvođača i potrošača konzumnih jaja u Srbiji o zabrani konvencionalnih baterija i prelasku na obogaćene kaveze i alternativne sisteme proizvodnje

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Rezime

Cilj anketnog istraživanja je da se utvrdi kako u sektoru proizvodnje jaja teče usaglašavanje sa zakonskom regulativom o dobrobiti životinja, koja nalaže zabranu konvencionalnih kaveza i prelazak na dozvoljene sisteme gajenja kokoši - obogaćene kaveze i alternativne sisteme, kao i kakavi su stavovi proizvođača i potrošača po pitanju uticaja dozvoljenih sistema na proizvodnju/kupovinu jaja i dobrobit kokoši. Ispitivanjem su obuhvaćeni proizvođači (50), koji gaje 1000 i više kokoši, pri čemu je u uzorku, procentualno učešće proizvođača bilo prema kapacitetu - veličini farme, odnosno shodno učešću u ukupnoj proizvodnji jaja u Srbiji. Anketnim ispitivanje potrošača (261) utvrđeni su njihovi stavovi o uticaju dobrobiti kokoši i sistema gajenja pri kupovini jaja. Prema rezultatima ispitivanja može se konstatovati da: pri prelasku na dozvoljene sisteme gajenja izbor svih anketiranih proizvođača (100%) su obogaćeni kavezi i da je prelaznom periodu od 2014. do jeseni 2020. godine sa konvencionalnih kaveza na obogaćene prešlo 16,3% anketiranih (2% u potpunosti i 14,3 delimično). U planu da pređu sa konvencionalnog kaveznog sistema na neki drugi sistem gajenja ima 80,49% od kojih je 60,98% izjavilo da će to učiniti ako budu imali finansijske mogućnosti, dok oko 19% proizvođača smatra da će biti prinuđeni da napuste proizvodnju. Svi proizvođači očekuju da će prelazak na obogaćene kaveze dovesti do smanjenje njihove proizvodnje, a njih 83,33% smatra da će to biti od 20 do 40%. Anketirani proizvođači (87,18%) očekuju da će prelazak sa konvencionalnih kaveza povećanje troškova proizvodnje, a 65,11% smatra da to neće uticati na dobrobit kokoši. Ispitivanjem stavova potrošača utvrđeno je da je pri kupovini jaja sistem proizvodnje za 19,91% potrošača je veoma važan, a za 19,41% potrošača nije uopšte važan, dok je dobrobit kokoši veoma važna za 26,84% i nije uopšte važna za 10,32% potrošača. Zaključak istraživanja je da u Srbiji prelazak na dozvoljene sisteme gajenja odvija sporo, da je usaglašavanje sa zakonskom regulativom veliki izazov i da se u narednom periodu očekuju promene koje mogu imati dalekosežne posledice po sektor proizvodnje konzumnih jaja.

Ključne reči: kokoši, dobrobit, sistemi gajenja, potrošač, jaja, anketa, Srbija

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CELL WALL COMPONENTS AND IN VITRO DRY MATTER DIGESTIBILITY OF ENSILED PEA: OAT MIXTURES

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Abstract: Cell wall content generally is regarded as the most important factor affecting forage utilization because it comprises the major fraction of forage dry matter and is correlated with forage intake and digestibility. The experiment was carried out to evaluate the cell wall constituents of ensiled pea – oat mixtures sown at five different seeding rates. Three effects were studied: application of bacterial inoculant, seeding rate of pea and oat in the mixtures and stages of plant development. The NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber), hemicellulose, lignin and DMD (Dry Matter Digestibility) were determined in silages. Pea and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac – Serbia, and were tested at five different mixture rates: pure pea and pure oat crop, 25% pea + 75% oat, 50% pea + 50% oat, 75% pea + 25% oat. Application of bacterial inoculant affected lower content of NDF and hemicellulose ($P < 0.05$). Content of cell wall constituents decreased with increasing pea content in the mixtures, but DMD increased. On the other hand, content of cell wall constituents increased with plant growth and development, but DMD decreased.

Key words: pea – oat silages, cell wall, digestibility

Introduction

Good quality silage and hay are important for the nutrition of ruminants, as well as for the quality and safety of dairy products. Silage quality depends on many factors. In terms of the nutritive value of the forage, the crop composition at harvest has a major impact on the ensiling process and quality of silage (*Buxton and O'Kiely, 2003*). The quality of grasses and legumes decreases with the growth

and plant development, primarily due to changes in the leaf to stem ratio, as well as due to a decrease in the content of nutrients in the stem. For that reason, it is necessary to cut these plants when the leaf to stem ratio is high. On the other hand, the quality of leguminous plants such as pea, cereals and combined crops of these legumes with cereals does not change in the same way with growth and development as those changes occur in grasses and other legumes, due to the influence of seed chemical composition and seed yield on quality (Salawu et al., 2001). Therefore, it is difficult to determine the time for using these plants in animal nutrition, especially ruminant nutrition. Several researches reported that annual legume – cereal mixtures resulted in high yields and high nutritional quality as compared with cereal alone (Yolcu et al., 2009; Balabanli et al., 2010; Kocer et al., 2012). One anticipated advantage of feeding bi-crop mixtures of cereal and legumes is an improvement in the efficiency of nutrient utilization due to the possible synchronous supply of readily fermentable energy and protein in the rumen (Blagojević et al., 2017). Pea forages generally have higher crude protein content and *in vitro* dry matter digestibility and lower NDF and ADF than oat (Marković et al., 2017).

The aim of the present study was to evaluate the cell wall components of pea-oat silages depending on application of inoculant, different seeding rate in the mixtures and stage of plant development.

Materials and Methods

Pea and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac-Serbia (21° 19' 35" E, 43° 34' 58" N). The experiment was designed with three replication according to a randomized complete block. The pea:oat mixtures were ensiled in the experimental containers holding 130 dm³, with three replications. After compaction, silomass was covered with plastic wrap, and covered with a layer of sand thickness of about 10 cm as the main load. Bacterial inoculant *BioStabil Plus* which contained homo-fermentative lactic acid bacteria (*Enterococcus faecium* and *Bacillus plantarum*) and hetero-fermentative lactic acid bacteria (*Bacillus brevis*) with a concentration of 5×10^{10} CFU per gram was added, and ensiled in containers for 45 days (a₁ – treatment with bacterial inoculant; a₂ – treatment without bacterial inoculant). The pea and oat were tested at five different mixture rates: b₁) 100% pea + 0% oat; b₂) 0% pea + 100% oat; b₃) 25% pea + 75% oat; b₄) 50% pea + 50% oat and b₅) 75% pea + 25% oat. Plant samples were taken at beginning of pea flowering and forming green seeds in 2/3 pea pods.

Neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicelluloses (HCL) and lignin content were determined according to Van Soest et al. (1991). Two stage pepsin-cellulase method was used for *in vitro* Dry Matter Digestibility - DMD (De Boever et al., 1986).

The experimental data were analyzed by a three-way analysis of variance for silage samples using a model that accounted for the main effects of addition of inoculant, pea:oat mixtures and stage of plant development. A computer program STASTICA -7.1 was used for data processing. Effects were considered significant at $P < 0.05$ level by Fisher test. The significance of differences between arithmetic means was tested by LSD test.

Results and Discussion

All three research factors had a significant impact on the NDF content in pea and oat silages (Table 1). The use of inoculants contributed to a significantly lower content of NDF ($588.8 \text{ g kg}^{-1} \text{ DM}$) compared to the treatment without the use of inoculants ($604.2 \text{ g kg}^{-1} \text{ DM}$). Pure oat silage contained the highest amount of NDF and compared to pure pea crop silage was richer in this cell wall constituent by 46.5%. The silage that contained the lowest proportion of pea in the mixture yielded silage with the highest NDF content ($646.9 \text{ g kg}^{-1} \text{ DM}$). With increasing pea proportion in the mixtures the NDF content decreased by 6.85% (pea proportion in the mixture was 50%), and 14.53% (pea proportion in the mixture was 75%). The NDF content in silage increased significantly from the pea flowering stage to the stage of forming green seeds in 2/3 of pea pods ($p < 0.05$).

Statistical analysis of the data showed that the application of bacterial inoculant had no effect on the ADF content. Contrary, the structure of the mixture had a significant effect on the ADF content. Due to the fact, that the highest ADF content was found in silage from pure oat crop, it is evident that with increasing seeding rate of pea in the mixtures content of ADF significantly decreased from 459.6 to $425.9 \text{ g kg}^{-1} \text{ DM}$. The results of investigation showed that higher content of ADF was found in silage when pea was in flowering stage of growth ($443.6 \text{ g kg}^{-1} \text{ DM}$), and with plant growth and development content of ADF decreased to $437.3 \text{ g kg}^{-1} \text{ DM}$, but differences between treatments were not significant.

The hemicellulose content in the silage with inoculant application was significantly lower ($146.0 \text{ g kg}^{-1} \text{ DM}$) compared to the silage without inoculant application ($167.7 \text{ g kg}^{-1} \text{ DM}$). The highest content of hemicellulose was found in silage contained pure pea crop. With increasing pea content in the mixtures the hemicellulose content decreased from 187.5 to $138.5 \text{ g kg}^{-1} \text{ DM}$. The silages prepared in the earlier stage of plant development had a significantly lower content of hemicellulose than silage prepared in the later stage of plant development.

The lignin content in pea and oat silage was also most influenced by the structure of the mixtures and stages of plant growth. The highest content of lignin was found in silage from pure oat crop ($94.5 \text{ g kg}^{-1} \text{ DM}$), whereas the lowest lignin content was found in silage from pure pea crop ($68.2 \text{ g kg}^{-1} \text{ DM}$). Increasing pea ratio in the mixtures caused a decrease in lignin content from 86.1 to 82.9 g kg^{-1}

DM. Utilization of plants in later stages of development caused an increase in lignin content (Table 2).

The DMD of pea and oat silages was significantly influenced by the structure of the mixtures and the stage of plant development. DMD is greatly influenced by the ADF and lignin content, so silages contained less lignin and ADF showed higher DMD compared to silages contained more lignin and ADF.

Table 1. Cell wall components of ensiled pea-oat mixtures

Parameter	Average	b ₁	b ₂	b ₃	b ₄	b ₅	Average AC	A	
NDF, g kg ⁻¹ DM	a ₁	c ₁	476.5 ^g	700.5 ^b	628.5 ^d	562.8 ^e	535.5 ^f	580.8 ^c	588.8 ^b
		c ₂	457.7 ^h	650.8 ^c	642.0 ^c	642.1 ^c	591.3 ^d	596.8 ^b	
	AB	467.1 ^e	675.6 ^b	635.2 ^{bc}	602.4 ^c	563.4 ^d	AC		
	a ₂	c ₁	491.1 ^g	688.7 ^b	636.9 ^c	569.3 ^e	564.6 ^e	590.1 ^b	604.2 ^a
		c ₂	465.7 ^h	730.9 ^a	680.5 ^b	647.3 ^c	567.8 ^e	618.4 ^a	
	AB	478.4 ^e	709.8 ^a	658.7 ^b	608.3 ^c	566.2 ^d			
	BC		483.8 ^f	694.6 ^a	632.7 ^c	566.1 ^d	550.0 ^e		C
			461.7 ^g	690.8 ^a	661.2 ^b	644.7 ^c	579.6 ^d		585.4 ^b
	B	472.8 ^e	692.7 ^a	646.9 ^b	605.4 ^c	564.8 ^d		607.6 ^a	
	ADF, g kg ⁻¹ DM	a ₁	c ₁	414.7 ^h	525.1 ^a	466.1 ^d	427.9 ^g	397.2 ⁱ	446.2 ^a
c ₂			360.5 ^k	483.0 ^c	436.0 ^f	479.0 ^e	453.4 ^e	442.4 ^a	
AB		387.6 ^f	504.0 ^a	451.0 ^d	453.5 ^d	425.3 ^e	AC		
a ₂		c ₁	377.4 ^j	484.7 ^c	474.2 ^{cd}	417.6 ^h	451.3 ^e	441.0 ^a	436.6 ^{ns}
		c ₂	352.2 ^k	510.0 ^b	462.0 ^d	434.7 ^f	401.9 ⁱ	432.2 ^b	
AB		364.8 ^g	497.4 ^b	468.1 ^c	426.2 ^e	426.6 ^e			
BC			396.0 ^e	504.9 ^a	470.1 ^b	422.7 ^d	424.3 ^d		C
			356.3 ^f	496.5 ^a	449.0 ^c	456.9 ^e	427.6 ^d		443.6 ^{ns}
B	376.1 ^e	500.7 ^a	459.6 ^b	439.8 ^e	425.9 ^d		437.3 ^{ns}		
Hemicellulose, g kg ⁻¹ DM	a ₁	c ₁	61.9 ^e	175.3 ^c	162.5 ^d	135.0 ^f	138.3 ^f	134.6 ^c	146.0 ^b
		c ₂	112.8 ^g	167.8 ^d	206.0 ^b	163.1 ^d	138.2 ^f	157.5 ^b	
	AB	84.7 ^g	171.5 ^d	184.3 ^c	149.1 ^e	138.3 ^e	AC		
	a ₂	c ₁	113.7 ^g	204.1 ^b	162.7 ^d	151.7 ^e	113.2 ^g	149.1 ^{bc}	167.7 ^a
		c ₂	113.6 ^g	220.9 ^a	218.5 ^a	212.6 ^b	165.8 ^d	186.3 ^a	
	AB	113.7 ^f	212.5 ^a	190.6 ^b	182.2 ^c	139.5 ^e			
	BC		87.8 ^g	189.7 ^b	162.6 ^c	143.3 ^e	125.7 ^f		C
			113.2 ^f	194.4 ^b	212.3 ^a	187.8 ^b	152.0 ^d		141.8 ^b
B	100.5 ^d	192.0 ^a	187.5 ^a	165.6 ^b	138.9 ^c		171.9 ^a		

a₁ - treatment with bacterial inoculant; a₂ - treatment without bacterial inoculant; b₁ - 100% pea + 0% oat; b₂ - 0% pea + 100% oat; b₃ - 25% pea + 75% oat; b₄ - 50% pea + 50% oat; b₅ - 75% pea + 25% oat; c₁ - 10% of pea flowering; c₂ - forming the green seeds in 2/3 pea pods; NDF - Neutral Detergent Fiber; ADF - Acid Detergent Fiber; Different letters denote significantly different means (P < 0.05)

Table 2. Lignin content and DMD of ensiled pea-oat mixtures

Parameter	Average		b ₁	b ₂	b ₃	b ₄	b ₅	Average AC	A	
Lignin, g kg ⁻¹ DM	a ₁	c ₁	70.9 ^e	102.7 ^a	81.3 ^d	72.7 ^e	67.7 ^e	79.0 ^b	84.3 ^{ns}	
		c ₂	69.0 ^c	93.9 ^c	84.4 ^d	98.1 ^b	102.5 ^a	89.6 ^a		
	AB		69.9 ^c	98.3 ^a	82.8 ^b	85.4 ^b	85.1 ^b	AC		
	a ₂	c ₁	64.3 ^f	81.7 ^d	77.0 ^e	68.3 ^e	76.1 ^e	73.5 ^b		81.5 ^{ns}
		c ₂	68.4 ^c	99.9 ^a	102.0 ^a	91.9 ^b	85.1 ^d	89.5 ^a		
	AB		66.4 ^c	90.8 ^a	89.5 ^a	80.1 ^b	80.6 ^b			
	BC			67.6 ^d	92.2 ^b	79.1 ^c	70.5 ^d	71.9 ^d	C	
				68.7 ^d	96.8 ^a	93.2 ^b	95.0 ^a	93.8 ^b	76.3 ^b	
	B		68.2 ^d	94.5 ^a	86.1 ^b	82.7 ^c	82.9 ^c		89.5 ^a	
	Lignin, g kg ⁻¹ NDF	a ₁	c ₁	148.8 ^b	146.6 ^{bc}	129.3 ^d	129.1 ^d	126.4 ^e	130.6 ^b	143.2 ^a
c ₂			150.7 ^b	144.3 ^c	131.5 ^d	152.8 ^b	173.3 ^a	150.5 ^a		
AB		149.8 ^a	145.5 ^b	130.4 ^d	140.9 ^c	149.9 ^a	AC			
a ₂		c ₁	130.9 ^d	118.6 ^f	120.9 ^{ef}	119.9 ^f	134.8 ^d	125.0 ^b	135.0 ^b	
		c ₂	146.8 ^c	136.7 ^{cd}	149.9 ^b	141.9 ^c	149.8 ^b	145.0 ^a		
AB		138.9 ^{cd}	127.7 ^f	135.4 ^e	130.9 ^d	142.3 ^c				
BC				139.9 ^c	132.6 ^d	125.1 ^e	124.5 ^e	130.6 ^d		C
				148.8 ^b	140.5 ^c	140.7 ^c	147.3 ^b	161.5 ^a		130.5 ^b
B		144.3 ^a	136.5 ^b	132.9 ^c	135.9 ^b	146.0 ^a		147.8 ^a		
Dry Matter Digestibility, g kg ⁻¹ DM		a ₁	c ₁	659.2 ^{bc}	540.0 ^f	544.7 ^{ef}	600.2 ^d	632.5 ^c	595.3 ^a	560.5 ^{ns}
	c ₂		672.9 ^{ab}	476.7 ⁱ	472.6 ⁱ	490.0 ^h	516.4 ^g	525.7 ^d		
	AB		666.0 ^a	508.4 ^{de}	508.7 ^{de}	545.1 ^c	574.5 ^b	AC		
	a ₂	c ₁	681.6 ^a	514.4 ^g	554.9 ^e	588.0 ^{de}	555.0 ^e	578.8 ^b	560.1 ^{ns}	
		c ₂	666.9 ^b	478.0 ⁱ	472.6 ⁱ	491.0 ^h	598.5 ^d	541.4 ^c		
	AB		674.3 ^a	496.2 ^e	513.8 ^d	539.5 ^c	576.7 ^b			
	BC			670.4 ^a	527.2 ^d	549.8 ^c	594.1 ^b	593.7 ^b		C
				669.9 ^a	477.3 ^e	472.6 ^e	490.5 ^e	557.4 ^c		587.0 ^a
	B		670.2 ^a	502.2 ^d	511.2 ^d	542.3 ^c	575.5 ^b			533.5 ^b

a₁- treatment with bacterial inoculant; a₂- treatment without bacterial inoculant; b₁- 100% pea + 0% oat; b₂- 0% pea + 100% oat; b₃- 25% pea + 75% oat; b₄- 50% pea + 50% oat; b₅- 75% pea + 25% oat; c₁- 10% of pea flowering; c₂- forming the green seeds in 2/3 pea pods; Different letters denote significantly different means (P < 0.05)

The highest DMD was found in silage from pure pea crop (670.2 g kg⁻¹ DM), whereas the lowest DMD was found in silage from pure oat crop (502.2 g kg⁻¹ DM). Silage with the highest pea ratio in the mixture was characterized by the highest DMD (575.5 g kg⁻¹ DM), whereas silage with the lowest pea ratio in the mixture showed the lowest DMD (511.2 g kg⁻¹ DM). Crop utilization in the later stages of development influenced lower DMD.

Similar results were obtained in the investigations of pea:oat mixtures quality depending on the stage of growth (Marković et al., 2018). Mustafa and Seguin (2004) found out that NDF and ADF content were not affected by harvest date in whole-crop pea-cereal mixture silages harvested 8 or 10 weeks after seeding. These authors indicated that lignin content was affected by harvest date. In pea plants, the greatest changes in chemical composition occur during the pouring of grains into pods (Åman and Graham, 1987). Nutrients, especially crude protein and carbohydrates are translocated from the vegetative parts of plants into the grain, while cellulose, hemicellulose and lignin are deposited in the leaves and especially in the stem. For this reason, the chemical composition of whole plants was approximately constant with plant growth and development, whereas soluble sugars are transformed to starch and the content of cell wall constituents increased (Åman and Graham, 1987). The content of dry matter, crude protein, starch, NDF, as well as organic matter digestibility in pure pea crop increases with plant growth and development, while the content of these components in the combined pea and oat crop depends on the seeding rate of individual crops in the mixtures and plant stage of development (Salawu et al., 2001).

Dry matter digestibility is higher in pure pea crop than in silage of combined pea and cereal crop (Salawu et al., 2002b). In addition, the intake of silage from the combined crops of legumes and cereals is higher than the intake of grass silage with a similar content of nutrients (Salawu et al., 2002a; Adesogan et al., 2004). The effect of pea silage or combined pea silage with cereals compared to cereal silage (Mustafa et al., 2002), or grass silage (Salawu et al., 2002a) on milk yield and milk composition is very different. According to investigations by Mustafa et al. (2002) feeding silage to animals has a similar effect on milk yield and milk composition as when animals are fed with barley or alfalfa silage.

Conclusions

A significant influence of proportion pea and oat in the mixtures and stage of plant development on cell wall components in silages was established in this investigations. On the other hand, the inoculant application did not significantly influenced content of cell wall components except on content of NDF and hemicellulose. In general, it can be concluded that silage of pure oat crop contained significantly higher content of cell wall components than silage of pure pea crop and their mixtures. The structure of mixtures significantly affected lignin content in

silages, and the highest value of lignin was obtained in silage of pure oat crop (94.5 g kg⁻¹ DM).

Komponente ćelijskog zida i *in vitro* svarljivost suve materije silaža graška i ovsu u smeši

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Rezime

Sadržaj ćelijskih zidova se generalno smatra najvažnijim faktorom koji utiče na iskorišćavanje krme, jer predstavlja najvažniju komponentu suve materije i utiče na konzumiranje i svarljivost hrane. Eksperiment je sproveden kako bi se utvrdio sadržaj konstituenata ćelijskog zida u silažama smeša graška i ovsu u pet različitih odnosa ove dve biljne vrste. Ispitan je uticaj tri faktora: primena bakterijskih inokulanata, udeo graška i ovsu u smeši i faza razvića biljaka. Sadržaj NDF-a, ADF-a, hemiceluloze, lignina i svarljivost suve materije je utvrđen u ispitivanim silažama. Grašak i ovas su uzgajani na oglednom polju Instituta za krmno bilje Kruševac: čist usev graška, čist usev ovsu, 25% graška + 75% ovsu, 50% graška + 50% ovsu i 75% graška + 25% ovsu. Primena bakterijskih inokulanata je uslovlila niži udeo NDF-a i hemiceluloze u ispitivanim silažama. Sadržaj konstituenata ćelijskih zidova se smanjivao sa povećanjem udela graška u smeši, ali se svarljivost suve materije povećavala. S druge strane, rastenje i razviće biljaka uslovio je povećanje sadržaja komponenti ćelijskog zida, dok se svarljivost suve materije smanjila.

Ključne reči: silaža graška i ovsu, ćelijski zid, svarljivost

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TECHNICAL AND ECONOMIC PERFORMANCE OF DAIRY CATTLE FARMING IN MOUNTAIN AREAS IN TIZI-OUZOU, ALGERIA

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Abstract: The aim of this study is to explore the technical and economic aspects of cattle farms in mountain areas and to identify their technical constraints and potentialities. One hundred dairy cattle farmers were surveyed for seven months. The results show that the average farm size is 13 dairy cows and shows considerable variability at the sample level. For one-third of the farms, stabling is almost permanent and feed concentrate used as supplement (on average 7 kg /cow/day). 85% of the factors of production (useful agricultural area and possession of tractor) are owned by 45% of the breeders. Cow productivity averages is around 10.5 kg / day with two milkings / day. In addition, the average self-consumption of milk is 6 kg / day, which represents 2.8% of milk production. Feed expenses represent 90% of production costs. Annual income range from 99 909 AD / livestock unit (LU) nearly 148 421 AD / livestock unit. This variation is a function of the endowment of production resources. Subsidies for milk production represent 58% of the average income of farmers, what shows the low yield of dairy cattle farms. Today, with the drastic reduction in financial resources, dairy production development policies should focus on strategies to improve cow productivity and profitability in those areas.

Key words: dairy cattle, farm management, milk production, profitability, mountain area.

Introduction

In Algeria, milk is an important part of the food intake of populations, especially young ones. National production is still unable to cover a growing demand. Indeed, in 2007 the production reached 2.2 billion kilograms of milk equivalent, with a growth rate of 8% (*Temmar, 2007*). However, milk production

has averaged evolution of 2.6% since 2000 (FAOSTAT, 2011). This production has increased to just over 3 billion kg in 2012 (Makhlouf, 2015). Nevertheless, imports remain the only solution to the problem of national demand for milk. Dairy cattle farming is considered one of the most important in the agricultural sector in mountain areas. For a certain class of farmers, it is the main source of household income.

In terms of numbers, cattle breeding ranks second after sheep in the District of Tizi-Ouzou (DSA, 2013). Cattle farming accounts for 50% of agricultural value added (MADR, 2007). Its role in employment is significant in a region where unemployment reaches 18% of the active population (DPAT, 2009). In a region where the useful agricultural area (UAA) is insufficient (0.27 ha/inhabitant) (Ferrah, 2005), forage culture is undeveloped. Feeding cows, based mainly on purchased concentrate, constitutes a constraint to the development of these farms (Kadi and Djellal, 2009; Mouhous et al., 2014). Few scientific works is carried out with the aim of better understanding the economic performance of farms in mountain areas. Precisely, our study was to explore the technical and economic aspects of cattle farms. The main objective of this study is a technical-economic characterisation of dairy cattle farms, and the identification of the constraints and potentialities of current farming systems.

Materials and Methods

The study area (Tizi-Ouzou) is located in the northern part of the country (www.tiziouzou-dz.com/). It covers an area of 2 975.79 km² that is 0.13% of the national territory (The surface of the study area consists of 5 physical sets that give the region its mountainous character). The district of Tizi-Ouzou has a population of 1,133,349 inhabitants. In numerical terms, dairy cattle is ranked second after sheep, with 40,477 cows (local and improved cattle). Its production capacity would have reached more than 100 million kg (DSA, 2013). A stratified sample of 100 farms, based on the physical strata of the study area, was collected. Of the 100 questionnaires completed, 3 were eliminated and 97 were exploited. The analytical methods used are the descriptive analysis method (frequencies, standard deviations, etc.) and the discriminant analysis method. The aim is to determine the similarities that characterize the farms and to identify the factors of differentiation. It is performed by analyzing K-Means clustering on a set of variables relating to: useful agricultural area (UAA), Dairy cattle number, annual work unit (AWU) and possession of tractor. The results of this analysis were used to compare the management, production and economic performance of dairy farmers groups. To estimate the economic performance of milk production, some parameters were calculated as variable costs (feed, veterinary products, labor), estimation of the monetary value of milk production and gross margins according to Desbois (2006).

Results and Discussion

All of the respondents have dairy cattle farming as their main activity. Less than 5% have a secondary activity (as an electrician, shopkeeper, etc.). The average age of farmers is 35 years. *Benidir et al. (2017)* reported the same results in the region of Sétif (Algeria). The number of permanent workers on the farm does not exceed 2 AWU (Table 1). In Moroccan dairy cattle farms, *Srairi (2004)* reports a similar number of AWU. It should be noted that among those exercising farming activities, 52% have an educational level that does not exceed the second level. In addition, 65% of our respondents cultivate their fodder. The most cultivated species are sorghum, clover, oats and the association vesce-oats. 30% of farmers are mowing only spontaneous fodder. The remaining 5% who do not make fodder crop, due to low UAA owned, proceed with the purchase of all fodder consumed by animals.

Table 1. Socio-economic profiles of farms

	Statistics	Standard error
Number of farms	97	
Average age of household head (years)	34.97	1.00
Average household size (persons)	8.85	0.42
Number of assets (greater than 15 years)	3.05	0.16
Number of permanent workers	1.72	0.11
School level (% of sample):		
Unlettered	5.15	
Primary and middle levels	51.55	
Secondary level	38.14	
University level	3.09	
Professional training	2.07	

Animals feeding

Feed is the biggest expense item in dairy farms in the mountain area. The poverty of the region in soil constitutes a constraint for the extension of the forage-cultivated areas. Therefore, to meet the feed needs of the animals, farmers are forced to fall back on animal feed markets for supplies. The results show that 35% of breeders practice permanently tied stabling due to lack of pasture or steep slope. The basic ration is strongly supplemented by the concentrate, that is distributed daily and throughout the year, with an average of 7 kg/head/day (Table 2). In the same study area but in 2007, *Kadi et al. (2007a)* reported that this amount could reach 10 kg/cow/day. In the western region, *Yerou et al. (2019)* report that the concentrate distribution varies from 5.5 to 10 kg/cow/day. But in the eastern region of India, *Gupta et al. (2014)* reports that cows receive small amounts of concentrate, on average less than 3 kg / head / day. To a small degree, pastures are

an important source of feed, especially in spring and summer. On average, a herd passes on pasture 3.5 hours/day.

Table 2. Share of different sources in the feeding of cattle herds in Tizi-Ouzou area

	Autumn	Winter	Spring	Summer
Concentrated (kg / head / day)	7.46 (0.31)	7.62 (0.31)	5.45 (0.37)	7.36 (0.33)
Hay (boots / flock / day)	6.24 (0.57)	6.4 (0.57)	1.46 (0.29)	5.03 (0.55)
Straw (boots / flock / day)	1.12 (0.20)	1.13 (0.20)	0.93 (0.17)	1.1 (0.20)
Natural pastures (hours / d)	2.33 (0.30)	1.67 (0.27)	5.93 (0.45)	4.11 (0.45)

(): Standard error

Typology of farms

Discriminant analysis of farms

To test the hypothesis of inequality in allocation of factors of production, we used the following variables: AWU, total UAA (ha), tractor ownership and dairy cattle numbers. The discriminant analysis showed that the variables used in the analysis contribute significantly to the classification of farmers (Table 3). There is some heterogeneity in the population of dairy cattle farmers.

Table 3. Wilks' Lambda and Univariate Fischer Test of Discriminant Analysis

Variables	Wilks	F	Signification
Possession of tractor	0.775	12.018	0.000
AWU	0.846	7.549	0.001
UAA	0.838	8.042	0.001
Number of dairy cattle	0.784	11.465	0.000
DDL	2	83	

AWU: annual work unit; UAA: useful agricultural area; DDL = degree of freedom (the first value corresponds to DDL1, and the second value to DDL2; Wilks and F are Lambda statistics from Wilks and Fisher.

The dynamic clustering analysis (Table 4) showed 5 different types of breeders compared to product factors endowment. The different types can be grouped into 3 groups. The first group consists of types 1 and 2. Their factor endowment is below the sample average, accounting for nearly 70% of the farms in the sample.

Table 4. Average Breeders resource classes

Classes/variables	AWU	UAA total (ha)	Possession of tractor (Number)	Number of dairy cattle
Sample	1.73	12.09	0.71	12.950
N=97	(0.11)	(1.62)	(0.08)	(1.52)
Type 1 (N=43)	1.326	6.456	0.000	6.907
	(0.07)	(1.04)	(0.00)	(0.51)
Type 2 (N=24)	2.333	7.250	0.917	11.708
	(0.12)	(1.19)	(0.06)	(1.30)
Type 3 (N=18)	1.000	10.833	1.111	13.889
	(0.00)	(1.77)	(0.08)	(3.24)
Type 4 (N=9)	2.000	48.167	2.111	21.000
	(0.17)	(8.69)	(0.26)	(3.91)
Type 5 (N=3)	6.333	31.000	2.667	80.000
	(0.67)	(8.96)	(0.33)	(5.77)

AWU: annual work unit; UAA: useful agricultural area; () : Standard error

The second group is represented by type 3. Nearly 19% of the sample is considered to be average farmers. Finally, the last group consists of types 4 and 5 which represents more than 12% of the sample and are the most affluent breeders. Their factor endowment level is well above the sample average.

In addition, the Lorenz curve (Figure 1) shows the distribution of product factors on the breeders of the sample. With the exception of labor, nearly 85% of the resources (UAA and tractor ownership) are owned by 45% of the farmers. The remaining 15% of resources are shared on 55% of breeders.

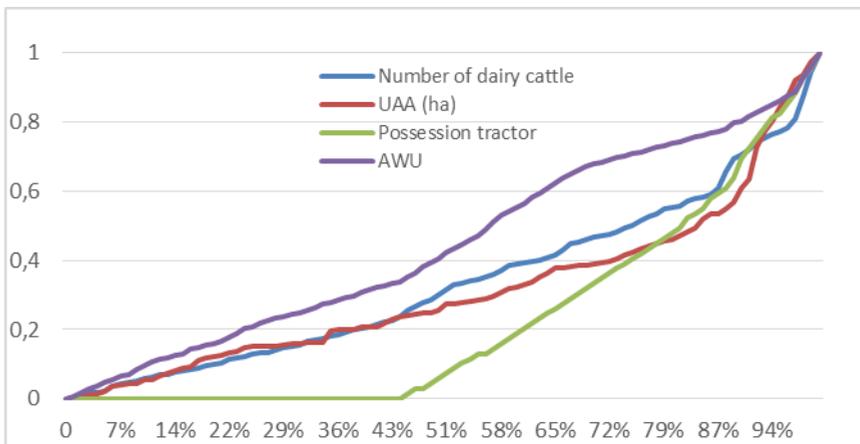


Figure 1. Distribution of production factors (Lorenz curve).

For cow numbers, 85% are held by 55% of breeders. While the remaining 15% are spread over the 45% of breeders. Indeed, these results show an inequality in the distribution of product factors on the breeders of the sample.

Livestock structure

The analysis of the structure indicates the breeding strategies followed by the breeders to ensure the sustainability of their activity. For breeding females, on average in a herd, there are nearly 13 breeding females (12.96) (Table 5). This result is similar to that reported by *Belkheir et al. (2011)* which is 13.9. Crescendo, herds of type 1 have 6.9 breeding females and those of the type 5 reach 80 females.

Table 5. Comparative average structure of the cattle herd in head count

Categories Type	Dairy cows	Heifers	Bulls	Bull calves
Type 1	6.9 (0.51)	3.95 (0.43)	2.32 (1)	2.44 (0.28)
Type 2	11.7 (1.3)	8.37 (1.15)	3.37 (0.85)	4.04 (0.51)
Type 3	13.88 (3.24)	6.88 (0.82)	2.66 (1.08)	5.05 (1.17)
Type 4	21 (3.91)	14.55 (2.73)	2.77 (2.17)	7.33 (1.35)
Type 5	80 (5.77)	30 (2.89)	14.66 (7.42)	8 (4.16)
Sample	12.96 (1.52)	7.38 (0.69)	3.07 (0.63)	3.95 (0.36)

() : Standard error

In addition, the results show that there is a difference in the herd structure between types 1, 2, 3, 4 and type 5. Indeed, breeding females represent 45% of the herd of the first four types, while they constitute 60% of type 5. For breeding males, there is no difference and contribute to the herd structure up to 11% for the 5 types. This is explained partly by the development of the practice of artificial insemination incited and subsidized by the government. Nevertheless, in the state of Khartoum, *Mohamed et al. (2014)* report that more than 78% of the farms have one or no breeding bull.

Some parameters of the production and management of milk.

Milk productivity is estimated at 10.52 kg / cow / day for all sample breeders (Table 6). This yield doesn't seem to evolve since it is similar to that recorded in the same mountainous region 10 years ago by *Kadi et al. (2007b)* and 15 years ago by *Adem (2003)*. In addition, *Kučević et al. (2015)* report that milk yield is influenced by factors such as year of birth, length of lactation, calving season, and breeding area. However, in a dairy basin constituted by plains located in the study

area, *Si Tayeb et al. (2015)* reported a higher yield of 15 kg/cow/day. In addition, it should be noted that nearly 55% of the farms have productivity below average; it seems that types 2 and 3 mark the lowest levels of productivity and represent 42% of farms that are below average. Type 5 has the highest productivity at 13.5 kg/day. However, in Central Uganda *Nalubwama et al. (2016)* recorded a productivity of crossbred cows that do not exceed 8 kg / cow / day.

Table 6. Interclass comparison of some milk production parameters

Class/Parameters	Type 1	Type 2	Type 3	Type 4	Type 5	Sample
Productivity (kg/cow/day)	10.54	9.93	9.69	12.66	13.5	10.52
	(0.58)	(1.00)	(0.68)	(1.68)	(1.80)	(0.42)
Number milking/day	1.88	1.92	1.89	1.89	2	1.9
	(0.05)	(0.06)	(0.08)	(0.11)	(0.00)	(0.03)
Milk exploitation time (months)	9.56	9.33	9.11	9.22	9.33	9.38
	(0.13)	(0.10)	(0.14)	(0.15)	(0.33)	(0.07)
Concentrate feed kg / cow / day	6.83	6.33	7.22	8	10	6.99
	(0.50)	(0.60)	(0.48)	(1.11)	(2.00)	(0.31)

() : Standard error

What should also be mentioned is the productivity of type 3 which is 9.69 kg/day, the lowest level of productivity of the 5 types. Nevertheless, it records a consumption level of the concentrate of 7.22 kg/cow/day. This is probably due to the difference in the mastery of production techniques with a waste of feed.

Sale and self-consumption of milk

The general average of self-consumption is 2.85% of milk production (Table 7). Daily consumption represents 5.22% among breeders of type 1 (less affluent). For types 2, 3 and 4, the share of self-consumed production varies between 2 and 3% of production. This self-consumption reaches its highest level among breeders of type 5 (10 kg/day) which represents only 0.6% of self-consumed milk from daily production.

Table 7. Household self-consumption and sale of milk (kg / day / household)

Class/Parameters	Type 1	Type 2	Type 3	Type 4	Type 5	Sample
Self-consumption	5.44	6.25	6.39	6.67	10.33	6.08
	(0.38)	(0.5)	(0.58)	(0.97)	(0.88)	(0.27)
Consumption per capita	0.7	0.84	0.86	0.8	1.73	0.81
	(0.06)	(0.10)	(0.12)	(0.16)	(0.64)	(0.05)
Size of households	8.76	8.88	8.67	10	7.66	8.86
	(0.58)	(0.75)	(1.35)	(1.49)	(2.33)	(0.42)
Sale	104.86	180.83	211.22	322.56	1666.67	211.9
	(8.97)	(22.35)	(40.85)	(54.31)	(166.67)	(29.83)

() : Standard error

In others, farmers sell 211.9 kg/day of milk. We note that 70% of breeders (type 1 and 2) cannot reach this average sales. Indeed, the lowest amount of milk sold is registered in type 1 farms (104.9 kg/day). Only types 4 (322.6 kg/day) and 5 (1666.7 kg/day), which are the most endowed with production resources but which represent only 12% of the sample, come to exceed the average (211.9 kg/day).

Economic performance of dairy cattle farms.

Proposed analysis focuses on the structure of production costs, incomes and gross margins. Estimated cost of production indicates that food expenses represent 90% of the production cost for all farmers. The same situation is described throughout the Maghreb by *Srairi et al. (2013)*. However, *Ghozlane et al. (2009)* report much lower food expenditures than our results on farms with more than 100 ha of UAA. Local production of fodder and concentrate seems to cover a small part of the livestock feed. Therefore, breeding expresses a very dependent relationship market (*Kadi and Djellal, 2009*). For veterinary care and labor, there is no significant difference between the different types (Table 8). They represent each of them on average 5% of the total cost of production. But type 2 shows relatively high health expenditures that are close to 18% of total expenditures.

Table 8. Cost structure of milk production (AD)

	Type 1	Type 2	Type 3	Type 4	Type 5
Food (%)	89.25	79.32	96.05	90.14	90.80
Veterinary products (%)	4.28	17.87	2.01	2.34	1.37
AWU (%)	6.47	2.80	1.94	7.52	7.82
Total variable costs (AD / kg of milk)	34.55 (1.95)	36.38 (2.3)	39.26 (2.3)	37.19 (3.8)	27.53 (3.02)

AD: Algerian Dinar. 1 AD= 0.0075 Euro

In addition, the average production cost of one kg of milk is 35 AD / kg. This cost is similar to that reported by *Yerou et al. (2019)* in the western region of Algeria, which is 37.1 AD/kg. Farmers of type 5 (more affluent) report the lowest cost of production compared to other groups at only 27.5 AD/kg. This level of performance is striving for a distributed more rational consumption, and benefits of economy of scale. Also, the size of the budgets is significantly different between the classes of breeders identified on the basis of their endowment of production resources (Table 9). The richer farms (type 5) invest the most and get the best results. They are followed respectively by farms of types 4, 3, 2 and 1. The latter being the least resource-rich.

For gross margin, the allocation of production resources determines the economic performance of the breeders. Gross margin values are positively correlated with the investments allocated to production. The same observation is made for income, type 1 breeders have an average annual income of (666 100.3

DA, or 103 001.95 DA/LU), it is the lowest income of the 5 types. The highest income is received by breeders of type 5, which averages 11.95 million DA/year, or 148 421.56 AD/LU.

Table 9. Gross margin released by the different groups of farms (AD)

	Type 1	Type 2	Type 3	Type 4	Type 5
Total expenditure (AD*10 ⁶)	0.65 (68 021.35)	1.05 (185 708.33)	1.11 (332 243.5)	1.83 (553 995.5)	8.51 (1 762 244.6)
Total sale (AD*10 ⁶)	1.21 (104 678.8)	2.18 (271 752.2)	2.21 (477 201.9)	3.92 (653 862.9)	20.47 (1 922 400.8)
Gross margin (AD*10 ⁶)	0.66 (70 065.35)	1.13 (193 329.9)	1.09 (216 790.08)	2.09 (423 165.3)	11.95 (1 520 367.9)
Gross margin AD / livestock unit (LU)	94 909.70 (6 448.12)	101 600.49 (10 598.72)	116 568.53 (9 632.97)	103 129.05 (17 791.30)	148 421.56 (11 044.09)
Share subsidy of milk in incomes (%)	62.48	66.94	51.74	61.67	48.15

AD: Algerian Dinar. 1 AD= 0.0075 Euro

In addition, among the milk production incentive policies that the government has established, there is mention of milk production subsidies (15 AD/kg) for approved breeders. This grant contributes significantly to total income. Indeed, subsidies for milk production account for 58% of total income. In addition, breeders of type 2 report the share of the largest subsidy among the 5 types, which amounts to close to 67%. This proportion is close to that reported by *Mouhous et al. (2014)*, which is 71%. Type 5 breeders report the lowest proportion of the subsidy (48%) in the formation of their income.

Farms Dairy cattle remain family, medium sized (13 females/livestock). There is also an unequal distribution of the means of production. The succession is assured since young breeders manage these farms. Cow productivity remains low with concentrate distribution not exceeding 7 kg/cow/day.

The analysis of production costs indicates that food expenditures represent 90% of production costs. With the weakness of UAA in mountain areas, a large part of livestock feed is bought at the market. The estimated income is based on the investments made in the farms. The average annual income is 103 001.95 AD/LU.

Finally, subsidies for milk production represent more than half of the average income of breeders. For this purpose, the share of subsidies in income goes from 48% (the most affluent) to nearly 67% (less affluent). It seems that the government, through the transfer of wealth, provides half of the income of breeders through subsidies.

Tehničke i ekonomske performanse mlečnog govedarstva u planinskim predelima Alžira - Tizi-Ouzou

Azeddine Mouhous, Farid Djellal, Hocine Guermah, Si Ammar Kadi

Rezime

Cilj ovog istraživanja je bio da se ispituju tehnički i ekonomski aspekti mlečnog govedarstva na farmama u planinskim predelima i da se identifikuju njihova tehnička ograničenja i potencijali. Tokom sedam meseci anketirano je sto farmara muznih krava. Rezultati pokazuju da je prosečna veličina farme 13 muznih krava i pokazuje značajnu varijabilnost na nivou uzorka. Za trećinu farmi grla se gotovo stalno drže u objektima, a koncentrat za životinje se koristi kao dodatak (u proseku 7 kg/krava/dan). U pogledu proizvodnih faktora (korisna poljoprivredna površina i posedovanje traktora), 85% je u vlasništvu 45% uzgajivača. Prosečna produktivnost krava je oko 10,5 kg/dan uz dve muže dnevno. Pored toga, prosečna samopotrošnja mleka je 6 kg/dan, što predstavlja 2,8% proizvodnje mleka. Troškovi stočne hrane predstavljaju 90% proizvodnih troškova. Godišnji prihod kreće se od 99.909 do 148.421 AD/uslovnom grlu (LU). Subvencije za proizvodnju mleka predstavljaju 58% prosečnog dohotka poljoprivrednika, što pokazuje nizak prinos mlečnih farmi. Danas, sa drastičnim smanjenjem finansijskih sredstava, politika razvoja mlečnog govedarstva treba da se usredsredi na strategiju za poboljšanje produktivnosti i profitabilnosti krava u tim oblastima.

Ključne reči: mlečna goveda, upravljanje farmama, proizvodnja mleka, profitabilnost, planinsko područje.

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POTENTIALS OF SERBIAN LIVESTOCK PRODUCTION – OUTLOOK AND FUTURE

Milan M. Petrović¹, Stevica Aleksić¹, Milan P. Petrović¹, Milica Petrović², Vlada Pantelić¹, Željko Novaković¹, Dragana Ružić-Muslić¹

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Review paper

Example 2

EFFECTS OF REARING SYSTEM AND BODY WEIGHT OF REDBRO BROILERS ON THE FREQUENCY AND SEVERITY OF FOOTPAD DERMATITIS

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia

Corresponding author: Zdenka Škrbić, e-mail address

Original scientific paper

Original scientific paper should contain following paragraphs with single spacing (title of paragraphs should be in Times New Roman 14 **bold**, except for **Abstract** and **Key words** where font size is 11 **bold**):

Abstract: up to 250 words, Times New Roman, font size 11, justify. Abstract should contain a brief overview of the methods and the most important results of the work without giving reference. Abstract submitted in English language.

Key words: not more than 6. The selection carried out by relying on widely accepted international source such as a list of keywords Web of Science.

Introduction – present the review of previous research and objective of the paper.

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Results and Discussion – present investigation results separately from discussion or together in one paragraph. Presentation of the results should be precise and without repetitions, and include the evaluation of significant differences and other parameters.

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Milan M. Petrović, Stevica Aleksić, Milan P. Petrović, Milica Petrović, Vlada Pantelić, Željko Novaković, Dragana Ružić-Muslić

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Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 6885.

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In scientific journals:

PETROVIĆ M. M., SRETENOVIĆ LJ., BOGDANOVIĆ V., PERIŠIĆ P., ALEKSIĆ S., PANTELIĆ V., PETROVIĆ D. M., NOVAKOVIĆ Ž. (2009): Quantitative analysis of genetic improvement of milk production phenotypes in Simmental cows. *Biotechnology in Animal Husbandry*, 25,1-2, 45-51.

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FITZGERALD M. (1994): Neurobiology of Fetal and Neonatal Pain. In: Textbook of Pain. 3rd edition. Eds Wall P. and Melzack R. Churchill Livingstone, London, UK, 153-163.

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ŠKRBIĆ Z., LUKIĆ M., BOGOSAVLJEVIĆ-BOŠKOVIĆ S., RAKONJAC S., PETRIČEVIĆ V., DOSKOVIĆ V., STANOJKOVIĆ A. (2015): Importance of farm management in reducing broilers skin lesions. Proceedings of the 4th International Congress “New Perspectives and Challenges of Sustainable Livestock Production”, October 7 – 9, Belgrade, 145-158.

Citations in the text are presented in italic form, examples: ...results of *Petrović (2009)*; *Petrović et al. (2009)*; *Webb and O’Neill (2008)*....; (*Škrbić et al., 2015*); (*Ružić-Muslić, 2006*); (*Webb and O’Neill, 2008*)

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**13th International Symposium
“Modern Trends in Livestock Production”
6th – 8th October 2021, Belgrade, Serbia**

Organizer

**INSTITUTE FOR ANIMAL HUSBANDRY,
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SECOND ANNOUNCEMENT

On behalf of the International Scientific and Organizing Committee, it is our pleasure to invite you to participate at the **13th International Symposium on Modern Trends in Livestock production**, which will be held **from 6th to 8th October 2021, in Belgrade**.

We invite you to take part with an oral or poster presentation. You also have the opportunity to present your institution or company at the Symposium.

At the Symposium, the experts from Serbia and abroad will present the results of their research in order to enable a better transfer of scientific achievements in all fields of animal husbandry and science and making them available to the scientists, researchers and practitioners in livestock production, as well as students, in the private sector and to the general public.

The aim of the scientific meeting is to establish better cooperation between researchers in the field of animal science from different institutions, and experts from the industry, trade and other related fields, as well as producers from Serbia, Western Balkans, EU and other parts of the world in the field of science, education and good livestock production practice.

MAIN TOPICS OF THE SYMPOSIUM

1. Breeding, Selection, Genetics, and Reproduction of Farm Animals
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6. Protection of the Environment and Biodiversity in Animal Production
7. Livestock Production and Food Security in a Context of Climate Change
8. Livestock Feed and Ecology

OFFICIAL LANGUAGE

The official language of the Symposium is English.

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REGISTRATION FEE	Before 30th June 2021 (Early registration)	After 30th June 2021 (Late registration)
Registration Fee, covers publishing of paper, Symposium material, participation in all sessions of the Symposium, coffee/tea break	80 €	100 €
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The Symposium will be held in Hotel Tulip Inn Putnik Belgrade, Palmira Toljatija street 9, 11070, Belgrade, Serbia

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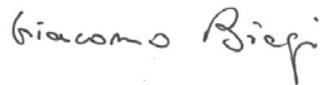
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Dr Čedomir Radović,
Senior Research Associate
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