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REVIEW ARTICLE

THE HORSE IN THE LIGHT OF VETERINARY MEDICINE AND THE MUSLIM TRADITION - A COMPARATIVE STUDY

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ABSTRACT

Of all the animals that accompany humans through almost all stages of human development, horses have the most prominent place. In addition to a presentation of the role and significance of horses in human life throughout history until today, taking the Bosnian mountain horse as a paradigm, a presentation is highlighted of the relationship of the Islamic religion to horses, which are the most valued domestic animals. In Islamic theology, caring for the animal world is mandatory, primarily as an expression of doing deeds that bring us closer to God, where the well-being of horses is especially important.

Keywords: Bosnia and Herzegovina, Bosnian mountain horse, Islam, mule, hinny

INTRODUCTION

It is assumed that the human population began domesticating horses six thousand years ago, although there are indications that this happened much earlier, but at the same time it occurred much later than the domestication of some other animals (Jansen et al., 2002; Lönker et al. 2020; Vučevac-Bajt, 2012). Over thousands of years, herds of horses gradually merged with human societies. The act of joining two species is referred to by modern scientists as "kinetic empathy" due to their similar and compatible social structures, where the relationship between the two species develops in their common niche (Gustafson, 2023). The cradle of the first domesticated horses were the steppes of today's western Kazakhstan, southwestern Russia, and Ukraine (Warmuth et al. 2012; Vučevac-Bajt, 2012).

The wild ancestors of today's domestic horse are considered to be the Asian wild horse, mentioned above (*E. caballus przewalskii*) and the tarpan from Eastern Europe and the Ukrainian steppes, as well as the forest horses of Northern Europe. Research based on sequencing of mitochondrial genomes indicated the possibility that horses were

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domesticated several times by crossing local wild horses and domesticated mares (Lippold et al., 2011). Throughout history, horses performed many functions, and played an irreplaceable role in shaping ancient civilizations. Although hunting or finding food was the initial motivation for people in the early stages of the domestication of horses, their role as a basic means of transport and the main trump card in warfare grew progressively throughout the subsequent periods (Orlando, 2020).

Considering the inestimable benefits that man achieved by using horses for his needs, concern for their well-being progressively increased, and special attention was paid to their health. The success of an individual and/or community depended primarily on their horses' health. Vučevac-Bajt (2012) highlights the time in Arab veterinary medicine over a period of 600-1200 years, where considerable work was done on training veterinary staff in charge of the health care and treatment of horses and camels. Horse breeding and training were also approached in a similar way.

The use of horses largely depends on the cultural origin of the people and their affiliation to a certain religion, which often shapes the approach to and perceptions of this noble animal. Cattle, for example, play an important role in Hinduism (Pawshe et al., 2016), which directly affects human mental health (Koenig, 2009), but horses are not part of any religion (Lönker et al., 2020). There is a deficit of published works dealing with this issue, especially when it comes to the attitude of Islam towards this animal population.

The aim of this article is to present the basic features of the horse through the paradigm of the indigenous breed of the Bosnian mountain horse, in the context of veterinary medicine and the Muslim tradition.

Historical review

In ancient cultures, horses were used extensively in warfare, for transporting loads, pulling carts, and cultivating the land. The Babylonians introduced the use of four-wheeled chariots, and the early empires of the Assyrians, Hittites and Hurrians were created with the help of horses (Budimir et al., 2009). Horses were the main driving force of numerous Egyptian conquests, and high-quality horses were bred at numerous horse stud farms (Džaja and Severin, 2019). During the time of the ancient Jews, thoroughbred horses were bred, and the development and treatment of war horses, which were used for draft and riding, were encouraged (Turudić, 1977). In ancient China, horses were considered a symbol of fire, speed, perseverance and good intentions, and in this period, during the greatest military reforms, chariots were replaced by cavalry (Vučevac-Bajt, 2012; Džaja et al., 2022). In addition to warfare, the ancient Greeks also used horses extensively in sports, and well-developed horse breeding in that period also led to the training of special specialists who treated civilian and military horses, and were called hippieatres (Gr. *Hyppos*=horse, *iatros*=doctor) (Hadžiomerović and Šatrović, 2004; Džaja, 2017). Apart from their versatile use, horses were associated with various deities, and were the most popular and most present animals in the rich ancient mythology. Centaurs (gr. Κένταυροι, *Kéntauroi*), hybrid creatures from Greek mythology, with the upper parts of the human body and the lower parts of the body of a horse, are nowadays the symbol of numerous veterinary organizations (Hausmann and Jöchle, 1988; Džaja et al., 2022). The ancient Romans bred several horse breeds that they used in wars, in their economy and in circuses, that had great social significance. Horses were also used to breed mules, which were highly valued due to their endurance (Vučevac-Bajt, 2012). In the Roman Empire, horses were also a religious symbol, and were used for religious rites and ceremonies (Budimir et al., 2009; Džaja et al., 2022). The cavalry also had great significance for the spread of Islamic culture and religion. Islamic scientists made a significant contribution to the development of Arab medicine and veterinary medicine, which was based on the scientific knowledge of older cultures. The main task in veterinary medicine was primarily the treatment

of horses, which was most often performed by horse farriers (Hadžiomerović and Šatrović, 2004). The most famous Arab veterinarian, Abu Bakr ibn Badr, in his work "Kamilus-sana'atejn" (that is, Perfection in two skills (horse breeding and horse treatment)) described over 200 disease conditions in horses and methods of treatment, horse breeding and keeping, horse shoeing, and other procedures related to horse breeding in that period (Hadžibajrić, 1982).

In Muslim empires a special form of animal care was practiced, based on a foundation (*vakuf*), derived from religious and theological reflection, specializing in animal care. Most often, it was about exhausted draft and riding animals such as horses and camels, which were protected by the foundation, and kept free, that is, they were not tied. Also, they were not used for food purposes, and in the winter period they would be provided with sufficient amounts of food (Jašić, 2019). In Bosnia and Herzegovina, as well as in other areas under the administration of the Ottoman Empire, there were many of these foundations (Zaimović, 2010; Jašić, 2019).

Wild horses

Wild horses (*Equus caballus*) can be found on all continents of the Earth except Antarctica (Lönker et al., 2020). In Bosnia and Herzegovina, wild horses are present in several localities (Katica et al., 2010). A large number of wild horses inhabit the Kruzi karst plateau, which is located at the foot of the Cincar mountain in western Bosnia. According to independent estimates and data from the non-governmental sector in this area, more than 1,000 horses live and reside freely, without direct human control, which are actually the descendants of abandoned domestic horses that served livestock farmers for transport, work in the fields or other activities until the development of mechanization. Moreover, the aggression against Bosnia and Herzegovina was one of the significant reasons for the appearance of a large number of abandoned horses, of which the most are of the Bosnian mountain horse breed, with some of the Arabian breed, and the rest are similar to these two breeds or crosses (Katica et al., 2010).

Apart from the fact that free-living horses all over the planet Earth are a spectacular sight and represent a kind of tourist attraction, wild horses are also credited with a far more significant influence. Through the control of plant growth and the preservation of natural diversity, horses play a major role in the overall balance of the ecological system (see Figure 1). By fertilizing the soil and spreading plant species, they have a positive effect

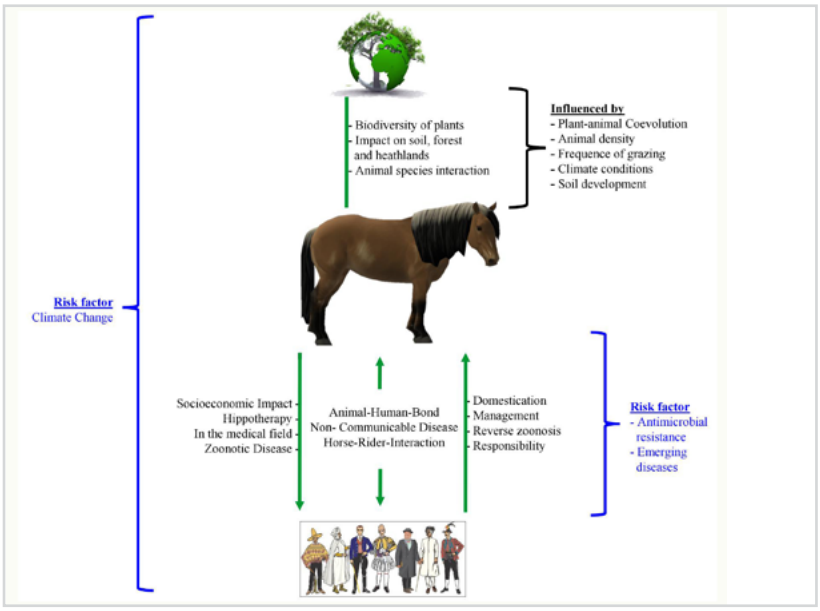

Figure 1 Forms of interaction between horse, man and environment (Lönker et al., 2020)

on the biodiversity of animal species, especially reptiles and small mammals, ants, herbivores and some species of birds (Lönker et al., 2020). Wild horses also contribute to the indirect protection of domestic animals, representing one of the most important sources of food for indigenous wild animals, especially wolves (Katica et al., 2010). The population of free-living horses can also have negative impacts on the environment, such as trampling the soil, which can directly or indirectly lead to soil erosion, and through its compaction, reduce water infiltration and reduce the supply of plant vegetation with nutrients (Ostermann-Kelm et al., 2009). In the same way, horses, as animals that reproduce naturally and roam freely, are in some way in competition with the native fauna (Boyce et al., 2021).

However, the population of wild horses in Livno has been recognized as a problem for the human community for many decades. Namely, in search of food, salt or water, herds cover long distances, and they often cause traffic accidents on busy highways. In addition to the danger to human life in such incidents, the suffering of wild horses is also noted, which, along with frequent attempts to trap, steal and/or kill, significantly calls into question their well-being and survival in general (Katica et al., 2010).

Infectious and non-infectious diseases of horses

It is evident that there are several infectious diseases, or zoonoses, that affect both horses and humans (see Figure 1). They occur sporadically, most often moving from the population of wild horses to humans (Reperant et al., 2016). Zoonoses that are transmitted from horses to humans by direct contact (e.g. Hendra virus) (Middleton et al., 2014) or indirectly by infection through food products (e.g. botulism) (Johnson et al., 2016), then by vectors such as ticks (e.g. Lyme borreliosis) (Lehmann et al., 2017) and mosquitoes (e.g. West Nile fever) (Reperant et al., 2016).

The group of non-infectious horse diseases includes cardiovascular disorders, bone and joint diseases, psychological problems, and metabolic disorders (see Figure 1). Non-communicable diseases are the result of genetic, physiological, environmental and behavioral factors, and persist over a long period of time (Lönker et al., 2020). Psychological disorders can be linked to the inappropriate accommodation of horses (Cooper and Albentosa, 2005). Horses can develop stereotyped behavior, sucking and/or biting a fence or surrounding objects in their box, or walking about in the box, which often happens when horses are housed in poor and inappropriate stables (Waters et al., 2002). Also, unbalanced and poor-quality nutrition can cause diseases such as equine metabolic syndrome (Morgan et al., 2015). Other non-communicable diseases of horses can be caused by poor zoohygiene conditions in stables with poor air quality, they can often develop respiratory problems as well as chronic obstructions, for which the owners or horse breeders are directly responsible (Saastamoinen et al., 2015).

Domesticated horses - benefits for people

Unlike wild horses, domesticated horses live in close contact with humans (see Figure 1). The use of horses is highly dependent on the cultural background. In high-income countries, horses are primarily used for sports, breeding or as companions in free time, while in some poorer countries, horses are still primarily needed for work, thus affecting the owner's economic status (Lönker et al., 2020). In addition to this, horses are used as performers in circuses, rodeos, carnivals, and parades (Katica et al., 2010). Horses played a key role in the development of the first antidote to diphtheria (*Corynebacterium diphteriae*) in humans (Kaufmann, 2017). As an animal model, horses have been used in hepatitis C research, since that virus has a great similarity with equine hepacivirus (Tegtmeyer et al., 2019). Furthermore, horses have been used as a model for diseases of the respiratory system (human allergic neutrophilic asthma) (Klier et al., 2019). In addition, horse meat is a common food source, especially in France, Mexico, Argentina, Kazakhstan, etc. (Pawshe et al., 2016).

We are witnessing that, in the postmodern age, a kind of moral deformity is noticeable, namely in

economically developed countries where many homeless people live, rich households are home to pets who, due to their "social status", enjoy the many benefits of earthly life, in contrast to the traditional exploration of the phenomenon of dialogue and coexistence with the animal world and the environment (Jašić, 2019). Furthermore, in this same postmodern age, horses are often sold by their owners, when they are unable to meet their criteria and expectations, which is not the case with pets (Hausberger et al., 2008).

The Bosnian mountain horse

It is evident that the Bosnian mountain horse is internationally recognized, ancient and the most famous breed in the Balkans, and at the same time the only indigenous breed in Bosnia and Herzegovina (Maletić et al., 2018; Rukavina et al., 2021). The Bosnian mountain horse came to the Balkans with the migration of the Slavs. Its exterior characteristics were probably influenced by the Asian types of horses that appeared in this area with the arrival of the Huns and Avars. When the Ottomans arrived in the Balkans in the 15th century, they brought with them the Arabian thoroughbred, which had a significant influence on the formation of the body structure and the characteristics of the Bosnian mountain horse, because it was a typical representative of the Tarpan type of oriental horse. Under the influence of ecological factors in different areas of Bosnia and Herzegovina, different local types were created (Katica et al., 2010). The Bosnian mountain horse is a warm-blooded oriental indigenous breed, a product of the hot karst and strict selection. It belongs to the group of small horses, it is very hardy, resistant and modest in its diet. It is irreplaceable on rocky and hilly terrain, and its gait is very safe. It is mostly brown, reddish and black in color. It serves as a pack horse that, under a load of 100 to 120 kg, reaches a speed of 5-6 km per hour on a flat track, and can travel up to 40 km during the day. It is also used for towing and riding (Srebočan and Gomerčić, 1996). According to Katica et al. (2010) the total number of Bosnian mountain horses in the territory of Bosnia and Herzegovina was 99,803 in 1990, but by 2008 the number was drastically reduced to 22,326. The reason for the extreme decrease in the population of Bosnian mountain horses was the aggression against Bosnia and Herzegovina from 1992 to 1995, where a large part of the population was lost. Unfortunately, the trend of decreasing population continues.

Mules and hinnies

Mules and hinnies, although they are descendants of the same species of ungulates, horse (*Equus caballus*) and donkey (*Equus asinus*), have unique characteristics that make them special. A mule is a cross between a male donkey, a jack, and a female horse, a mare, while a hinny is a cross between a male horse and a female donkey. These crossbreeds differ from their parents in anatomical characteristics, nutritional needs and habits, behavior, and genotypic (number and structure of chromosomes) (McLean et al., 2019). Most mules and hinnies are sterile, although there are also documented reports of individual cases of fertile hybrids (Ryder et al., 1985; Rong et al., 1988; Henry et al., 1995).

Thanks to their physical strength and endurance, more modest nutritional requirements, better adaptation to extreme climatic conditions and other advantages, mules and hinnies have been used since ancient times for various purposes, including transporting people and transporting goods in inaccessible areas, as a labor resource in agriculture, for carrying ammunition and explosives during wars, for recreation, and in competitions in different disciplines. The estimated number of hinnies and mules in the world is about 14 million (McLean et al., 2019), and of the total number present, over 95% of donkeys and mules are found in developing countries (Fielding, 1991).

As crosses between donkeys and horses, hinnies and mules combine the virtues of their parents (Jackson, 2004). For example, mules have the physical strength and endurance, intelligence, patience and perseverance of a donkey, as well as the beauty, speed and enviable athletic abilities originally from horses. Hinnies, which inherit

their form of movement from donkeys, are slower and more meticulous in their movements than mules, which makes them especially suitable for use on inaccessible, steep and rocky terrain. This is enhanced by the physiognomy of their hooves, which are firmer and narrower in hinnies than in horses. Due to the nature and prevailing temperament of the donkey, hinnies are considered more docile than mules (Proops et al., 2012). On the other hand, it has been shown that compared to horses, mules have a greater ability to reason (Pritchard et al., 2005). Also, when assessing their cognitive abilities, mules were rated as very intelligent animals with better mental performance compared to both parents, and even dogs (Osthaus et al., 2013).

In terms of feeding habits, mules and hinnies are less fussy and have more modest requirements than horses. In terms of digestive efficiency (e.g. large bites, longer gastrointestinal transit time, etc.) hinnies and mules are more like donkeys, require less grazing and are adapted to consumption of fiber-rich food, which makes them more suitable than horses for keeping in extreme environments and areas with scarce vegetation (McLean et al., 2019)

Mules and hinnies have for a long time been considered as a species of ungulates that suffer less often or not at all from laminitis, colic and other frequent health disorders characteristic of horses, although today it is clear that these crossbreeds are also susceptible to the pathologies of other ungulates and that, probably due to their greater tolerance to pain, they manifest disease states less, which is why they often go unrecognized (McLean et al., 2019). As is the case with horses, injuries to the skin and locomotor system, poor nutrition, lameness, respiratory and gastrointestinal disorders, dental diseases and other health problems are common in these crossbreeds, which are used as a work and transport resource in various sectors of human activity, especially in poor communities (Burn et al., 2010).

Mules and hinnies tend to form close bonds with other ungulates, and the company of other mules and ponies is recommended for keeping mules, while hinnies form strong bonds with other hinnies and donkeys (Pritchard et al., 2005). Also, it has been suggested that young mules and hinnies show stronger attachment or affection towards their female parents, that is, mules towards mares and hinnies towards donkeys. Keeping hinnies and mules in such a way that they are isolated and closed in for a long time can lead to changes in behavior and the development of pathological forms of behavior (stereotypes) (McLean et al., 2019). Mules and hinnies are quite distrustful of strangers, and it is not uncommon for them to react to the presence of a stranger by showing clear signs of aggression (e.g. threatening to bite or hit), while this behavior is less likely towards known persons. For this reason, the process of training and sensitization of hinnies and mules should begin from an early age, and this is one of the basic prerequisites for the well-being of these animals and the safety of owners and veterinary professionals in situations of providing health care (McLean et al., 2019).

Aspects of the Muslim tradition

Horses in pre-Islamic Arabia

In pre-Islamic Arab society, the horse was a symbol of war, hunting and wealth, and owning a horse was a sign of prestige. The horses were taken care of with great care so that they were almost considered part of the family. When the mares foaled, celebrations were organized, just like celebrating the birth of a child, or when they won a poetry competition (Cengiz, 2021). The love and attachment expressed to horses is evidenced by the statements of some authors that pre-Islamic Arabs would rather leave their families hungry than allow their horses to lack food (Safuri, 2016). The most powerful expression of the pre-Islamic Arabs was expressed through poetry, in which, among other things, horses were described. The famous pre-Islamic poet Imru´al-Qays, describing his horse says:

"He jumps, bounces, appears, steps back in an instant, you see: a rock rolled away, salivated by rain" (Ramić, 1999).

Importance was also attached to the horse's origin, and in order to avoid the mixing of subspecies and blood relations, which was the reason for the existence of genealogies of purebred horses (Cengiz, 2021). It is also stated that Arabs preferred to use mares for riding because they could withstand fatigue, hunger and thirst better than male horses, and did not neigh during an ambush, thus revealing the position of the fighters. Khalid ibn al-Walid, a companion of the Prophet Muhammad, peace and blessings be upon him, and a famous military leader, only rode mares in battles for these reasons (Hattema, 2021). The practice of naming horses according to their characteristics and appearance was present both in the pre-Islamic era and in the Muslim tradition. Gundidžani (2007) recorded more than 800 horse names, as well as basic information about who owned them or the verses in which they were mentioned by that name.

Horses were highly valued in pre-Islamic Arabia, and their status did not change even in the early Muslim community, and the care and love for horses acquired other dimensions, such as an otherworldly reward for the one who keeps horses, which we will talk more about below.

The mention of horses in the Holy Qur'an

Horses are mentioned several times in the Qur'an, usually in the plural form (*el-hajl)* - horses, which in Arabic language does not have the same singular root (*el-džem'u-l-mufterik)* (Mujić, 2019).

They were created with a purpose and in them there is a benefit for people: both horses and mules, and donkeys - to ride them, and as decoration (An-Nahl, 6).

Horses are undoubtedly part of this world's blessings, and due to the strength of love for them that a person may feel, they can be the cause of forgetting the One who bestows these blessings and as such become a sign of passion and desire: *It seems to people that only what they desire is beautiful: wives, sons, piles of gold and silver, beautiful horses, cattle and crops...* (Ali Imran, 14) (Ibn Adžibe, 2015). In this list of human desires, horses are mentioned before livestock and crops, which indicates that among domestic animals, horses are the most valued and dearest to their owners, and the owner cannot imagine anything better and more valued, due to his heart-felt preoccupation with them. The phrase "wonderful horses" (*El-hajlu-l- musevveme*) can also mean horses that are singled out by a color or brand, or those that graze freely in pastures (Bursevi, 2018).

In the surah Al-Anfal, horses are mentioned in the context of preparing for war: *And against them, prepare as much strength and horses as you can for battle (ribatu-l-hajl), in order to frighten the enemies of Allah and yours...* (Al-Anfal, 60). Asad (2004) explains the phrase *ribatu-l-hajl* as "restraining the horses", i.e. "keeping mounted troops ready at all points exposed to enemy invasion". In this verse, the special value of horses is also pointed out, this time in the context of their importance in war, because they are mentioned after strength, although they are certainly included in it (Bursevi, 2020).

In addition to the term (*el-hajl)*, horses are also mentioned in the Qur'anic text with other names that describe certain characteristics of horses. Such is the case with the horses of Prophet Sulaiman, peace be upon him, which are mentioned in the Qur'an surah entitled "Sad": *When one evening thoroughbred horses were brought before him that stood on three legs and barely touched the ground with the fourth* (Sad, 31).

According to Et-Tusi (2016) Prophet Suleiman, peace be upon him, had three hundred pure bred Arab horses, the likes of which no ruler before or after him owned, which is also indicated by their special characteristics mentioned in the verse. These were extremely fast horses when galloping, and they would stand on three legs, while the fourth would touch the ground only with the tip of the hoof (Begavi, 1989). They are certainly horses of a noble breed to which Prophet Suleiman, peace be upon him, one day devoted so much of his attention that he missed the time of prayer. This affected him, so according to some interpretations, he slaughtered those horses, while other sources indicate that he showed them respect by asking

them to return them to him and caressing them, since they were horses that were used in battle on God's path (En- Nedždžar, 2008).

The first five Qur'anic verses (ayats) within the surah entitled "Al-Adiyat" reading: "By the galloping, panting horses, striking sparks of fire with their hoofs, launching raids at dawn, stirring up clouds of dust, and penetrating into the heart of enemy lines!" (Al-Adijat, 1-5.) demonstrate "the striking scene of the war cavalry in the midst of its evening gallop and the sudden morning incursion into the territory of another tribe" which is a war scene deeply engraved in the consciousness of the pre-Islamic Arabs. According to a large number of classic commentaries on the Qur'an, the cavalry mentioned in these verses is the cavalry of fighters on God's path, that is, their attack on enemy positions (Fatić, 2020). Bearing in mind the number of commentaries on the Qur'an, it should be noted that each of the previous examples could be the subject of a separate study. Here, however, in keeping with the topic, we will only focus on the previous short entries.

The wisdom of the Blessed Prophet Muhammad, peace and blessing be upon him, about horses

The Prophet Muhammad, peace and blessing be upon him, loved horses and was kind to them. He did not allow the cutting of the ears of the horses for marking purposes, nor the trimming of their tails and manes, and he recommended taking care of the cleanliness of horses and their nutrition (Es-Subhi, 2011), and that in general they be treated well and nobly (Es-Sidžistani, 2012). Beside that the Prophet, peace and blessing be upon him, warned his companions regarding the treatment of riding animals, not to sit on them unnecessarily (Ibn Džuzejj el-Garnati, 1986). A large number of oral traditions (Hadith) have been recorded of the Prophet Muhammad, peace and blessing be upon him, about the value of horses, the rewards of caring for them, and their importance to the Muslim community. It has been mentioned previously that owning a horse in pre-Islamic Arabia was a symbol of prestige and wealth that elevated one above others. Although the value of the horse is described in the oral traditions (Hadith) of Muhammad, peace and blessing be upon him, and their possession and care of them is a source of good both in this world and in the next, it should be noted that this good depends on the attitude of the owner himself towards unsuspected bounty, and his inner drive and intention that determine his attitude towards horses, which is discussed in the oral tradition (Hadith) of Muhammad, peace and blessing be upon him: "Horses are for three people: One will be rewarded, another will be protected, and the third will be punished. The reward will be given to the one who keeps them with the intention of fighting in the way of Allah, so if he grazes them in a meadow or a garden, whatever he grazes in that meadow or garden will be recorded in his good deeds. If they break the rope by which they are tied, and run to a hill or two, the owner is credited with good deeds for their feces and feet. If they pass by a river and drink from it, and he did not intend to give them water, that counts as good deeds for him. They are a protection to the one who keeps them for profit and sustenance and does not forget God's right in their necks and backs, and they are a sin and punishment to the one who takes them out of boasting, hypocrisy and enmity towards Muslims" (Es Sujuti, 2022). Special emphasis is placed on owning and training a horse to fight in God's way, which will be a reason for the owner's protection from fire, and all his effort and care for the horse will be on the scale of his good deeds on the Day of Judgment (Es Sujuti, 2009.) The fact that the Muhammad, peace and blessing be upon him, determined one share of the spoils of war for the infantry and two for the horsemen speaks of the value of these horses and horsemen in battle in general (El Munziri, 2008). "Blessings are found in the manes of horses" (El Munziri, 2008), and all that a man invests in a horse is like charity distributed to the needy. A person's wealth is tied up in their horse's mane until the Day of Judgment, and he who spends his wealth on horses is like one who stretched out his hand with charity and did not withdraw it." (Es Sujuti, 2022).

Furthermore, in the oral tradition (Hadith) there are statements describing certain characteristics of horses, for which the Muhammad, peace and blessing be upon him, had sympathy, especially for horses of a reddish color who possess "God's blessing" (Tirmizi, 2013) or black horses with a white spot on the nose, which are described as the best (Tirmizi, 2013).

Training horses and organizing horse races

The Prophet Muhammad, peace and blessing be upon him, had previously ensured that horses gained weight before starting exercise (Es Sujuti, 2020). There was also a specific way of training horses (muzemmer) to make them gallop faster. First, the horses would be subjected to an enhanced diet, and then the quantity of rations would be reduced. During that period, they were subjected to sweating in such a way that they were covered with blankets in closed spaces. In this way, sweating and fat loss were stimulated. In this way, the horses lost excess weight, and the prerequisites for achieving higher speeds were created (Cengiz, 2021).

The Prophet Muhammad, peace and blessing be upon him, also organized horse races, which was a special incentive for his companions to improve their horse riding skills. A companion of the Prophet Muhammad, peace and blessing be upon him, Ibn Omer, reports that the Prophet, peace and blessing be upon him, organized a race for trained horses between some locations in the wider area of Medina, specifically, from Hafja to Senijjetu-l-Veda´a and for untrained horses a race with a shorter distance from Senija to Masjid Banu Zureyq (El Munziri, 2008).

If the horses of the Prophet, peace and blessing be upon him, participated in these races, then the winners would not be rewarded, and if there were no horses belonging to the Prophet, peace and blessing be upon him, then he would determine the prizes for the winners of the race. The most common prizes were Yemenite robes. The horses would line up at the designated starting point and the race would begin after the third takbir (Cengiz, 2021). The prohibition is also mentioned of releasing an additional horse that is not racing, or releasing another horse alongside the horses in the race that would stimulate them to run (Es Sujuti, 2020). The practice of organizing races was continued by the Prophet's, peace and blessing be upon him, close companion Ali (Cengiz,2021).

Teaching children to swim, ride and practice archery is especially recommended, and the Prophet's, peace and blessing be upon him, close companion Omer wrote to the residents of Sham that they should teach their children to swim and ride horses (Džumua, 2003). These recommendations should certainly be updated in the times we live in, bearing in mind the numerous benefits of riding for the development and improvement of the "emotional, mental, physical and social state" of every individual today (Itković, 2002).

The horses of Blessed Prophet Muhammad, peace and blessing be upon him

As stated, the Muhammad, peace and blessing be upon him, owned horses and his horses participated in the races he organized. In the literature, the number of the Prophet's, peace be upon him, horses is stated as seven, on which almost all authors agree (Cengiz, 2021). Basic information about the most famous Prophet's, peace be upon him, horses is provided:

Es-Sekb: the first of the Prophet, peace and blessing be upon him, a horse that he bought in Medina from the tribe of Benu Fezara for ten pieces of silver. Earlier, he was called Daris and the Prophet, peace and blessing be upon him, changed his name to Es-Sekb, because he was extremely fast, like falling water pouring out. Es-Sekbu rode in the battle of Uhud (Es-Subhi, 2011).

Murtazaj: a white horse that the Muhammad, peace and blessing be upon him, bought from the Bemu Murra tribe (Cengiz, 2021). It was named because of its beautiful hum that was like a song (Es-Subhi, 2011).

Sabha: a golden-yellow horse that the Prophet, peace and blessing be upon him, bought from the tribe of

Juhain for ten camels, after the horse Sabha had won a race (Cengiz, 2021).

Laheef: the horse that Rebia ibn abi Bera gave to the Prophet (peace and blessing be upon him), and was named Laheef because of its extremely long tail (Cengiz, 2021).

Lazaz: the horse given to the Prophet, peace and blessing be upon him, by Mukavkis, the ruler of Egypt. He was named for his pleasant nature and speed of movement, and he was also attached to people. Lizaz won one of the races that the Muhammad, peace and blessing be upon him, organized. Muhammad, peace and blessing be upon him, also rode it in several battles (Es-Subhi, 2011). According to some authors, Mukavkis gave the Prophet, peace and blessing be upon him, a mule called Duldul (Abu Khalil, 2008).

Al-Zarrab: one of the most famous horses of the Prophet, peace and blessing be upon him, which got its name because of its size and fatness, as well as its strength and solidity (Es-Subhi, 2011).

Verd: this horse was presented to the Muhammad, peace and blessing be upon him, by his companion Temim ed-Dari, and the Prophet, peace be upon him, later presented it to his great friend Omar (Cengiz, 2021).

From the Muslim tradition

The stories state, as Schimmel (2014) says, that horses were created by the swift south winds. It is interesting that some authors, such as the Muslim authority Sujuti, cite a longer tradition that speaks of the creation of a horse from the south wind that would be strength for those who please God and a humiliation for God's enemies, and an ornament for those who obey God. It is further stated that the Lord will place people on the horses' backs who will glorify, praise and glorify Him, so whenever they do so, the horses will glorify, praise and glorify their Creator with them (Es Sujuti, 2009). Dumejri (2004) records several traditions with a similar meaning, and in some he states that he is writing the Arabian horse, which is distinguished above all other animals. In addition, this author points out the prevalence of numerous stories and traditions among the people, about the value of horses, which are not credible or are traditions from the Judaic tradition (Dumejri, 2004). Ibn Juzeyy (1986) also pointed out the unreliability of these traditions. However, there is no doubt that these descriptions of the horse and its creation of images indicate to us the great respect and value that the horse had in the Muslim tradition, and its special status in relation to all other animals that benefit humans.

On the other hand, when reading these Muslim classic works, one realizes that the attitude of Muslims towards animals in general, primarily horses in this case, is not determined only by man's need for them, nor the good that is achieved through them, but above all by the effort to recognize and understand the right - (hakk), which animals, as creatures of God that point to Him, have in relation to men. "The divine wisdom that created human beings imposed on them the duty of giving everything that has a right its right" Chittick (2018) stated in a great text about the prudence of animals.

Animals are a special community and they have a special kind of relationship with God and knowledge about Him. Likewise, each animal species has its own vision and way of glorifying God (Tasbih), which corresponds to their knowledge of the Creator's dissimilarity to what is created (Ibn Arebi, 2018).

To show how horses glorify the Creator, Bursevi (2020) cites the words of the Prophet's companion Ibn Abbas: "When two armies meet, the horses say: Much Glorified, Pure, our Lord and Lord of angels and Spirith" (*Subbuhun, Kuddusun, Rabbuna ve Rabbul-melaiketi ver-ruh*). Animals know why they were created, even though they are subordinate to man. He is in greater need of them than they are of him. Therefore, man, in a certain way, even though he is not aware of it, is subordinate to animals because he must necessarily take care of their needs, feed them, and all this effort is due to man's dependence on them (Ibn Arebi, 2018). In addition to this aspect of glorifying and glorifying God (Tasbih), which

is a characteristic of the horse, in the authentic traditions of the blessed Muhammad (peace and blessing be upon him), there is also a mention of the prayer that horses send to the Creator: "There is no such Arabian horse that is not allowed to offer two supplications. It says: My Allah, You have entrusted me to the one to whom You have entrusted me among the people, so make me the most dear to him of his family and his property." (Es Sujuti, 2022).

The first to whom the horses were subordinated was the Prophet Ismail, peace be upon him. After he and the Prophet Ibrahim, peace be upon him, raised the foundations of the temple in Mecca, the Lord announced to him that he would give him a treasure that had not been given to anyone before him. After that, there was not a single horse left in the land of the Arabs that did not come to him and was subdued, so Ismail, peace be upon him, is mentioned as the first man who rode horses after they were untamable. (Ibn Džuzej, 1986). Es Sujuti (2009) also states the opinion that Kabil, son of Adem, peace be upon him, was the first to ride a horse.

Dumejri (2004) in this context mentions the possibility, since horses were created before Adem, peace be upon him, and domesticated and ridden before, but in some period only wild horses remained until the period of Ismail, peace be upon him, which were then subjugated again. Bearing in mind that Ismail, peace be upon him, is considered to be the ancestor of the Arabs, these traditions can also refer primarily to Arab horses.

Finally, in the Muslim tradition, the different meanings are recorded that are indicated by seeing a horse in a dream. A submissive horse generally indicates good, a galloping horse indicates the quick fulfillment of a wish, and a horse's trot indicates balance. However, an unbridled and unruly horse indicate a careless man (Ibn Sirin, 2008).

CONCLUSIONS

Although they were domesticated relatively late compared to other domestic animal species, horses have left a striking mark throughout history, and still do today. Humanity has not had so many benefits from any other animal species as is the case with horses, with which man has built a close and strong relationship throughout history. The endowment of the speed, strength and endurance of this magnificent animal has made it indispensable as a companion to men in peace and in war. Nowadays, horses serve humanity as a source of quality animal protein, a strong workforce, a means of transport, and apart from that, they have a much more significant, above all noble role in rehabilitation programs for people with various physical, mental, emotional and socialization difficulties.

Apart from the presentation of the role and significance of horses in human life throughout history until today, the presentation is highlighted of the relationship of the Islamic religion to horses, which are the most valued domestic animals. In Islamic theology, taking care of the animal world is mandatory, primarily as an expression of doing deeds that bring us closer to God, where the well-being of horses is especially taken care of. Thanks to the arrival of the Ottomans in the Balkans, in the tradition of today's Muslims from these areas, a refined, noble attitude towards this animal has been preserved, which in all aspects is fully compatible with contemporary principles related to the well-being of horses.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conception – MK, LŠ; Design – MK, LŠ; Supervision - PDŽ; Materials – VK, EŠ, ZD, PDŽ; Data Collection and/or Processing – MK, LŠ, VK, EŠ, ZD, PDŽ; Analysis and/or Interpretation – MK, LŠ, VK, EŠ, PDŽ; Literature Search – MK, LŠ, VK, EŠ, PDŽ; Writing Manuscript – MK, LŠ, VK; Critical Review – ZD, PDŽ

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KONJ U SVJETLU VETERINARSKE MEDICINE I MUSLIMANSKE TRADICIJE - KOMPARATIVNA STUDIJA

SAŽETAK

Od svih životinja koje čovjeka prate gotovo kroz sve etape razvoja čovječanstva, konjima pripada naistaknutije mjesto. Izuzev prikaza uloge i značaja konja u životu čovjeka kroz historiju do danas, uzimajući za paradigmmu bosanskog brdskog konja, istaknut je prikaz odnosa islamske religije prema konjima koji su najcjenjenija domaća životinja. U islamskoj teologiji skrb o animalnom svijetu je obavezna, ponajprije kao izraz činjenja djela koja približavaju Bogu pri čemu se za dobrobit konja posebno marilo.

Ključne riječi: Bosna i Hercegovina, bosanski brdski konj, Islam, mazga, mula

RESEARCH ARTICLE

HISTOLOGY OF THE ONE-HUMPED CAMEL'S UTERUS AND VAGINA DURING THE FOLLICULAR AND LUTEAL PHASES OF THE OESTROUS CYCLE

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ABSTRACT

The dromedary camel is one of the domestic animals that have not received adequate scientific attention. Unlike other domestic animals, the dromedary camel does not ovulate spontaneously until after a successful mating. Therefore, present study seeks to evaluate the histological features of the vagina and the uterus of dromedary camel during follicular and luteal phases of the oestrous cycle. A total of 86 one-humped camels were included, and their blood samples were collected for progesterone assay. The progesterone assay enabled us to divide the camels into the follicular and luteal phase groups, according to progesterone concentration standards for luteal and follicular phase of the oestrous cycle. In the specimen collected during the follicular phase, the mean serum progesterone level was 0.89±16 ng/ml, while its level was 1.61±0.81 ng/ml in the specimen during the luteal phase. Animals with P4 values <1 ng/ml (n=51, mean of 0.89 ± 0.16ng/ml) and those with P4 values >1 ng/ml (n=35, mean of 1.61 ± 0.81 ng/ml) were considered to be in follicular and luteal phase, respectively. Vaginal and uterine tissue samples were collected from all members of both groups. The histological features observed in the uterus during the follicular phase are presented with a layer of simple columnar epithelium with fewer elastic epithelial cells. In the uterine lamina propria, there are several visible tubular glands. Blood vessels are conspicuous and clogged. The inner circular and outer longitudinal layers of the muscle bands are thick, with numerous interstitial spaces. There are more intercellular gaps in the vagina than in the uterus. On the other hand, in the sample collected during the luteal phase, uterine tissue is consisted of simple columnar epithelium. The glands in the lamina propria are more coiled and tubular. Luteal phase is also characterized by the presence of congested blood vessels that are thick in appearance. Thick and clogged blood arteries in the uterus have also been observed. The uterine muscle layer consists of separate inner circular and outer longitudinal layers. It is also thicker than the one observed in the follicular phase. This detailed histological study of vagina and uterus will not only help to understand the reproductive status of camel, but it will also assist in pathological evaluation of the reproductive tract.

Keywords: Histology, one-humped camel, uterus, vagina

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INTRODUCTION

Camel is one of the most important animal species that is known to be adapted to arid and hot environments (Schwart, 1992; Beheiry, 2016). For centuries, the camel has been a very important animal in the desert region, because of its ability to provide milk, meat, and transport in harsh dry conditions. The improvement of the productivity of camel in Nigeria has necessitated the need for basic research on this animal species.

In the field of scientific research, the camel is one of the most neglected domestic animal species, which has not received adequate attention (Beheiry, 2016). This neglect could possibly be linked to poor nutrition and husbandry in arid and tropical areas of Africa and Asia (Sohail. 1983; Behery, 2016). Therefore, literature on the female reproductive tract of camels has been scantily evaluated compared to other mammalian species such as cows (Majama et al., 2018). Dromedary camels can also be found in arid regions of the Middle East, Northern India, and Africa, including the Sahara Desert. They have also been introduced to central Australia's arid regions, where wild populations still exist (Nowak 1991; Wilson, 1984). Some camels from Mali, Niger, and Chad were recorded in the southern Sahel states, particularly Upper Volta, Nigeria, and Cameroon. Pastoral groups in Sokoto, Katsina, Kano, and Borno, according to Ribadu (1988), own the majority of camels in Nigeria. Camels (*Camelus dromedarius*) are widely seen in Nigeria's northern borderlands, usually herded alongside cattle, sheep, goats, and donkeys, mingling freely at watering pools and marketplaces (El-Yuguda et al 2010; Markemann and Zarate 2010; Majama et al., 2023). Due to their sensitivity to cold and humidity, dromedary camels prefer desert environments with a long dry season and a short wet season **(**Nowak 1991). According to Khanvilkar (Khanvilkar et al., 2009), the female camel can breed as early as 2–5 years of age, and as late as 30 years of age. However, according to Marai (Marai et al., 2009), breeding can occur as early as 2–5 years of age. When compared to other ungulates, this animal has a distinct estrous cycle. Unless the female is bred and has ovulated, the phases of the cycle outlined for animals with spontaneous ovulation (estrous and di-estrous) do not exist in Camelidae. There is simply a sequence of follicular waves with a highly fluctuating rhythm in the absence of mating (Tibary and Anouassi, 1997). Camels are known to be induced ovulators, with ovulation occurring after mating in the non-pregnant animal (Marie and Anouassi, 1986; Ayoub et al., 2003; Skidmore et al., 2005; Ghazi, 2007). During the camelid's estrous cycle, four distinct uterine activity phases-high, falling, low, and increasing-were noted by Al-Eknah **(**Al-Eknah et al. 1993). The level of progesterone in the camel cow's blood is determined by her reproductive status and age (Kamoun and Jemmali, 2014; Majama et al*.,* 2018). The serum progesterone concentration in camel ranges from 0 to 0.41 ng/ml., with mean variations in the range of 0 to 0.380.04 ng/ml (Kamoun and Jemmali, 2014; Majama et al*.,* 2018).

Srikandakumar et al*.,* (2011) reported that the endometrial lining of the vagina consists of a single layer of columnar epithelium supported by a broad highly cellular connective tissue with simple tubular glands. These organs undergo tissue differentiation from prenatal, postnatal, and adult stages under varied physiological and hormonal influences.

Previous reports (Elias,1990; Srikandakumar et al., 2011) on the non-gravid uterus of an adult dromedary have been documented providing vivid gross descriptive features of the uterus in animals found in other parts of the world. The morphometric characteristics of the one-humped camel's reproductive system have been documented (Ribadu, 1988), with scarce information on histological features of the uterus and vagina of the dromedary camels during different reproductive phases. Determining the histological differences of the uterus and vagina in camels during the follicular and luteal phase was the goal of this investigation.

MATERIALS AND METHODS

Materials

A total of 86 samples of blood and intact genitalia were collected from one-humped female camels brought for slaughter at the Maiduguri metropolitan abattoir. Jugular venipuncture was used to obtain blood samples before slaughter, which were then transferred into sterile, clean vacutainer tubes.

Methods

The genitalia were collected as soon as the animals were slaughtered, and put into a clean plastic container. They were urgently transported to the Department of Veterinary Anatomy Postgraduate Research Laboratory, located at the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria, as previously described (Jaji et al. 2012; Gazali et al. 2023). Serum was extracted from the blood specimens by centrifugation at 2000–3000 RPM for 10 minutes, and it was then kept at -80 °C until it was utilized for the progesterone assay.

Progesterone assay (P4)

The Camel Progesterone (PROG) ELISA Kit (My Bio Science, USA) was used for the P4 assay. This assay was based on the Biotin double antibody sandwich technology, and was carried out according to the manufacturer's instruction. The results of the serum P4 profiles for all the camels examined ranged from 0.6 to 4.6 ng/ml. Those animals with the P4 values of < 1 ng/ml (n=51, mean of 0.89 ± 0.16 ng/ml) and those with values of > 1 ng/ml (n=35, mean of 1.61 ± 0.81 ng/ml) were considered to be in their follicular and luteal phases, respectively, as previously described (Kamoun & Jemmali, 2014). Using this method, we divided the specimen of 86 camels into two groups: the follicular phase group (N=51), and the luteal phase group (N=34).

Tissue preparation for histology

About 30-40 mm of the vaginal and uterine tissue was collected from each of the 86 intact genitalia. The tissue was fixed in Bouins solution for 72 hours, washed in running tap water to remove the fixatives, and then treated with ascending grades of ethyl alcohol (70%, 90%, and 100%) for dehydration. After dehydrating the tissue, the alcohol was removed and subsequently treated with xylene, and finally embedded with paraffin wax at 63 degrees. The tissue blocks were then mounted on wooden chocks, cooled, and cut at 5µm thickness. The tissue sections were then flattened on warm water at 45° C and placed on glass slides, smeared with egg albumin, and dried in the oven at 45° C. The sections were then stained with H&E for histological examination using a light microscope (Drury et al, 1976).

RESULTS

Serum Progesterone (P4) Assay

Table 1 displayed the results of the progesterone assay. During the follicular phase, the female camel's mean serum progesterone concentration was 0.89 ± 0.16 ng/ml, and during the luteal phase, it was 1.61 ± 0.81 ng/ml. Animals with P4 values < 1 ng/ml (n=51, mean of 0.89 ± 0.16 ng/ml) and those with P4 values > 1 ng/ml (n=35, mean of 1.61 ± 0.81 ng/ml) were considered to be in follicular and luteal phase, respectively, as previously described (Kamoun and Jemmali, 2014).

Table 1 One-humped camel (*Camelus dromedarius*) from Maiduguri, Nigeria, serum progesterone (P4) levels during the follicular and luteal phase of the estrous cycle

Estrous Cycle Phases	Number of animals	P4 onc. (ng/ml)	
		Mean ± SD	Range
Follicular phase	51	0.89±0.16	0.6-1.0
Luteal phase	34	1.61±0.81	1.1-4.6

Histology

The histological features of the uterus and the vagina of the one-humped camel in the follicular and luteal phases are presented below in Figures 1 and 2.

Figure 1 presents the photomicrographs of the

uterine and vaginal tissue during the follicular phase. Throughout the follicular period, the epithelial layer of uterus is consisted of simple columnar epithelium with less elastic epithelial cells. The lamina propria shows numerous tubular glands. There are prominent and highly congested blood vessels. The muscular layer is thick and edematous, with collagen fibers showing distinct inner circular and outer longitudinal layers. The histological features of the vagina during the follicular phase are presented with stratified squamous epithelium which is more folded than the one in the uterus. The lamina propria of the vagina is observed to be devoid of glands but has few blood vessels scattered. The muscular layer is thick and consisted of inner circular and outer longitudinal layers with more interstitial space. The intercellular spaces observed in the vagina are more spacious than those in the uterus.

Figure 2. exhibits the histological features of the uterus and the vagina during the luteal phase. The uterus is lined with simple columnar epithelium. The lamina propria shows more coiled and tubular glands. It is characterized by the presence of congested blood vessels which are thick in appearance. The muscular layer of the uterus is thicker during the luteal than in the follicular phase, with distinct inner circular and outer longitudinal layers. Histologically, during the luteal phase

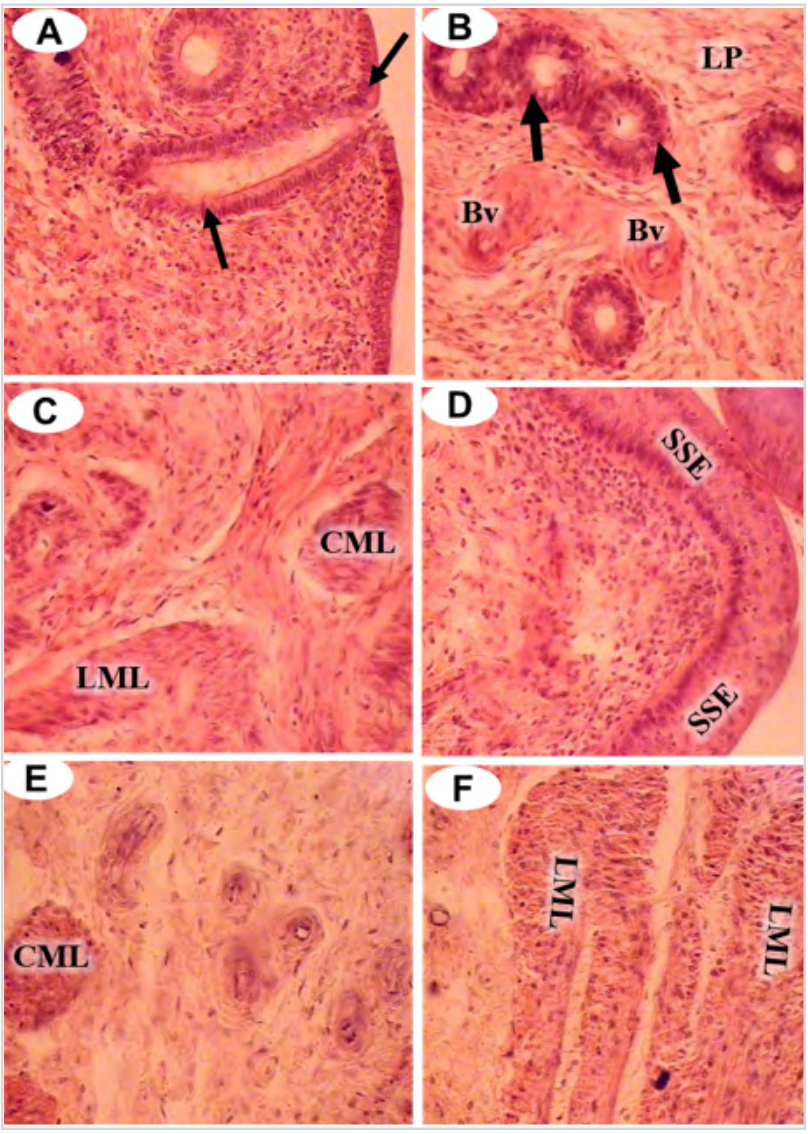

Figure 1 Photomicrograph of the uterine and vaginal tissues of the one-humped camel (*Camelus dromedarius*) during the follicular phase showing: (A) Uterus consisting of simple columnar epithelium with less elastic epithelial cells (thin black arrows), (B) Lamina propria (LP) of the uterus showing numerous tubular glands (thick black arrows), and congested blood vessels (Bv), (C) Myometriun showing thick musculature with distinct inner circular muscular layer (CML) and outer longitudinal muscular layer (LML), (D) Vaginal tissue showing stratified squamous epithelium (SSE) that is more folded than the uterus, (E) Vaginal lamina propria with scattered blood vessels (Bv) as shown in (B), (F) Vaginal muscular layer consisting of inner and outer longitudinal muscular layers (LML)

the vagina is characterized by the presence of stratified squamous epithelium with prominent blood vessels. There are more intercellular spaces observed in the lamina propria than in the tunica adventitia. The tunica muscularis of the vagina consists of inner circular and outer longitudinal layers with muscle bundles, reticular fibers, and scanty nerve fibers.

DISCUSSION AND CONCLUSION

The vagina and uterus are parts of the female reproductive system of the one-humped camel with very important role in fertilization. Unlike other domestic animals, the camel does not ovulate spontaneously. As a result, the luteal phase appears only following a successful mating (El Allal et al., 2017; Majama et al., 2018). Therefore, progesterone in high concentration can only occur during pregnancy in the camelids (Majama et al., 2018), what is consistent with the present study, as the progesterone concentration was higher during the luteal phase (0.1-1.0 ng/ml) than in the follicular phase (1.1-4.6 ng/ml).

Furthermore, since the cyclical patterns observed in other animals are not consistent with the Camelids, the histological features of the utero-vagina have not been adequately described in this species (Ahmadi et al. 2005; Majama et al., 2018).

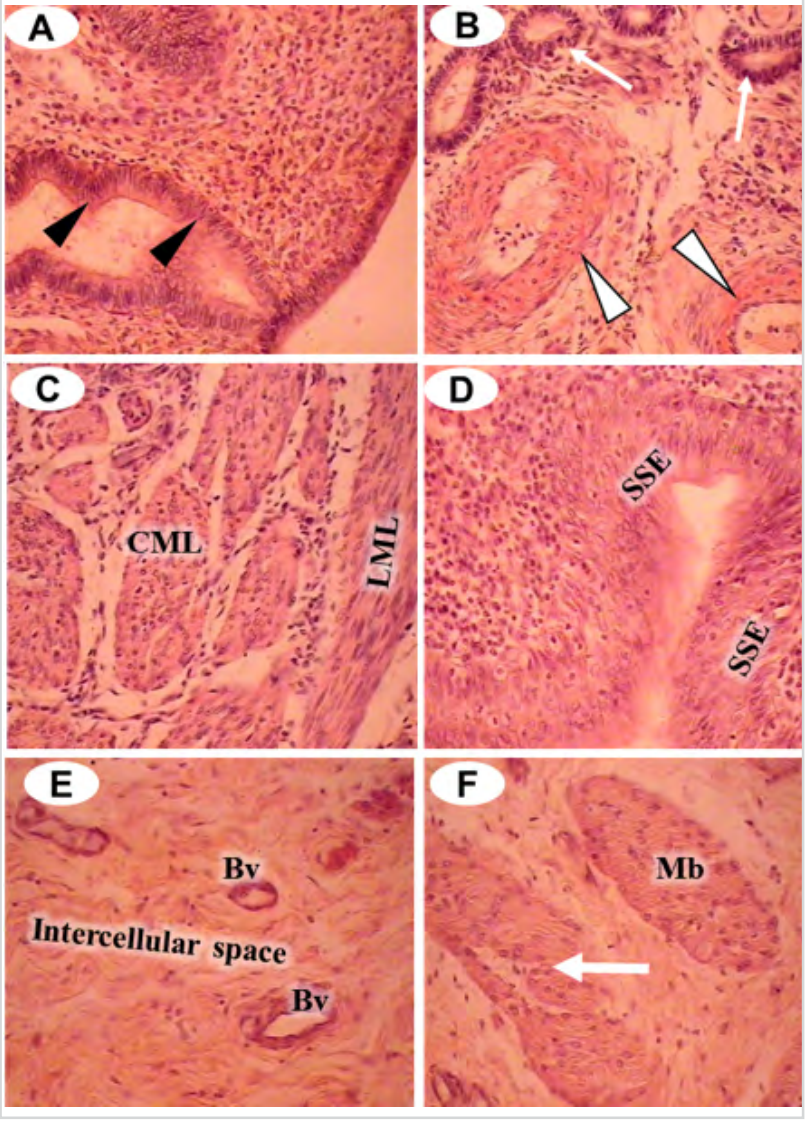

Figure 2 Photomicrograph of the uterine and vaginal tissues of the camel (*Camelus dromedarius*) during the luteal phase showing (A) Uterus characterized by simple columnar epithelium(black arrow heads), (B) Lamina propria with more coiled and tubular glands (thin white arrows) larger tubular glands with congested blood vessels (white arrow heads) (C) Myometrium showing thick muscular with distinct inner circular muscular layer (CML) and outer longitudinal muscular layer (LML), (D) Vaginal stratified squamous epithelium (SSE), (E) Vaginal lamina propria with intercellular space and blood vessels (Bv), (F) Tunica muscularis consisting of inner and outer longitudinal muscular bands muscle bundles (Mb) and scanty reticular nerve fibers (white arrow). H&E × 400

Present study revealed more prominent mucosal folds in the uterine epithelium during the follicular phase than in the luteal phase, as it was previously observed in the cow (Mokhtar, 2014). Similarly, the uterine tissue was characterized by simple columnar epithelium with less elastic epithelial cells during the follicular phase. The lamina propria revealed numerous glands that were tubular with prominent blood vessels. These differences could possibly be due to the varying secretion rates of ovarian hormones as were previously observed in bovine species (McDanie et al., 1968; Mokhtar, 2014), and camelids (Srikandakumar et al*.,* 2011). The muscular layer was thick and edematous with distinct collagen fibers showing inner circular and outer longitudinal layers during this phase. The mucosa showed irregular raised longitudinal folds, which agrees with a previous report on Arabian camels (Srikandakumar et al*.,* 2011).

During the luteal phase, there were no marked deviations in the histological features observed during the follicular phase, except that the glands became more coiled and tubular. The histological features of the vagina during the follicular phase were characterized by stratified squamous epithelial layers which were more folded than the uterus. These cyclic changes in the vagina and uterus showing hypertrophy and atrophy of their epithelial tissues could be due to the status of the endocrine function; as a result, the tissues may undergo some changes during the estrous cycle, as previously reported (Yániz et al., 2000; Mokhtar, 2014).

Interestingly, the lamina propria was devoid of glands but with few scattered blood vessels. The muscular layer was observed to be thick and consisted of inner circular and outer longitudinal layers with more intercellular space. As observed during the follicular phase, there was also no marked difference in the histology of the vagina during the luteal phase; this could be due to limited activities of the vagina compared to the uterus during embryo nutrition and development. Djang et al., 1988, showed that the vaginal lumen was lined with many longitudinal folds. In conclusion, the changes in the vagina and uterus of dromedary camel during follicular and luteal phases are similar to other domestic animals, though the luteal phase can only come following successful mating, which is remarkably different from other domestic species.

In conclusion, the progesterone levels observed throughout the oestrous cycle are very important in determining the reproductive status of camels. This detailed histological study of the vagina and uterus will not only help to understand the reproductive status of camel but will also assist in pathological evaluation of the reproductive tract.

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CONFLICT OF INTEREST

According to the authors, there are no conflicts of interest that might be thought to compromise the objectivity of the research presented.

CONTRIBUTIONS

Conceptualization and writing of the draft manuscript: YBM, writing and editing of the manuscript: MZ, HMBM, statistical analysis: LA and Supervision and critical review: HDK.

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HISTOLOGIJA UTERUSA I VAGINE JEDNOGRBE KAMILE U FOLIKULARNOJ I LUTEALNOJ FAZI ESTRUSA

SAŽETAK

Dromedar kamila je vrsta domaće životinje kojoj se ne posvećuje adekvatna znanstvena pažnja. Za razliku od ostalih domaćih životinja, dromedar ne ovulira spontano, već nakon uspješnog parenja. Stoga je cilj našeg istraživanja procijeniti histološke karakteristike vagine i uterusa kod dromedara za vrijeme folikularne i lutealne faze estrusa. U istraživanje je uključeno ukupno 86 jednogrbih kamila čiji su uzorci krvi prikupljeni za određivanje progesterona. Razina progesterona je omogućila podjelu kamila u dvije grupe, jednu u folikularnoj, drugu u lutealnoj fazi, a prema referentnim vrijednostima progesterona u lutealnoj i folikularnoj fazi estrusa. U uzorku prikupljenom u folikularnoj fazi, srednja vrijednost progesterona je iznosila 0.89±16 ng/ml, a u lutealnoj fazi 1±0.81ng/ml. Za životinje sa vrijednostima P4 < 1 ng/ml (n=51, srednja vrijednost od 0.89 ± 0.16 ng/ml) i one sa P4 vrijednostima >1 ng/ml (n=35, srednja vrijednost od 1.61 ± 0.81 ng/ml) je smatrano da se nalaze u folikularnoj, odnosno lutealnoj fazi. Uzorci vaginalnog i uterinog tkiva su uzeti od svih životinja iz obaju grupa. Uočene histološke karakteristike uterusa u folikularnoj fazi su predstavljene slojem jednostavnog cilindričnog epitela i manjim brojem elastičnih epitelnih ćelija. U uterinoj lamini propriji je vidljivo nekoliko tubularnih žlijezda. Krvni sudovi su uočljivi i začepljeni. Unutrašnji cirkularni i vanjski longitudinalni mišićni slojevi su zadebljani sa širokim intersticijalnim prostorom. Vagina ima više intercelularnog prostora nego uterus. S druge strane, u uzorku prikupljenom u lutealnoj fazi tkivo uterusa se sastojalo od jednostavnog cilindričnog epitela. Žlijezde u lamini propriji su vijugave i tubularne. Lutealna faza je također karakterizirana prisustvom kongestiranih krvnih žila koje su se doimale širim. U uterusu su uočene i proširene i začepljene arterije. Sloj uterinih mišića se sastoji od unutarnjeg cirkularnog i vanjskog longitudinalnog sloja koji su međusobno odvojeni. Ovaj sloj je deblji od sloja u folikularnoj fazi. Ovako detaljna histološka studija vagine i uterusa će biti od koristi ne samo u razumijevanju reproduktivnog statusa kamile, nego i u patološkoj evaluaciji reproduktivnog trakta.

Ključne riječi: Histologija, jednogrba kamila, uterus, vagina

RESEARCH ARTICLE

THREE-DIMENSIONAL MODELS OF HUMAN SKULLS AND THEIR APPLICATION IN SEX DIFFERENCES ANALYSIS OF MIDSAGITTAL LINE

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ABSTRACT

The development of technology and different imaging techniques has contributed to the development of science in general. Imaging has also found application in anatomy. Aim of this research was the application of three-dimensional models of the human skulls in analysis of sex differences of the midsagittal line. The research was performed on 211 three-dimensional models (3D) of human skulls from the Bosnian population (139 male and 72 female skulls). 3D models were obtained by scanning the skulls using a laser scanner. On 3D, seven nonpaired landmarks in the area of the midsagittal line were marked using Landmark Editor program. The position of the marked landmarks was used for analysis of sex differences in the shape of the midsagittal line and for analysis of sex differences of maximum sagittal diameter in the area of the midsagittal line (maximum cranial length, glabella-opisthion). Geometric morphometrics was performed to analyze shape differences of the midsagittal line between the sexes, and the results of correct classification test showed statistically significant differences. Sex determination using the shape of the midsagittal line was possible with 66.19% accuracy for male skulls and 62.5% for female skulls ($p<0.001$). Results of regression analysis showed a statistically significant effect of size of the midsagittal line on its shape. Results of univariate analysis showed statistically significant differences between the sexes ($p<0.001$). The application of three-dimensional models of the skulls for sex determination based on the midsagittal line showed statistically significant differences between the sexes.

Keywords: 3D models, geometric morphometrics, sexual dimorphism, skulls, univariate analysis

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INTRODUCTION

The development of technology and different imaging techniques have contributed to the development of science and humanity in general. Many scientific disciplines are based on imaging or the analysis of an image content. Imaging is an indispensable technique in many branches of medicine. Imaging parts of the body is the basis of radiology, the results of which are used for procedures in almost all areas of medicine. Techniques used in medicine for imaging have developed over time, which has contributed to better and faster disease diagnosis as well as the treatment process itself (D'Errico et al, 2017).

Imaging of body parts can be based on the application of computed tomography (CT), digital radiography (RTG), magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT) and other imaging technologies that have gradually developed and are widely used. In addition to being used in diagnostics and treatment, new imaging methodologies are also widely used in scientific research in almost all areas of medicine (Almutairi et al., 2017; Hidrovo et al., 2017; Liu et al., 2018; Sun et al., 2018; Pottle, 2019).

Imaging has also found its application in anatomy, where X-ray images have been used from the earliest studies of anatomy to the newer techniques of 3D modelling of the human body. New 3D modelling method has proven to be excellent in understanding interrelationships of organs and organ systems in anatomy. Over the past few years, advanced 3D modelling technology has provided the ability to create realistic printed models of organs, including the bones. Thanks to the precise scanners used for 3D scanning, the three-dimensional models are morphologically indistinguishable from the scanned part of the body. The three-dimensional models obtained in this way have a significant role in education, but also in scientific research (Vaccarezza, 2015). The printed models have significant educational value, where parts of the body can be viewed from all sides, and the interrelationship of organs does not have to be imagined by looking at a 2D image (Hadziomerovic et al., 2023). 3D printed models have shown advantages in anatomical education (Ventola, 2014; Smith et al., 2018; Smerling et al., 2019). In 2020, the results of meta-analysis showed that students who studied on three-dimensional models had better results on anatomy exams compared to students who used the traditional method of studying anatomy (Fleming et al., 2020).

The use of three-dimensional modelling has brought new approaches and technologies to the healthcare industry. Data obtained by CT or MRI can now be processed and converted into 3D models. 3D models can be printed. In addition to the application of 3D modelling for printing organs that will be used for educational purposes, 3D modelling is used for printing and manufacturing of customized prosthetics and implants (Beculic et al., 2023).

Scanning parts of the human body and saving 3D models enable the digitization of data that can be saved for a long period of time, unlike the biological materials. Also, parts of the human body are less accessible for study and scientific research, having their own duration and some important anatomical features destroyed with use. Therefore, their scanning and storage of 3D models enable permanent storage from which 3D models can be obtained over a longer period of time, which facilitates the process of learning and research (Ye et al., 2020).

Three-dimensional models of human body parts in anatomy are used for education and scientific research. All research on macroscopic morphological characteristics of organsperformed on organs themselves, is also performed on three-dimensional models of the examined organs (D'Errico et al., 2017). Thanks to the development of precise statistical programs, the application of three-dimensional models enables much more complex and precise research than classical methodologies used in research directly on parts of the human body.

The aim of this research was to use three-

dimensional models of human skulls for the analysis of gender differences in the area of the midsagittal line.

MATERIAL AND METHODS

The research was performed on 211 three-dimensional models of human skulls from the Bosnian population of known sex and age (139 male and 72 female skulls) after an Ethical approval by the Ethics Committee of the Medical Faculty (Number 02-3-4-2377). Three-dimensional models were obtained by scanning the skulls using a laser scanner (HP 3D Structured Light Scanner Pro S2 (DAVID SLS-2), resolution accuracy of 0.05%.). After setting up and aligning the scanner according to the manufacturer's instructions, three-dimensional models of the skulls were taken. The skull for scanning was placed on a rotating stand, and the number of scans to be made was set on a computer connected to the scanner. The distance of the object from the scanner is defined in the manufacturer's instructions and was determined through calibration on the rotating stand. The skull was placed in the Frankfurt horizontal plane and 15 scans were taken, and the stand was rotated 24˚ between two adjacent scans (24x15=360, 360˚- full circle). After scanning in the Frankfurt horizontal plane, the skull was placed on the right side to make 3 scans of the skull base and 1 scan of the skull roof named calvaria, and the process was repeated after the skull was placed on the left side. In this way, a total of 23 scans of the scanned skull were obtained. The scanner contains its own software for automatic or manual scan assembly.

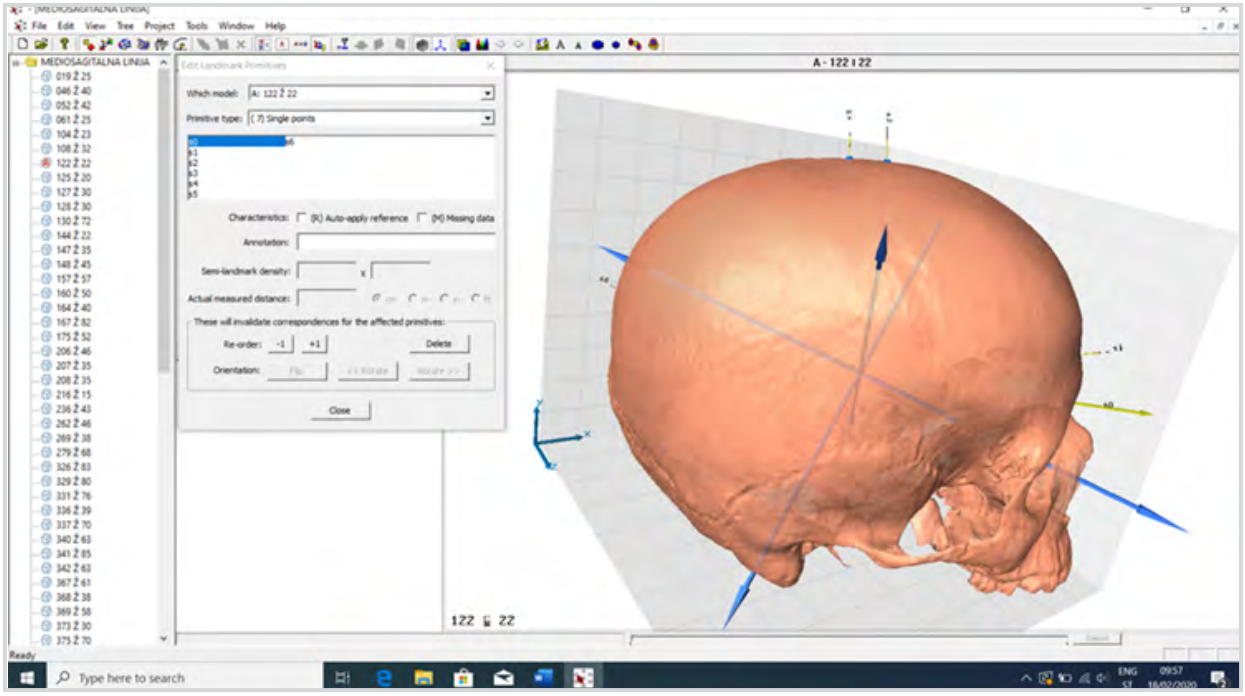

Figure 1 Designation of landmarks in Landmark Editor program on the midsagittal line

The position of the marked landmarks was used for:

1. Analysis of gender differences in the shape of the midsagittal line,

2. Analysis of gender differences of maximum sagittal diameter in the area of the midsagittal line. This diameter is named as maximum cranial length, and that is the distance between two landmarks, glabella and opisthion.

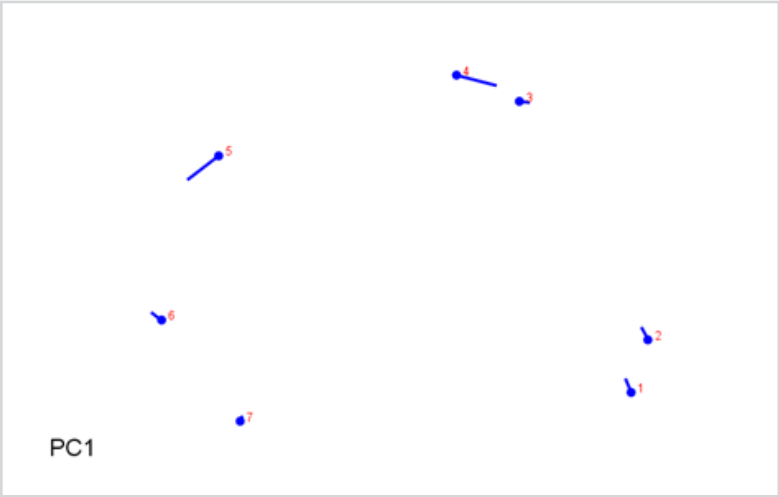

Figure 2 Position changes of landmarks on the midsagittal line described by principal component 1 (PC1) *Blue circles represent mean values of landmarks* Blue lines represent the direction and intensity of changes from the mean values of landmarks (1-nasion, 2-glabella, 3-bregma, 4-vertex, 5-lambda, 6-inion, 7-opisthion)

We provided manual scan assembly. The resulting scans were then cleaned of the artifacts, i.e. everything was removed from the scan except the skull scan itself. The scans were then assembled in such a way that clearly recognizable identical points were observed on two adjacent scans that were joined, which ultimately resulted in the assembly of the entire skull model from the captured scans. After merging the scans, a three-dimensional model was obtained. The procedure was repeated for all skulls from the tested sample. All skull models were given a uniform name, where the first three numbers indicate the serial number of the skull, in the middle is the letter M or Z which indicates the sex of the skull (M- male and Z-female), and the last two numbers indicate the age of the skull (for example 010 M 15). After obtaining three-dimensional models of all skulls from the examined sample, the models for each skull were saved in two formats, a .ply file and a .stl file.

On the three-dimensional models, seven nonpaired landmarks in the area of the midsagittal line were marked using Landmark Editor program (Wiley, 2005). Landmarks are: nasion, glabella, bregma, vertex, lambda, inion and opisthion (Figure 1).

RESULTS

Analysis of gender differences of midsagittal line shape on three-dimensional models of human skulls using geometric morphometric method

Position of the skulls based on the shape of the midsagittal line described by Principal component 1 (PC1) was presented on Figure 2.

Procrustes distances were calculated in MorphoJ program (Klingenberg, 2014). As classifier was entered sex, according to which the differences in the shape and size were analysed anda covariance matrix was generated. The analysis of the principal components (PCs) showed that the first two components (PC1 and PC2) described 52.918% of the total variability of the shape of the midsagittal line (Table 1).

Table 1 Eigenvalues and percentage of shape variability of the midsagittal line described by principal component analysis (PCs)

Number of PCs	Eigenvalues	% Variance	Cumulative %
1.	0.00117335	28.483	28.483
2.	0.00100656	24.434	52.918
3.	0.00070537	17.123	70.041
4.	0.00037235	9.039	79.080
5.	0.00033326	8.090	87.170
6.	0.00026760	6.496	93.666
7.	0.00013078	3.175	96.841
8.	0.00008526	2.070	98.910
9.	0.00002962	0.719	99.629
10.	0.00001526	0.370	100.000

Correct classification tests compare two groups. The test analyzes the mean values of two groups in the form of Procrustes distances or Mahalanobis distances.

Correct classification test was performed, and we analyzed the differences in the shape of the midsagittal line between the sexes. The calculated Procrustes distance was 0.0225, and the p value was less than 0.0001, which showed statistically significant differences between genders. Variability of the skull according to the shape of the midsagittal line defined by the first two principal components was presented on Figure 3.

Table 2 presented the results of correct classification test which correctly classified 92 out of a total 139 male skulls, i.e. as male skulls, which is 66.19% accuracy for male skulls. The correct classification test correctly classified 45 skulls out of a total 72 female skulls, i.e. as female skulls, which is 62.5% accuracy for female skulls.

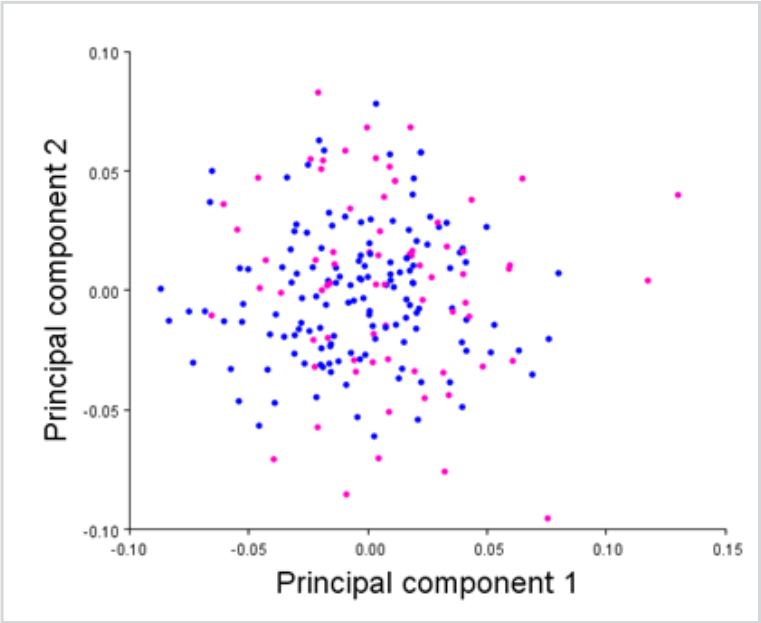

Figure 3 Variability of skulls according to the shape of the midsagittal line defined by first two principal components

Table 2 Results of correct classification test of sex using shape of the midsagittal line

Skulls	Male skulls	Female skulls	Total
Male skulls	**92**	47	139
Female skulls	27	**45**	72

Analysis of effect of size of midsagittal line on its shape using geometric morphometric method

A regression analysis was applied to examine the influence of size on the shape of the midsagittal line. The results showed an influence of 3.5216%. The results of regression analysis was presented in Figure 4.

The interval of the shape changes of the midsagittal line was presented on Figure 5.

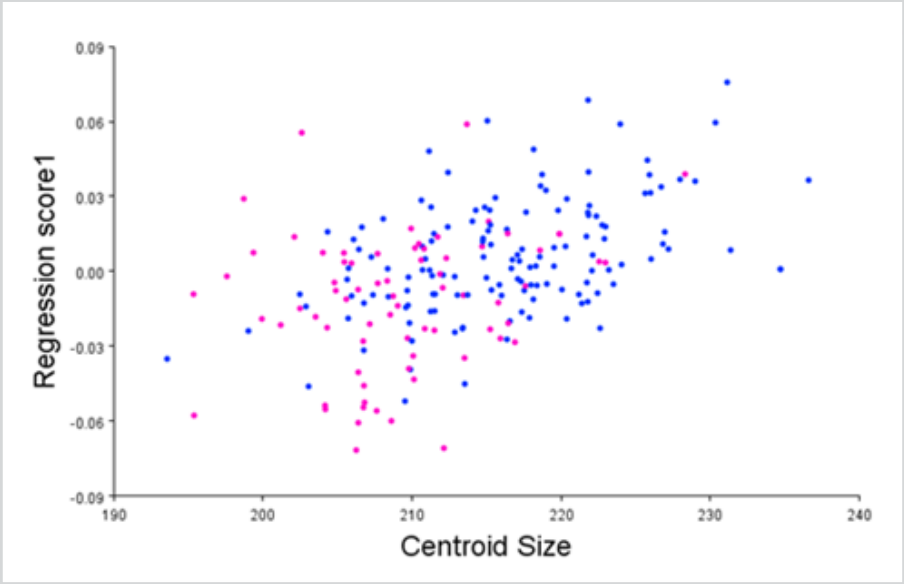

Figure 4 Influence of the size of a region in the area of the midsagittal line on sexual dimorphism of its shape

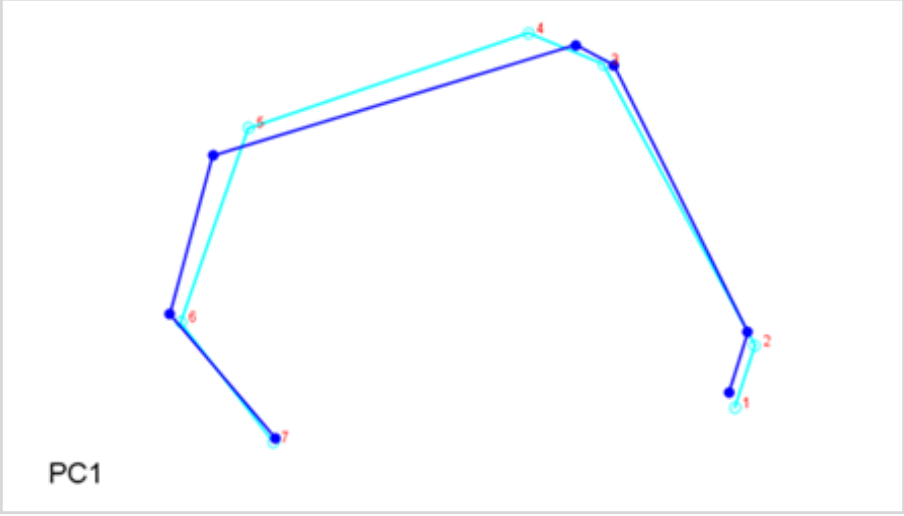

Figure 5 Iinterval of the shape changes of the midsagittal line

Analysis of sexual dimorphism of midsagittal line size on three-dimensional models of skulls using univariate analysis

Gender differences of the size of the midsagittal line on three-dimensional models were assessed by measuring linear diameter maximum cranial length on each three-dimensional model. Maximum cranial length is a diameter between landmarks glabella and opisthion.

Each landmark used to calculate the diameter was marked on three-dimensional models of the examined skulls. Landmark glabella and landmark opisthion have their x, y and z axis values in the coordinate system, based on which their mutual distances are calculated, which represent the linear diameter maximum cranial length (L1). The values of the maximum cranial length for all 211 three-dimensional models of the examined skulls were presented tabularly in Excel, after which statistical processing of the obtained data was performed.

Univariate analysis of gender differences of linear diameter L1 was performed in STATISTICA 10 program.

The results of univariate analysis of sex differences in the length of L1 diameter showed statistically significant sex differences (p value was less than 0.01), Table 3.

Table 3 Univariate analysis of sexual dimorphism of linear diameter L1 (glabella-opisthion)

	B	S.E.	Wald	df	p	Exp(B) DG	95% Confidence interval	
							GG	
L1	-1.641	0.325	25.434	1	**0.000**	0.194	0.102	0.367

DISCUSSION AND CONCLUSION

The study was performed on 3D models of 211 adult human skulls of known sex and age. The skulls belong to the Osteological Collection, Faculty of Medicine, University of Sarajevo. They belonged to individuals who lived on the territory of Bosnia and Herzegovina during the Second World War. One of the goals of this study was to scan the skulls of the osteological collection. Bone material has been exposed to damage over time and due to use. Some of the important anatomical features are missing or have fragmented over time. Therefore, the scanning of existing anatomically preserved skulls, whereby their three-dimensional models are obtained, enables their digitization and permanent preservation. In this way, the osteological material was digitized. Thanks to the precision of the scanner used (resolution accuracy of 0.05%), the obtained three-dimensional skull models are analogous to the recorded skulls. The obtained three-dimensional models can be used for scientific research, and they can also be printed, whereby the printed material can be used for education in anatomy and related disciplines.

In this study, three-dimensional models were used to analyze gender differences in the midsagittal line. In the first part of the research, geometric morphometry was used to analyze gender differences in the shape of the midsagittal line.

After the Procrustes superimposition, generation of the covariance matrix and the introduction of sex as a classification variable, a discriminant functional analysis was applied, which determined the prediction for the male gender with 66.19% accuracy and for the female gender with 62.5% accuracy. The size effect of this region on its shape was examined. Result showed a statistically significant effect of the size on its shape, and that was 3.5216% of the sex variability of the shape of a region in the area of the midsagittal line.

In the second part of the research, maximum cranial length was calculated on the three-dimensional models based on the x, y, and z axis values of two landmarks between which this diameter was measured.

After obtaining the values of the diameter of maximum cranial length for all three-dimensional models of the examined sample, the statistical processing of the data was started. The results of univariate analysis showed statistically significant differences in the length of this diameter between the sexes. Maximum cranial length is significantly longer in three-dimensional models of male skulls.

The area of the midsagittal line is the area of interest for a number of researchers who analyzed the gender differences.

In the investigation of sexual dimorphism of the midsagittal line, Bigoni et al. (2010) used a geometric morphometrics method. Gender determination based on the shape of the midsagittal line was possible with 100% accuracy for males and 98.4% accuracy for females. Our results showed a lower percentage of accuracy in determining sex (66.19% accuracy for the male gender and 62.5% accuracy for the female gender). In their study, the shape of the mediosagittal line was used, which was analyzed using semilandmarks that are more applicable for the analysis of the mediosagittal line, given that the mediosagittal line has only nonpaired anthropometric points in the mediosagittal plane.

We used a geometric morphometrics program based on the analysis of landmarks, not semilandmarks.

In a study conducted in 2016 on the skulls from the Greek population using geometric morphometry, the accuracy of sex determination based on the shape of the mediosagittal line was 68.8%, while the accuracy of sex determination based on the shape of the mediosagittal line (size and shape) was 79.4%. In this study, the authors used the same program as in our study, and the results are approximately equal to our results (Chovalopoulou et al., 2016).

The analysis of gender differences of maximal cranial length showed that there were statistically significant differences in the length of this diameter between the sexes. These results coincide with the results of other studies that conducted similar research.

Supporting the accurate and high-quality scanned three-dimensional models of the examined skulls were the results of this research in comparison with the results of the study conducted directly on the skulls from the examined sample. The results of the examination of gender differences of maximum cranial length of three-dimensional models showed statistically significant differences between the sexes, as did the results of the examination of the gender differences in maximum cranial length of the skulls that were scanned for the current study (Ajanovic, 2017).

Abdelnasser et al. (2017) used three-dimensional skull models obtained from postmortem CT scans of 87 skulls from Malaysia for sex determination. They measured 22 diameters on the obtained three-dimensional models. The results of the study showed that all examined diameters were longer in male skulls, except for the height of the orbit. The accuracy of gender determination was 85.1% in the mentioned research.

Results of the study performed on CT scans of the skulls of patients from Sudan showed that all linear diameters were larger on CT scans of male skulls then the same linear diameters on CT scans of female skulls (Altayeb et al., 2011).

Results of the study provided on the sample of the skulls from Brasilian population showed that all measured linear diameters were statistically significantly longer on male than on female skulls (Zavando et al., 2009).

The results of the studies conducted on skull samples from different populations showed that the linear diameters of the skulls were statistically significantly longer in male skulls compared to the same diameters in female skulls, which is in agreement with our results (Marinescu et al., 2014; Vidya et al., 2012; Saini et al., 2011; Lopez et al., 2009; Franklin et al., 2005).

The application of three-dimensional models

of the skulls for sex determination based on the midsagittal line showed statistically significant differences between the sexes in this region with similar results to research conducted directly on the skulls.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – ZA; Design – ZA; Supervision – ASH; Resources –ED, AL; Materials – ZA, UA; Data Collection and Processing – UA, HH; Analysis and Interpretation – ZA, ASH; Literature Search – LD; Writing Manuscript – ZA; Critical Review – ASH.

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TRODIMENZIONALNI MODELI LJUDSKE LOBANJE I NJIHOVA APLIKACIJA U ANALIZI SPOLNIH RAZLIKA SREDNJE SAGITALNE LINIJE

SAŽETAK

Razvoj tehnologije i različitih slikovnih metoda snimanja su doprinijeli razvoju znanosti u cjelini. Snimanje je pronašlo svoju primjenu i u anatomiji. Cilj ovog istraživanja je primjena trodimenzionalnih modela ljudske lubanje u analizi spolnih razlika srednje sagitalne linije. Istraživanje je provedeno na 211 trodimenzionalnih modela (3D) ljudskih lubanja pripadnika bosanske populacije (139 muške i 72 ženske). 3D modeli su kreirani skeniranjem lubanja korištenjem laserskog skenera. Na 3D modelima je obilježeno sedam neuparenih tačaka u području srednje sagitalne linije korištenjem Landmark Editor programa. Položaj obilježenih tačaka je korišten za analizu spolnih razlika u obliku srednje sagitalne linije i analizu spolnih razlika u maksimalnom sagitalnom dijametru u području srednje sagitalne linije (maksimalna kranijalna dužina, glabela-opistion). Izvršena je geometrijska morfometrijska analiza kako bi se procijenile razlike u obliku srednje sagitalne linije između spolova, a rezultati testa ispravne klasifikacije su pokazali statistički signifikantne razlike. Određivanje spola prema obliku srednje sagitalne linije je izvedeno sa preciznošću od 66.19% za muške lubanje i 62.5% za ženske lubanje ($p<0.001$). Rezultati regresijske analize su pokazali statistički signifikantan utjecaj veličine srednje sagitalne linije na njen oblik. Rezultati univarijantne analize su pokazali statisitički signifikantne razlike između spolova ($p<0.001$). Primjena trodimenzionalnih modela lubanja pri određivanju spola na osnovu srednje sagitalne linije je pokazala statistički signifikantne razlike između spolova.

Ključne riječi: 3D modeli, geometrijska morfometrija, spolni dimorfizam, lubanje, univarijantna analiza

RESEARCH ARTICLE

HISTOMORPHOMETRIC PARAMETERS AS INDICATORS OF NERVE TISSUE DAMAGE AFTER PERINEURAL AND INTRANEURAL APPLICATION OF LIPOSOMAL BUPIVACAINE IN RAT MODEL

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ABSTRACT

Liposomal bupivacaine is a long-acting local anesthetic agent and it is thought to be one of the safest local anesthetics. However, there has been detailing possible neurotoxic effects. At present, the exact molecular mechanism of liposomal bupivacaine-mediated neurotoxicity is unknown. We postulated that intraneural injection of 1.33% liposomal bupivacaine resulted in greater nerve injury than perineural injection, and this would be proved by objective quantitative histological analysis.

A rat sciatic nerve block model was used. The study was conducted in accordance with the principles of laboratory animal care and was approved by the Laboratory Animal Care and Use Committee. Thirty adult Wistar rats of both sexes were studied. After induction of general anesthesia, the sciatic nerve was exposed unilaterally. Sciatic nerves were randomly assigned by the method of sealed envelopes to recieve: 2 mL perineurally 1.33% liposomal bupivacaine, 2 mL intraneurally 1.33% liposomal bupivacaine, 2 mL perineurally saline or 2 mL intraneurally saline. Quantitative histological examination was followed to determine the potential damage to nerve tissue. All intraneural injections showed significantly smaller number of nerve fibers ($p<0.001$). There was no statistical significance in myelin thickness ($p>0.005$) and nerve fiber diameter ($p>0.005$) between the groups. Intraneurally administrated liposomal bupivacaine showed reduction in axon diameter ($p<0.005$) comparing to perineurally administrated liuposomal bupivacaine and saline.

Keywords: Liposomal bupivacaine, peripheral nerve blocks, neurotoxicity

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INTRODUCTION

Pain is a protective mechanism that has an adaptive value, and the inability to feel pain is associated with early mortality from accidental injuries or joint damage (Shuang and Leigh, 2022; Sandhu et al., 2021). However, the pain that came as a result of surgical intervention is not desirable. The benefits of optimal control of postoperative pain include: improved cardiac, respiratory and gastrointestinal functions, fewer thromboembolic complications, demonstrated longer duration of arterial grafts, fewer septic complications, lower incidence of developing chronic pain conditions, and reduced mortality in high-risk patients (den Bandt, 2019).

The application of local anaesthetic in the connective tissue around the nerve will cause a loss of sensation and / or paralysis in the area that the nerve innervates. Peripheral nerve stem injury results in varying degrees of injury and nerve fascicles. The decisive factor that determines the degree of injury and recovery after injury is axonal injury. After a peripheral nerve suffers an injury, complex pathophysiological, morphological, and metabolic changes occur at the site of injury. These complex changes also occur within the body of the nerve cell, but also proximal and distal to the site of nerve injury (Kadioglu, 2004; Park et al., 2019; Suaid Hen, 2022).

Liposomal bupivacaine is a prolonged-release formulation of bupivacaine, designed to allow drug diffusion for up to 72 h after a single application at the end of surgery (Sandhu et al., 2021). To date, the only approved indications for the clinical use of liposomal bupivacaine are postoperative wound infiltration after various surgical procedures and interscalenic blockade of the brachial plexus for postoperative analgesia. There is currently no experimental study in the world that would define the possible neurotoxic effect of liposomal bupivacaine. The aim of our study is to define the safety profile of liposomal bupivacaine using quantitative histological analysis of nerve tissue after perineural and intraneural application (Malik et al., 2017).

MATERIAL AND METHODS

After approval of the Ethics Committee of the Faculty of Medicine and Veterinary Medicine of the University of Sarajevo (02-3-4-2819/17) and by the Principles of Care and Preservation of Laboratory Animals (Bethesda, 1985), 30 adult Wistar rats of both sexes with an average weight of 300 g were included in the study. The animals were introduced to general anaesthesia by intraperitoneal injection of Nembutal - sodium pentobarbital (50 mg / kg), respecting the rules of strict asepsis. An incision was then made on the skin and gluteal muscle to access the sciatic nerve. All procedures were performed between 08.00 and 14.00.

Total of 60 nerves (30 rats) were randomly assigned to one of four groups (15 per group) using a computer-generated sequence with sealed envelopes. The first group received an injection of 4 ml liposomal bupivacaine 1.3% perineurally, the second group received 4 ml liposomal bupivacaine 1.3% intraneurally, the third group (control) received 4 ml saline intraneurally, whereas the fourth group (control) received 4 ml saline perineurally. The injections were performed under direct vision. Perineural injections were applied on the right side, while intraneural injections were applied on the left side. For perineural injections, the needle bevel was placed outside the external epineurium to inject liposomal bupivacaine around the nerve, whereas for intraneural injections, the needle was inserted under the external epineurium.

Insulated 27-G, 5-cm-long, long-beveled nerveblock needles (Terumo Europe NV, Leuven, Belgium) were used. Drugs were injected by an automated infusion pump (PHD 2000; HarvardApparatus, Holliston, MA, USA) at a 4 ml/min speed. Data were acquired with an in-line manometer (PG5000; PSI Tronics Technologies, Inc., Tulare, CA, USA) coupled to the computer via an analog-digital conversion board (DAQ card 6023; National Instruments, Austin, TX) and placed proximal to the needle in line with a non-distensible high-durometer polyvinylchloride injection tubing (2.1-m arterial pressure tubing, Abbott Critical Care Systems; Abbott Laboratories,

North Chicago, IL, USA). After application, the wounds were closed with a stitch.

After monitoring the animals for 3 days, the animals were euthanized with an overdose of sodium pentobarbital and potassium chloride. Samples of the examined nerves were then excised bilaterally (site of application, 1 cm proximal and distal to the site of application). After fixation in 10% formalin and dehydration in ethylene alcohol, the samples were treated with xylene as an intermediate and embedded in paraffin. Then, the tissue blocks were cut with a microtome (Leica „RM 2165) into incisions 3 - 4 μm thick. The sections were then passed through a series of alcohol of decreasing concentration and stained with the Hematoxylin Eosin method. After obtaining the appropriate sections and preparations, they were subjected to quantitative histological analysis, by light microscopy. Quantitative histomorphological analysis of all samples was performed by an experienced pathologist who did not know from which group of experimental animals the samples originated. Nerve fibrehistomorphometry was performed using a light microscope (Eclipse E400, Nikon) with a digital camera installed and a computer on which image processing and analysis software was installed (Modular software for interactive image processing and analysis - ELLIPSE Version 2, 0, 8, 1). Half of the preparations were selected from each group. Nerve field samples were selected as described by Geuna et al. (Geuna et al., 2001). To avoid potential errors in histomorphometry, we first divided the nerve into 12 large fields and then each large into 9 smaller ones. Only 1 of the 9 smaller fields were randomly selected (medium). In order to overcome the "marginal effect", a method based on the counting of fibres whose cross sections covered the upper and lower boundary of the observed field, was used (Geuna et al., 2004).

The parameters we determined in each selected field were: total number of nerve fibers (N), diameter of nerve fibers (D), axon diameter and myelin thickness, which was calculated by subtracting axonal diameter from the total nerve fiber diameter.

Statistical analysis

Total of 60 nerves were required to obtain relevant results to detect a significant difference in the proportion of nerve injury between intraneural and perineural injections $\alpha=0.05$. Statistical analysis was performed using SPSS program (Statistical Package for Social Sciences) version 19.0 (Chicago, IL, USA). For histomorphometry, a statistical comparison of the quantitative data was subjected to a one-way ANOVA test. The P value <0.05 was considered statistically significant. The results are expressed through the methods of standard descriptive statistics: mean ($\bar{X}$), standard deviation (SD), standard error of the mean (SEM), median (Me) and percentiles (25th, 50th and 75th), minimum (Min.) and maximum (Max.) value, absolute frequency (N) and relative frequency (%).

The Shapiro - Wilk test was used to estimate the normal distribution of continuous variables. The significance of the difference for the independent continuous variables that did not follow the normal distribution was tested by the Kruskal-Wallis test if there were more than two examined groups. The nonparametric Mann - Whitney U test was used to examine the differences between the two groups in cases where the data distribution deviated from the normal, while the Friedman test was used to examine the difference between repeated measurements within the group. Student's t-test for independent samples was used to examine the difference between the two groups. In order to examine the difference in the results of repeated measurements, the ANOVA multiple comparison test with post-hoc Bonferroni correction was used. The P value <0.05 was considered statistically significant.

RESULTS

All animals successfully completed the experiment and had uneventful post-surgical recovery and weight gain. There were no signs of local or systemic infection in any of the animals.

There were statistically significant differences in number of nerve fibers between intraneural liposomal bupivacaine and perineural saline

(p=0.002), and between perinaural and intraneural saline (p=0.039).

There were statistically significant differences in axon diameter between perineural and intraneural applied liposomal bupivacaine (p =0.017), and between intraneural liposomal bupivacaine and perineural saline (p=0.0001).

The groups did not differ in myelin thickness values and nerve fiber diameter (p>0.05).

Table 1 Number of nerve fibres in each group

Group	N		SD	SEM	95% CI*		Min.	Max.
					Lower limit	Upper limit		
l.b.intraneural	15.000	1.402	0.327	0.084	1.221	1.583	0.832	2.032
l.b.perineural	15.000	1.624	0.316	0.082	1.449	1.799	0.949	2.229
s.intraneural	15.000	1.521	0.394	0.102	1.303	1.739	0.999	2.506
s.perineural	15.000	1.870	0.311	0.080	1.697	2.042	1.416	2.458

l.-liposomal bupivacaine
s.-saline

Table 2 Differences in the number of nerve fibres between each group

(I) group	(J) group	Mean difference (I-J)	SEM	p	95% CI	
					Lower limit	Upper limit
l.b.intraneural	l.b.perineural	-0.222	0.124	0.463	-0.560	0.116
	s. intraneural	-0.119	0.124	1.000	-0.457	0.219
	s. perineural	-0.468(*)	0.124	**0.002**	-0.806	-0.130
l.b.perineural	l.b.intraneural	0.222	0.124	0.463	-0.116	0.560
	s. intraneural	0.104	0.124	1.000	-0.234	0.442
	s.perineural	-0.245	0.124	0.312	-0.583	0.093
s. intraneural	l.b.intraneural	0.119	0.124	1.000	-0.219	0.457
	l.b.perineural	-0.104	0.124	1.000	-0.442	0.234
	s. perineural	-0.349(*)	0.124	**0.039**	-0.687	-0.011
s. perineural	l.b.intraneural	0.468(*)	0.124	**0.002**	0.130	0.806
	l.b.perineural	0.245	0.124	0.312	-0.093	0.583
	s. intraneural	0.349(*)	0.124	**0.039**	0.011	0.687

l.-liposomal bupivacaine
s.-saline

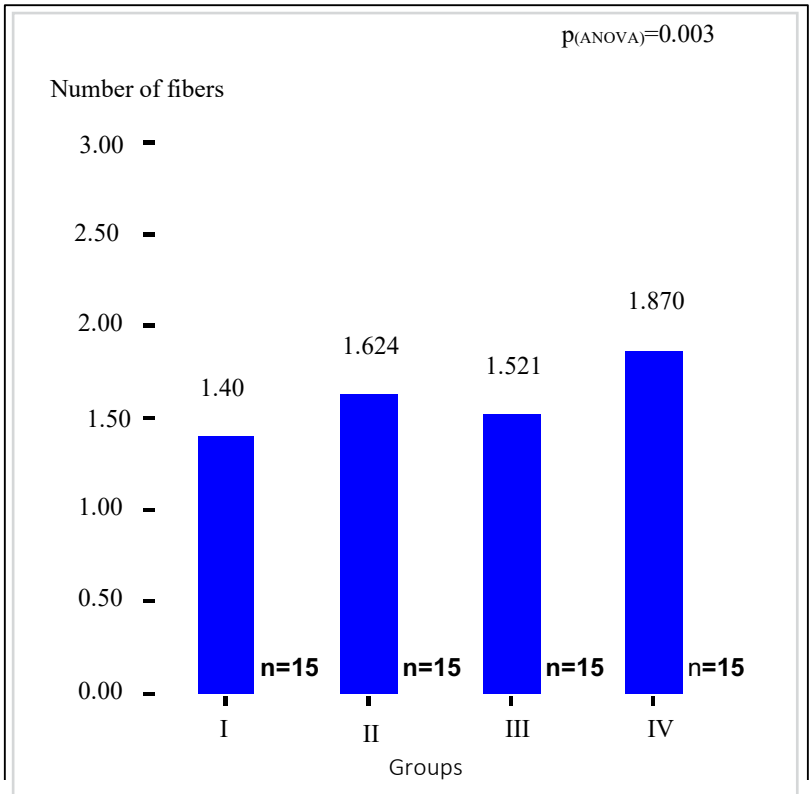

Figure 1 Values of the number of nerve fibres and differences between individual groups

I-liposomal bupivacaine intraneural; II-liposomal bupivacaine perineural; III-saline intraneural; IV-saline perineural

Table 2 Nerve fibre diameter

Group	N	*	SD	SEM	95% CI*		Min.	Max.
					Lower limit	Upper limit		
l.b.intraneural	15.000	6.168	0.471	0.122	5.907	6.429	5.491	6.906
l.b.perineural	15.000	6.359	0.370	0.096	6.154	6.564	5.901	7.339
s. intraneural	15.000	6.603	0.934	0.241	6.085	7.120	5.734	8.683
s. perineural	15.000	6.427	0.575	0.149	6.108	6.745	4.943	7.230

l.-liposomal bupivacaine
s.-saline

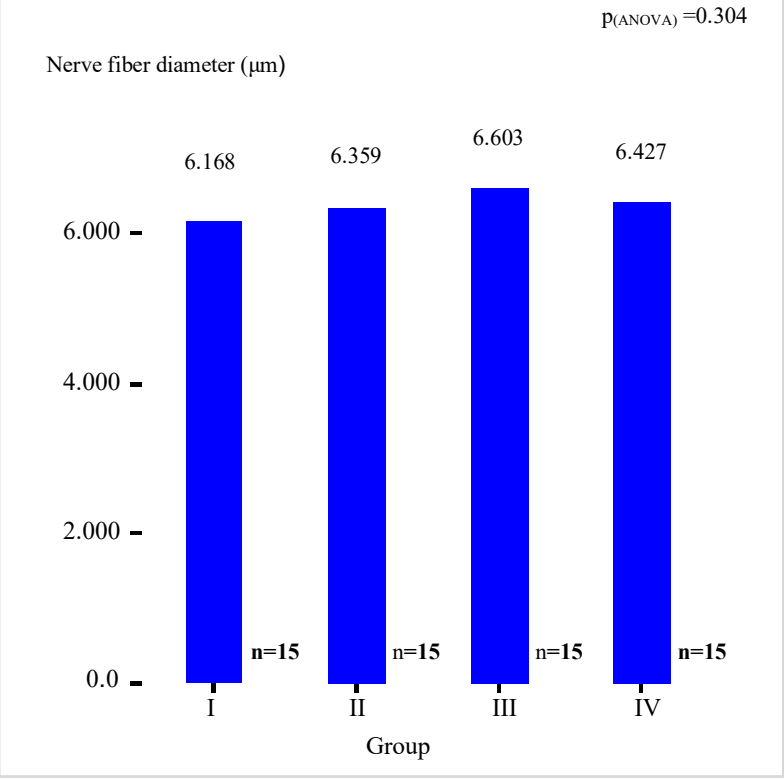

Figure 2 Nerve fibre diameter values and differences between individual groups

I-liposomal bupivacaine intraneural; II-liposomal bupivacaine perineural; III-saline intraneural; IV-salineperineural

Table 3 Axon diameter

Group	N		SD	SEM	95% CI*		Min.	Max.
					Lower limit	Upper limit		
l.b.intraneural	15.000	2.030	0.253	0.065	1.889	2.170	1.545	2.491
l.b.perineural	15.000	2.324	0.240	0.062	2.191	2.457	1.790	2.655
s.intraneural	15.000	2.482	0.613	0.158	2.142	2.821	1.809	3.933
s.perineural	15.000	2.549	0.323	0.083	2.370	2.728	1.956	3.005

l.-liposomal bupivacaine
s.-saline

Table 4 Differences in axon diameter between each group

(I) group	(J) group	Mean difference (I-J)	SEM	p	95% CI	
					Lower limit	Upper limit
l.b.intraneural	l.b.perineural	-0.294(*)	0.090	**0.017**	-0.548	-0.040
	s.intraneural	-0.452	0.171	0.089	-0.952	0.048
	s.perineural	-0.519(*)	0.106	**0.0001**	-0.819	-0.219
l.b.perineural	l.b.intraneural	0.294(*)	0.090	**0.017**	0.040	0.548
	fiz.intraneural	-0.158	0.170	0.920	-0.655	0.340
	fiz.perineural	-0.225	0.104	0.208	-0.520	0.070
s.intraneural	l.b.intraneural	0.452	0.171	0.089	-0.048	0.952
	l.b.perineural	0.158	0.170	0.920	-0.340	0.655
	fiz.perineural	-0.067	0.179	0.999	-0.583	0.448
s.perineural	l.b.intraneural	0.519(*)	0.106	**0.0001**	0.219	0.819
	l.b.perineural	0.225	0.104	0.208	-0.070	0.520
	fiz.intraneural	0.067	0.179	0.999	-0.448	0.583

l.-liposomal bupivacaine
s.-saline

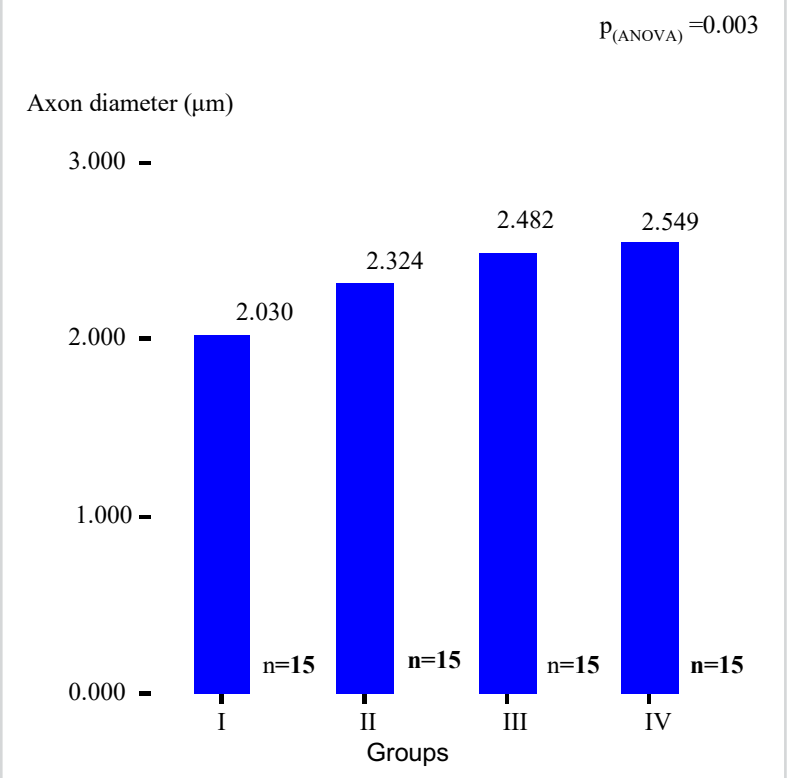

Figure 3 Axon diameter values and differences between individual groups

I-liposomal bupivacaine intraneural; II-liposomal bupivacaine perineural; III-saline intraneural; IV-saline perineural

Table 4 Myelin thickness

Group	N		SD	SEM	95% CI*		Min.	Max.
					Lower limit	Upper limit		
l.b.intraneural	15.000	2.039	0.212	0.055	1.921	2.156	1.675	2.306
l.b.perineural	15.000	2.025	0.183	0.047	1.924	2.126	1.830	2.373
s.intraneural	15.000	2.046	0.216	0.056	1.926	2.165	1.757	2.689
s.perineural	15.000	1.951	0.216	0.056	1.831	2.071	1.513	2.356

l.-liposomal bupivacaine
s.-saline

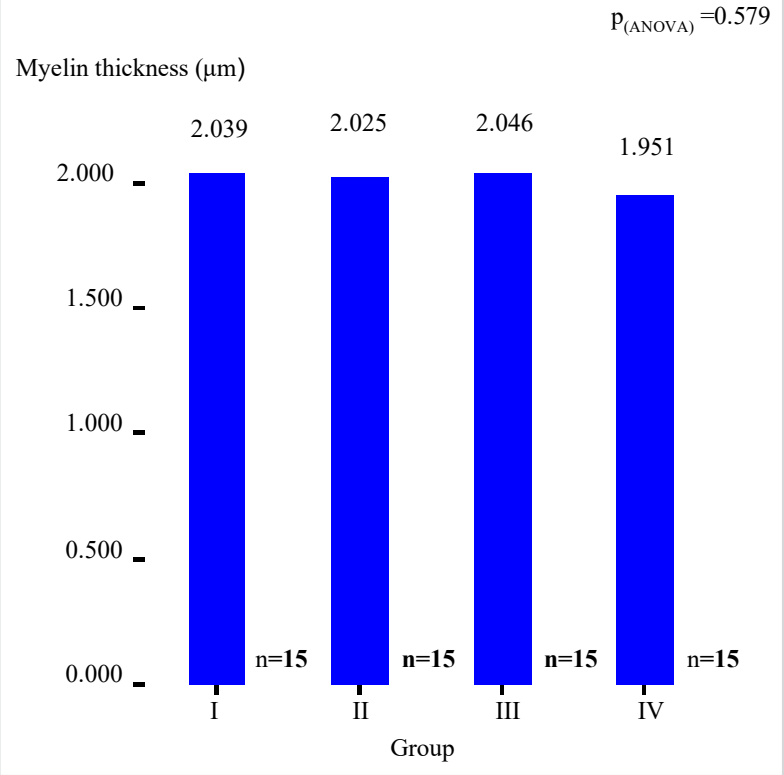

Figure 4 Myelin thickness values and differences between groups

I-liposomal bupivacaine intraneural; II-liposomal bupivacaine perineural; III-saline intraneural; IV-salineperineural

DISCUSSION AND CONCLUSION

Morphoquantitative stereological assessment of nerve fibres is a major research task in a number of biomedical disciplines, including neuroanatomy, neuropathology, neurosurgery, and reconstructive microsurgery. Nerve fibre histomorphometry has been shown to be particularly useful in studies of development, aging, regeneration, neurotoxicity, and various pathological conditions (Costa et al., 2013). Changes in the number of nerve fibres, especially the myelinated ones, are important for the assessment of various pathological nerve conditions, such as intoxication with some drugs. The use of histomorphometry in our study allowed us to objectively quantify the found structural changes in the nerves and identify minimal morphological differences in the examined groups.

In our study, we used an objective method of field selection on a nerve trunk, the Geuna method, which allowed us to have all fields within the nerves have equal chances of selection, that fibers had systemic distribution in different nerve areas, and ultimately had subjectivity in selection fields removed, so that we were given the most efficient approach to obtain relevant results of our study. The results of our study gave us a clear insight into the degree of nerve damage depending on the type of solution applied and the method of application.

The results of our study showed that there was a statistically significant difference in the number of fibres in the groups where liposomal bupivacaine was administered intraneurally and in the group where saline was applied perineurally. Decreased fibre number need not to be due solely to the neurotoxic effects of liposomal bupivacaine, as the same statistically significant difference in fibre number was found between the groups with intraneurally and perineurally administered saline. This finding may indicate that these lesions were due to mechanical trauma during intraneural application, because we found no difference in nerve fibre diameter and myelin thickness between the tested groups. The results of our study correlate well with the number of fibres after perineural and intraneural administration of traditional, already present formulations of local anaesthetics, which were found not to be neurotoxic after perineural administration in clinically permitted doses (Farber et al., 2013; Hasanbegović et al., 2013).

No statistically significant difference in nerve fibre and myelin thickness was found between the groups, which correlates with the results of Damjanovska et al. (2019), but also with the results of Zel et al. (2019), after subarachnoid application of liposomal bupivacaine and saline. However, the results of Damjanovska et al. show slightly higher absolute values of fibre thickness. We believe that this is due to the anatomical difference in fibre size between the nerve fibre of pigs and rats.

The statistically significant difference in axon diameter between intraneurally and perineurally applied liposomal bupivacaine may indicate a selective toxic effect of liposomal bupivacaine on nerve fibres. Perineurally administered liposomal bupivacaine has not shown significant differences comparing to saline in any of the histological parameters monitored so far, indicating its good safety profile.

Compared with the obtained histomorphometric results in our study, Damjanovska et al. found no differences in any parameters measured by histomorphometry after administration of liposomal bupivacaine intraneurally, ordinary bupivacaine HCl solution and saline (Damjanovska, 2015; Damjanovska, 2019). Fibre density and diameter and the ratio of axon diameter to myelin thickness were without significant differences between the examined groups.

Nevertheless, the Whitlock et al. showed similar changes and significantly reduced nerve fibre density after intraneural injection of ropivacaine in rat nerves, similar to our results. However, rats used in our and in the Whitlock study, because of their usually unifascicular structure, are more susceptible to injury than the polyfascicular nerve of pigs used in the Damjanovska study (Damjanovska, 2019; Whitlock et al., 2010).

In our study, the sciatic nerve was surgically exposed and isolated from the surrounding

connective tissue under direct visual monitoring. There is a possibility that this surgical isolation makes the nerve more susceptible to injury, because in everyday clinical practice the nerves are much more mobile in the surrounding tissue and there is less chance that the needle will actually penetrate the nerve during nerve blockade. The use of the results of our study in everyday clinical practice in performing peripheral nerve blockades would be important for patients and for the health system in general. Determining the number of myelinated nerve fibres and the thickness of individual nerve fibre ultrastructure provided us with a detailed and objective insight into the actual condition of the examined nerves after perineural and intraneural application of different test solutions. The size and number of nerve fibres provided a good basis for comparing fibres within and between experimental groups. Liposomal bupivacaine, compared to other short-acting anaesthetics, is a good choice for perioperative and intraoperative regional anaesthesia and analgesia. Providing a longer duration of anaesthesia shown in clinical studies in humans, liposomal bupivacaine is a superior, better and more comfortable choice for the patient, because it is adequately administered and devoid of neurotoxic effects.

In the course of numerous experimental sciatic nerve injuries, it has been shown that there are numerous deficiencies in the assessment of the neurological functions of laboratory animals. All this led to a greater interest in quantitative histological analysis, which proved to be an objective, reliable method to assess the venous toxic effect of local anesthetics on nerves. After the injury, the animal had its back extremity paralyzed and often bit its own limb, which could have led to the amputation of the toes on the injured extremity. This behavior and joint stiffness reduce the reliability of functional tests, such as assessment of sciatic nerve function. In some cases, the researcher may be willing to exclude the animal from the research, due to ethical reasons and the welfare of the animal itself. Henceforth, in awake animals it is very difficult to assess the function of the sciatic nerve after injury or it is very limited (Navarro, 2016). In our study, quantitative histological analysis enabled us to objectively assess and quantitatively describe the microstructures of nerve fibers, their size, and the parts of the endoneural space that occurred during the research.

Rats and mice are the most frequently used laboratory animals for evaluating the toxic effects of local anesthetics. However, when rats or mice are used in such research, we must be aware of the distinct differences that exist in the structure of the sciatic nerve of laboratory animals and the human sciatic nerve: (1) the voids that occur during the application of anesthetics are smaller than those that occur during sciatic nerve injuries in humans; (2) axonal regeneration occurs more rapidly in rats than in humans; (3) after the occurrence of an injury in rats, recovery is usually complete after some time, which is not the case in humans (Kaplan et al., 2015).

On the basis of these preclinical data, we conclude that liposomal bupivacaine poses no risk beyond that of classical local anaesthetics that are commonly used in everyday clinical practice. Morphometry enabled us to describe structural changes after intraneural and perineural application of 1.33% liposomal bupivacaine in quantitative terms and in particular revealed to us minimal morphological differences between the states of function.

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HISTOMORFOMETRIJSKI PARAMETRI KAO INDIKATORI OŠTEĆENJA NERVNOG TKIVA NAKON PERINEURALNE I INTRANEURALNE APLIKACIJE LIPOZOMALNOG BUPIVAKAINA NA MODELU ŠTAKORA

SAŽETAK

Lipozomalni bupivakain je dugodjelujući lokalni anestetik koji se smatra jednim od najsigurnijih lokalnih anestetika za koji su, međutim, opisani i mogući neurotoksični efekti. Trenutno nije poznat tačan molekularni mehanizam neurotoksičnosti posredovane lipozomalnim bupivakainom. Pretpostavili smo da intraneuralna injekcija 1.33% lipozomalnog bupivakaina rezultira većim oštećenjem nerva u odnosu na perineuralnu injekciju, što smo i dokazali objektivnom kvantitativnom histološkom analizom. Kao model je korištena blokada ishijadikusa kod štakora. Istraživanje je izvedeno u skladu sa principima zaštite laboratorijskih životinja i odobreno je od Odbora za zaštitu i korištenje laboratorijskih životinja. Istraživanje je obuhvatilo trideset odraslih Wistar štakora obaju spolova. Nakon uvođenja u opću anesteziju, unilateralno je isprepariran ishijadični nerv kojemu je randomizacijom metodom zapečaćenih koverti za aplikaciju dodijeljeno: 2 ml 1.33% lipozomalnog bupivakaina perineuralno, 2 ml 1.33% lipozomalnog bupivakaina intraneuralno, 2 ml fiziološke otopine perineuralno ili 2 ml fiziološke otopine intraneuralno. Nakon toga je izvršen kvantitativni histološki pregled kako bi se odredilo moguće oštećenje nervnog tkiva. Kod svih intraneuralnih injekcija je postojao signifikantno manji broj nervnih vlakana ($p<0.001$). Nije postojala statistički signifikantna razlika među grupama u debljini mijelina ($p>0.005$) i prečniku nervnih vlakana ($p>0.005$). Intraneuralno apliciran lipozomalni bupivakain je pokazao smanjenje prečnika aksona ($p<0.005$) u odnosu na perineuralno apliciran lipozomalni bupivakain i fiziološku otopinu.

Ključne riječi: Blokada perifernog nerva, liposomalni bupivakain, neurotoksičnost

RESEARCH ARTICLE

PROBIOTIC ENEMA PROTECTS INTESTINAL MUCOSA AND ALTERS PLASMA DIAMINE OXIDASE ACTIVITY AMONG CALVES WITH DIARRHEA

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ABSTRACT

Diamine oxidase (dAo) (alternatively histaminases,in under the old denomination), as a natural enzyme present in high levels, promotes the integrity and maturation of the small intestinal mucosa. In the present study, we sought to investigate whether the plasma levels of diamine oxidase activity denote mucosal injury during diarrhea among calves, and we attempted to use rectal enema probiotic therapy against it. Upon arrival at a private and commercial milk-fed calf facility, calves were subjected to scoring on fecal consistency on a scale from 0 to 3. Calves exhibiting a fecal score of 2 (loose feces)-or 3 (watery feces) were enrolled as diarrheic. As solely calves with diarrhea were enrolled. All diarrheic calves received rectal enema multi strain probiotic treatment (Farm Rumin Probiotic Powder). The mean dAo levels (ng/mL) among diarrheic calves before and after probiotic enema treatment were detected as 8.48±1.67 and 28.06±3.51, respectively, presenting statistically significant alterations ($p<0.001$). In summary, it should not be unwise to draw a preliminary conclusion that plasma dAo activity was decreased in response to intestinal mucosal injury in relationship with diarrhea, which was reversed with rectal enema probiotic treatment for 10 days, reflecting a feed-back regulation of dAo activity connected with mucosal recovery, as was proposed.

Keywords: Bovine health, growth performance, intestinal barrier function, intestinal biomarker

INTRODUCTION

With the development of novel gastrointestinal and antimicrobial drugs also involving molecular targeted drugs the usage of several uncontrolled and random drugs has been increasing. However, gastrointestinal mucosal damage might be conducted through administration of several drugs, being capable of consequently causing gastrointestinal toxicity among calves. There have been no data on the relationship with polypharmacy among calves experiencing diarrhea and/or other relevant gastrointestinal clinical signs. However, several drugs could influence diamine oxidase (dAo) activity, with an old-fashioned article reporting that the antimalarial drugs (amodiaquine, quinacrine and chloroquine) could be capable of inhibiting the catabolism of putrescine, which was attributed to action of the latter drugs on dAo (Ma and Sourkes, 1980). Another study denoted that chloroquine, clavulanic acid, cimetidine, verapamil isoniazid, metamizole, acetyl cysteine, amitriptyline, diclofenac, metoclopramide, suxamethonium and thiamine have varying degrees of inhibition potential efficacy of interaction on dAo (Leitner et al., 2014). There exist no relevant data on the efficacy of commonly used drugs among calves, even if interacting with dAo (however, our subsequent study would thus be aimed at this issue, probably). Neonatal diarrhea, a significant economic concern in the cattle industry, leads to mucosal inflammation and villous atrophy in calves, resulting in dehydration and electrolyte imbalances (Mosier and Oberst, 2000; Smith, 2009). While many aforementioned treatments rely on indirect markers, the state of the intestinal mucosa is still often overlooked. Diamine oxidase is a well recognized cytoplasmic enzyme present dominantly within the small intestine villi, playing a pivotal role in the degradation of histamine (Kitanaka et al., 2002). As well recognized data indicate, dAo acts within the mucosa, mostly in the top villi, while its activity occurrs increasedly in the small intestine (Biegański, 1983). There has been a pragmatic relationship between plasma dAo activity and the matureness/unity of the intestinal mucosa (Wolvekamp and de Bruin, 1994). Taking into account the latter data, as mucosal damage intensified, mucosal/plasma dAo activity exhibited decline. It should not be unwise to mention that regarding the prior investigations, plasma dAo activity plays a pivotal role in predicting intestinal mucosal injury (Tanaka et al., 2003; Miyoshi et al., 2015) and in rats (Akimoto et al., 2006). Fukuta et al. (2019a) indicate that plasma dAo activity might serve as a reliable biomarker for assessing intestinal mucosal disorders stemming from diarrhea.

In the present study the objectives were (1) to examine the action of rectal enema probiotic containing multistrain probiotic bacterial species, (2) to approve it for the treatment of unclassified diarrhea, at least clinically (as a substitute to frequently used empiric antibiotic therapy), and (3) to examine whether the serum dAo activity in calves is related to diarrhea responding to probiotic enema treatment.

MATERIAL AND METHODS

Approval of the ethics committee

This retrospective field study was performed at a commercial milk-fed calf facility in the Eagean Region of Türkiye and was approved by the local ethic committee of Aydın Adnan Menderes University Ethical Commitee on 27/10/21 under ref. no. 64583101/2021/146. All participated calves were enrolled in the present research with a written owner's consent.

Study design

In a total of 8 calves participating, 0.5 ml blood was drawn from *V. jugularis* into anticoagulated tubes. Following centrifuge, plasma was seperated. Commercially available dAo ELISA kit: Bovine Diamine Oxidase ELISA Kit (My Biosource, San Diego, United States) was purchased by RDA Group, Istanbul and became available. The latter assay exhibits elevated sensitivity and excellent specificity for detecting dAo. There has been no known cross-reactivity/interference between dAo and analogues, as was previously described. Plasma samples were analyzed with an available

assay Quantitative Competitive via Sandwich ELISA. Sensitivity was1.0 ng/mL with a detection range between 0.312-20 ng/mL. All samples were stored at suitable degree prior to analytes, and all reagents were kept at 2–8 degree C. Fecal scoring system was previously described (Graham et al., 2018) (Table 1). Diarrhea was unclassified due to the limited financial budget.

Table 1 Fecal scoring system adopted (Graham et al. 2018)

Fecal Score	
0	normal consistency
1	semiformed or pasty
2	loose feces
3	watery feces

Rectal enema probiotic usage

Rectal probiotic enema was shown in Figure 1. For this purpose, probiotic support (Prebio, Farm Rumin, Turkey) was applied by the way of the rectum roughly with a catheter once a day during 10 days . This product contains *Lactobacillus plantarum* (1k1604) 5 x 109 CFU, *Sacchoromyces cerevisiae* (CNCM-1-1077) (4b1711) 1 x 109 CFU, fermentated product of *L. acidophillus*, *L. helveticus*, *L. fermentum* 15 x 109 CFU, a protein-rich product derived from *Bacillus subtillis* 5 x 109 CFU. In addition to above content, the supplement also includes 470 mg/packet inulin, 20 mg/packet colloidal silica (E 551b) and 60 mg/packet calcium propionate (1a282).

Statistical analysis

Descriptive statistics was utilized to analyze the obtained data, and the resulting values were presented as the mean and standard error. The Mann-Whitney analysis was performed to compare the diamine oxidase levels between the different groups. Graphpad Prism software (v. 9.2, America) was used for conducting the statistical analyses and generating the graphs. Statistical significance was defined as situations where the p-value was less than 0.05.

RESULTS

All tabulated data along with statistical values were given in Figure 2 and Table 2 with box plot analytes. During ELISA analysis there was no error, and all samples were investigated. A well-educated laboratory staff performed ELISA and followed the instructions from the commercial kits. Mean dAo (ng/mL) values prior to (BT) and after treatment (AT) showed statistical significance ($p<0.01$).

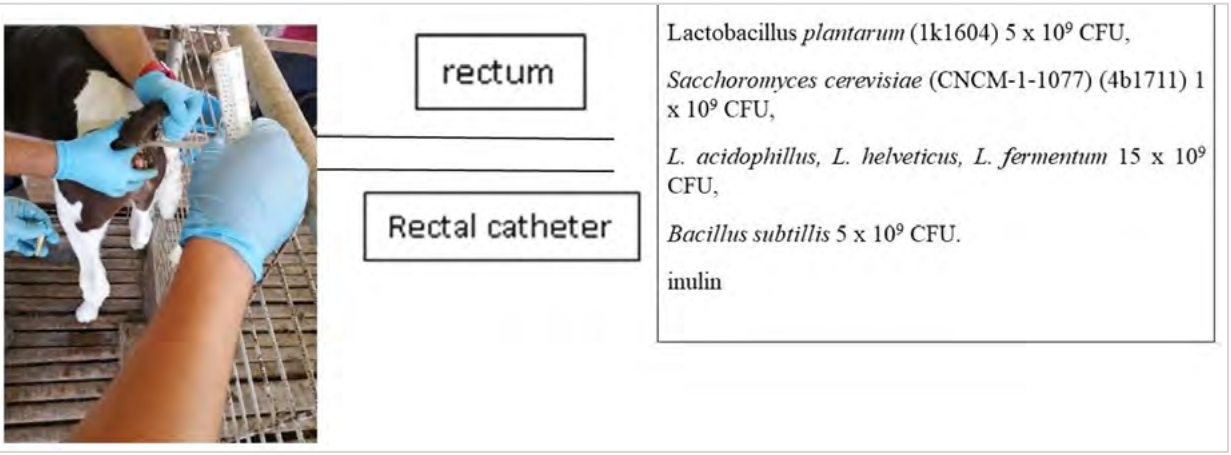

Figure 1 Photographic record and schematic representation of rectal enema probiotic (Farm Rumin) administered 15 cm within the rectum for each calf enrolled

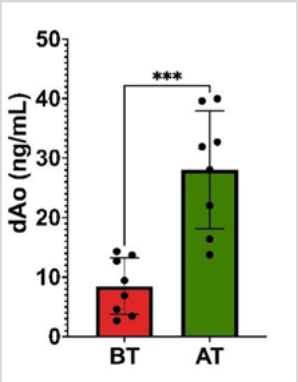

Figure 2 Box plot analytes for mean dAo values before (BT) and after treatment (AT)

Table 2 Statistical analytes for mean dAo values

	BT ($\bar{x}$)	AT	p value
	± SE	± SE	
dAo (ng/mL)	8.48 ± 1.67[a]	28.06 ± 3.51[b]	0.001

[a,b]: Values indicated with different letters on the same line are statistically significant. BT: before treatment, AT: after treatment.

DISCUSSION AND CONCLUSION

Diamine oxidase is expressed specifically within the intestine, kidney and placenta, where it is kept in vesicular structures for secretion activity (Luk et al., 1980; Schwelberger et al., 1998; McGrath et al., 2009). dAo is conveyed and stored in epithelial cells villi along the intestine, constituting a primary defense barrier against dietary histamine (Schwelberger et al., 1998). On the other hand, pending whether the existing intestinal dAo activity sufficiently degrades the selected amount of histamine, it can translocate into the circulation resulting with histamine-related clinical signs (Schwelberger et al., 1998; McGrath et al., 2009). The latter data should be discussed in depth. Histamine that is released within the gastrointestinal system might be quickly subjected to detoxification via amine oxidases in health, and it could also be produced by epithelial cells (Wagner et al., 2003). Hence, acid-induced alterations of epithelial barrier function induce translocation of histamine from the gastrointestinal route to the bloodstream (Aschenbach and Gabel, 2000).

In a prior research aimed to determine plasma dAo activity alterations, an attempt was made to mirror the severity of intestinal mucosal disorder in 36 out of 50 Holstein calves with diarrhea (14 calves without diarrhea were assigned to the control group). The plasma dAo activity was markedly diminished ($p < 0.01$) in the calves with severe or moderate diarrhea in contrast to control group and was significantly decreased ($p < 0.05$) in the severe group in comparison to the moderate group. Obtained results denoted that plasma dAo reflects the degree of intestinal mucosal disorder in association with diarrhea (Fukuda et al., 2019a). Another interesting article seeked the association among serum dAo activity, postnatal days and the plasma copper (Cu) values among diarrheic and healthy calves. In healthy calves, the serum dAo activity was markedly higher at 2 Postnatal Day in contrast to ≥ 7 Postnatal Day, without any significant alterations detected after 7 Postnatal Day. The serum dAo activity in 14 diarrheic calves (66.78 ± 14.37 IU/ml) was diminished significantly in contrast to 19 healthy calves (170.33 ± 97.83 IU/m, $p<0.01$) (Fukuda et al., 2020). In the present study, the mean dAo levels (ng/mL) among diarrheic calves before and after probiotic enema treatment were detected as 8.48 ± 1.67 and 28.06 ± 3.51, respectively, presenting statistically significant alterations ($p<0.001$). Altered dAo levels following rectal enema probiotic treatment could reflect mucosal healing and mucosal integrity restoration among calves enrolled.

Twenty-two diarrheic Japanese black calves, equally divided, received probiotics (n=11) or antibiotics (n=11) treatment limited to 8 days in which serum dAo activity markedly elevated only in probiotic treatment (64.4 ± 7.2 on Day 1 vs. 76.3 ± 5.1 IU/ml on Day 8). According to the latter study, probiotics could be capable of influencing serum dAo activity in diarrheic calves (Fukuda et al., 2019b). This finding was also supported by our study, as decreased dAo levels were restored and returned to elevated levels in contrast to the prior era of probiotic enema treatment.

Our valuable field data suggested that multistrain probiotic enema treatment involving *L. plantarum, S. cerevisiae, L. acidophillus, L. helveticus, L. fermentum* and *B. subtillis* could have easily substituted empiric antibiotic therapy and polypharmacy, specifically in non-bacterial infectious diarrheic calves. Some might speculate as to why, as an etiological algorithm was not available, we preferred probiotic usage. This may be briefly explained by our preliminary field investigation and fecal smear cytology (which was not necessary to be reported) providing us with preliminary evidence of proof of bacterial origin of enteritis. Furthermore, we also noted that serum dAo activity decreased in calves with diarrhea, in which rectal enema probiotic usage could have reversed serum dAo activity and cause its increase, Further warranted research is required in larger calf populations in an attempt to extrapolate the association between intestinal mucosal damage and serum dAo activity in diarrheic calves.

Following injury to the intestinal mucosa capable of affecting gut microbiota in relationship with the existence of great amounts of D-lactate, it could quickly send it off to circulation because of elevated intestinal permeability (Ficek et al., 2017). Furthermore, intestinal mucosal damage and elevated intestinal permeability influence dAo activity (Meng et al., 2016). In a prior study, D-lactate and dAo were among the selected biomarkers of intestinal permeability (Wu et al., 2019). In that study, the levels of D-lactate and dAo, both in the observation and control groups, were significantly diminished after treatment in contrast to prior values ($p<0.001$). According to that study, probiotic combination therapy reversed the stress response and intestinal permeability of term neonates (Wu et al., 2019). In the present study, restored dAo values could easily be attributed to altered intestinal permeability, toward which our subsequent study would be directed.

In conclusion, serum dAo activity sensitively indicated gastrointestinal mucosal damage prior to diarrhea onset (probably) and could be a useful biomarker of intestinal mucosal damage. Rectal enema probiotic usage could be capable of reversing this damage. Furthermore, decreased fecal scoring obtained could also reflect the efficacy of probiotic enema treatment and could be in relationship with altered dAo levels.

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The first (DAU) and the last authors were the second and first advisor, respectively, of the PhD student (ET).

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – KU, DAU.; Design – KU, DAU.; Supervision – KU, DAU.; Resources –HE, SE, NK, ET.; Materials – HE, SE, NK, ET.; Data Collection and/or Processing – HE, SE, NK, ET.; Analysis and/or Interpretation – KU, DAU, HE, NK, ET.; Literature Search – KU, SE.; Writing Manuscript – KU, DAU, HE, SE, NK.; Critical Review – KU, DAU.

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PROBIOTIČKA ENEMA ŠTITI INTESTINALNU SLUZNICU I SMANJUJE AKTIVNOST PLAZMATSKE DIAMINOOKSIDAZE KOD TELADI SA DIJAREJOM

SAŽETAK

Diaminooksidaza (DAO) (stari naziv histaminaza) je prirodni enzim prisutan u visokim koncentracijama koji djeluje na cjelovitost i sazrijevanje sluznice tankog crijeva. Cilj našeg istraživanja je ispitati da li aktivnost diaminooksidaze odražava stepen oštećenja crijevne sluznice kod teladi sa dijarejom i mogućnost upotrebe rektalne probiotičke eneme u liječenju iste. Nakon dolaska u privatni komercijalni objekt za uzgoj teladi na prehrani mlijekom, telad su ocijenjena prema konzistenciji fecesa na skali od 0 do 3. Telad ocijenjena sa rezultatom 2 (rijedak feces) ili 3 (tečan feces) su uključena u istraživanje kao telad sa dijarejom. Sva telad sa dijarejom su primila rektalnu enemu koja je sadržavala višesojnu probiotsku terapiju (Farm Rumin Probiotic Powder). Srednje vrijednosti DAO (ng/mL) kod teladi sa dijarejom prije i nakon terapije enemom su iznosile 8.48±1.67 i 28.06±3.51 i označene su statistički signifikantnim ($p<0.001$). U sažetku, nije nerazumno preliminarno zaključiti da je aktivnost plazmatske DAO snižena kao odgovor na oštećenje intestinalne sluznice kod dijareje koja je izliječena s enemom probiotika apliciranom rektalno u trajanju od 10 dana. Ovim se oslikava povratna regulacija DAO aktivnosti povezana s oporavkom sluznice, kao što je i pretpostavljeno.

Ključne riječi: Funkcija intestinalne barijere, intestinalni biomarker, performansa rasta, zdravlje goveda.

RESEARCH ARTICLE

ANATOMY OF THE AXIAL AND PELVIC LIMB BONES OF THE WEST AFRICAN BLACK-CROWNED CRANE *(BALEARICA PAVONINA PAVONINA)*

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ABSTRACT

The study presented gross morphological features of the axial and pelvic limb bones of adult black-crowned crane. The bones were macerated using a standard technique and structural details of the processed bones were highlighted. The skull comprised of the splanchnocranium and neurocranium, separated by a large bony orbit. The mandible presented a rostral dental bone having minute foramina and a caudal supra-angular bone. The cranial segment of the vertebral axis consists of fifteen cervical and seven free thoracic vertebrae. The caudal portion of the vertebral column fused into a single bony column comprising of notarium, synsacro-lumbar, three-fused primary sacral, and fused three synsacro-caudal vertebrae, respectively. There were six free coccygeal vertebrae, the last presented the pygostyle. The pelvic girdle was formed by the osseous fusion of the ilium, ischium, and pubis. The maximum length of the femur was 10.5 cm, whereas the tibiotarsus was 24.5cm. The tarsometatarsus comprised of fused metatarsal bones II, III, IV which articulates with the distal row of tarsal bone. There were four functional digits in black-crowned crane. The first digit consists of two phalanges, the second and third digits presented three and four phalanges and the fourth digit consists of five phalanges.

Keywords: Gross anatomy, skull, axial, pelvic limb bones, black-crowned crane

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INTRODUCTION

The Black-crowned Crane *Balearica pavonina* inhabits the Sahel and Sudan Savanna regions of Africa (Edet et al., 2018). There are two Black-crowned Crane subspecies: the West African Crowned Crane (*Balearica pavonina pavonina*), which occupies the western part of the Sahel, from Senegal to Chad and the Sudan Crowned Crane (*Balearica pavonina ceciliae*) that lives in Eastern Africa, with its largest concentration in Sudan (Boere, 2006). Black crane birds had been classified as near-threatened (IUCN, 2006). The gross morphology of the sternum, pectoral girdle and wing bones of the Black-crowned Crane has been described recently (Girgiri et al., 2022). Earlier, Hiragi et al (2014) documented the vertebral formula in red-crowned and Hooded Crane. The present study was aimed at describing the gross morphology of skeleton of the Black crowned Crane with emphasis on the axial, pelvic girdle and pelvic limb bones.

MATERIALS AND METHODS

The cadaver of an adult black-crowned crane bird (*Belearica pavonina pavonina*) was collected from the Department of Veterinary Pathology, University of Maiduguri following a postmortem examination and was processed for gross anatomical studies. The axial, pelvic girdle and limb bones were macerated and processed, as described previously (Girgiri et al., 2022). Morphology and structural details of the processed bones were studied, and metrical dimensions of long bones were measured in centimeters (cm). Photographs of the bones at different anatomical planes were taken using Nikon D90 digital camera.

RESULTS

Skeleton of head

The skull of Black-crowned Crane comprised of the splanchnocranium and neurocranium, separated by a large bony orbit. The facial part of the skull, which constituted the splanchnocranium, comprised of bones that formed movable articulation with one another and with the neurocranium. The paired premaxillary bone formed the rostral portion of the upper beak and presented three processes. The frontal process of the premaxillary bone formed the dorsal border of the nasal aperture, and extended caudally, where it fused with the nasal and frontal bone. The maxillary process constituted the basal border of the nasal aperture, whereas the platinum formed the basal plate (Figure 1A-C). The lacrimal bone was small and formed the nasal border of the eye orbit, dorsally fusing with the frontal bone. The paired platinum bone flanked the vomer and extended rostrally, where it fused with the processes of the maxillary bone and caudally with the pterygoid and basihyoid bone. The zygomatic bone was rod-like, and extended caudally for articulation with the quadrate bone. The pterygoid bone was short and situated between the palatine, vomer, and the quadrate bones (Figure 1A). The nasal aperture presented a triangular outline, the base of which was formed by the frontal process of the intermaxillary bone (Figure 1A).

The neurocranium was formed by the fusion of occipital, sphenoid, temporal, parietal and the frontal bone, respectively (Figure 1A-B). The occipital bone was situated basally and constituted the caudal surface of the skull. This bone presented a dorsal (supraoccipital), squamous, bilateral part and basal part which surrounded the large foramen magnum (Figure B-C). A small but prominent hemispherical occipital condyle was present at the basioccipital bone close to the margin of the foremen magnum. A jugular process was present caudolaterally to the foramen magnum (Figure 1C). The sphenoid bone was situated at the base of the skull having two distinct parts. The basisphenoid was larger, elliptical with a centrally located body and a pair of temporal wings. It articulated with the basilar part of the occipital bone caudally and extended rostrally to articulate with the presphenoid. The presphenoid articulated with the temporal wing of the basisphenoid caudolaterally and was lined by the paired palatinum bones rostrally (Figure 1C). The temporal bone formed the caudoventral portion of the lateral wall of the skull. It consisted of the ear capsule and the

squamous portion. The ear capsule articulated with the parietal, lateral part of the occipital and the basisphenoid bones, respectively. The squamous temporal contributed to the formation of the lateral wall of the skull and presented the orbital process dorsal to the temporal fossa. The squamous temporal articulated dorsally with the frontal bone, ventrally with the sphenoid bone and caudally with the parietal bone (Figure 1A and C). The parietal bone contributed to the formation of the dorsal part of the caudal wall of the skull. It was situated between the frontal bone and the supraoccipital bone. The frontal bone formed the dorsum of the neurocranium. It was divided into the nasal orbital and caudal part (Figure B-C). The nasal part of the frontal bone was a very prominent feature, convex and extended slightly forward (Figure 1A-B).

Mandible

The mandible constitutes the bones of the lower beak. It has a paired body that fused rostrally at the mandibular symphysis, presenting a V-shaped outline that conformed to the orientation of the upper beak. The mandible comprised of secondary bones that fused into a single column. The dental bone was prominent and encompassed the rostral end of the mandible. A supraangular bone was present at the dorsocaudal aspect of the body of the mandible. The articular bone was immediately caudal to the supraangular bone, and ventral to it was the angular bone. The angular bone presented two articular processes. The medial articular process was large with rounded ends, while the caudally directed articular process was narrow with pointed end. The rostral end of the mandible presented numerous pneumatic foramina (Figure 1D). The lateral aspect of the ramus of the mandible presented two fenestrae (rostral and caudal). The caudal fenestra was less prominent. *Fossa temporalis* subdivided by a crest was present at the same location of these fenestrae on the medial aspect of the ramus of the mandible. This fossa delineates the contributions of the individual bones that formed the mandible (Figure 1E).

Vertebral Column

There were fifteen cervical vertebrae in Black-crowned Crane. The atlas was the first cervical vertebra and articulated with the skull cranially via the occipital condyles. It was atypical, ring-shaped with the body bearing a prominent depression caudally for articulation with the odontoid process of the axis. The dorsal arch was delicate forming the dorsal rim of the large vertebral foramen. The ventral arch presented a cranial deep articular facet for articulation with the occipital condyle. The caudal boundary of the dorsal arch articulated with the cranial articular processes of axis (Figure 2A). The second cervical vertebra was the axis. It presented atypical spinous process which was elongated and ridge-like. The odontoid process was a prominent feature which articulated with the axis rostrally. There exists a small lateral vertebral foramen ventrolaterally to the base of the spinous process. The cranial and caudal articular processes had articular facets (Figure 2B). The remaining cervical vertebrae presented characteristic vertebral body arch and cranial and caudal articular surfaces (Figure 2C). There were seven free thoracic vertebrae in the present study. The caudal portion of the vertebral column fused into a single bony column, the synsacrum. Approximately five thoracic vertebrae fused to form the notarium. The number of fused lumbar vertebrae that form *synsacrolumbar* was difficult to establish in the present study. The primary sacral and *synsacrocaudal* (coccygeal) vertebrae comprised of three fused vertebrae, respectively. There were six free coccygeal vertebrae, the terminal (seventh) vertebra presented the pygostyle (Figure 3).

Bones of pelvic girdle

The pelvis comprised of two hip bones formed by the osseous fusion of three primary bones consisting of the ilium, ischium, and pubis.

Ilium

The paired ilium was elongated and presented with two parts, the long preacetabular part and a short, broad postacetabular part, which united cranially

with the synsacrum of the vertebrae. Cranially, this union was by osseous fusion and caudally with the transverse processes of these vertebrae via syndesmosis. Ilioneural canal was present where the ilium, the spinal and transverse processes of the synsacrothoracic vertebrae united (Figure 4A).

The dorsal surface of the ilium presented elongated shallow depression for muscle attachment, whereas the internal surface presented the *fossa renalis,* an excavation for the accommodation of the kidneys. These fossae presented a cranial, smaller part known as the ischiatic fossa, which contained the cranial middle portion of the kidney and the lumbosacral plexus.The larger, deeper caudal part of the renal fossa was the pudendal fossa, which accommodated the caudal potion of the kidney. The renal fossa extends caudally as an invagination between the postacetabular part of the ilium and the ischium to form the renal caudal recess. The lateral edge of the postacetabular ilium bears the dorsolateral iliac crest (Figure 4 A-B). The acetabulum was circular bony ring formed by the ilium and ischium. The floor of the acetabulum presented the large acetabular foramen. Caudoventrally to the acetabulum was a small, oval obturator foramen. The obturator process partially separates the obturator foramen from the ischiopubic fenestra. A strong bony prominence representing the antitrochanter was present caudodorsally to the acetabulum. (Figure 4A-C).

Ischium

The paired ischium was a long bony plate situated caudally and ventrally to the ilioischiatic foramen. Its cranial part contributed to the formation of the caudoventral half of the acetabulum, while its caudal part presented a broad bony plate. The bony plate was roughly quadrate- shaped, thus presented four borders: the cranial border from the caudal half of the ischiatic foramen dorsally and obturator foramen ventrally, he lateral border united with the shaft of the pubis at the pubiischiatic incisures, he medial border sloped inward presenting a U-shape outline when joined the opposite border, andthe caudal border that presented a blunt caudal process (Figure 4A-C).

Pubis

The paired pubis was a thin, rib-like bony rod. The middle portion of the pubis fused with the ventral border of the ischium at the pubiischiatic incisure, where it projected beyond the ischium and curved caudomedially. The cranial extremities of the pubis terminated at the oval obturator foramen (Figure 4A-C).

Skeleton of pelvic limbs

The bones of the pelvic limbs comprised of the femur and patella, tibiotarsus and fibula, and the tarsometatarsus and digits.

Femur

The femur was tubular and presented a strong cylindrical body. The distal extremity was more massive than the proximal ends. The maximum length of the femur was 10.5 centimeters (cm). The proximal extremity consists of a hemispherical head, a distinct neck and the trochanter. The head of the femur was directed medially for articulation with the acetabulum. A distinct margin marked the limit of the head (Figure 5). There exists a rough notch at the center of the head, the *fovea capitis*. The trochanter major of the femur is situated laterally, slightly higher than the head and articulates with the antitrochanter of the acetabulum. The trochanter major continues as a bony ridge on the proximal cranial surface of the shaft. The shaft is generally smooth, straight and consists of four surfaces. The medial surface is the distal continuation of the head and neck. The cranial surface presents the bony ridge, an extension of the trochanter major. At the caudal lateral margin of the surface, the trochanter major continues as a distinct line that runs almost two thirds of the shaft. Both the cranial and caudal surfaces presented irregular muscular lines. The distal extremity of the femur was large bearing on its cranial surface the trochlea of the femur. The medial and lateral trochleas were separated by a wide intercondylar sulcus. The lateral was partially divided by an intratrochlear groove. Each condyle bears a small epicondyle.

The medial condyle extended proximally as a thin ridge to form the medial supracondylar crest and continued proximally on the caudal surface as the caudal intermuscular line (Figure 5).

Tibiotarsus

It was the paired tubular bone. The distal extremities of the tibia articulate with the proximal row of the tarsal bones. The tibia was comparatively longer than the femur. The maximum length of the tibia was 24.5 centimeters (cm). The proximal epiphysis presented the medial and lateral condyles for articulation with the condyles of the femur. The lateral condyle bears a facet on its lateral surface for articulation with the fibula. On the cranial surface of the proximal end of tibiotarsus, there exists a prominent sharp tibial crest. This crest extended from the proximal extremity towards the shaft. The caudal surface of the proximal end of tibiotarsus has the flexor fossa distally to the lateral condyle. The proximal and distal interosseous foramen was a small elongated foramen between the fibula and tibiotarsus. The shaft of the tibiotarsus was smooth and three-sided, proximally comprising of medial, lateral and caudal surfaces. Distally, it presented the cranial and caudal surfaces. The distal extremity of the tibiotarsus was characterized by the medial and lateral trochlea. A deep passage on the cranial surface of tibiotarsus proximal to its distal condyles was the extensor canal (Figure 6 A and B).

The fibula lied parallel to the long axis of the tibiotarsus and presented a prominent head known as the *capitulum*. A fibular articular facet for articulation with the tibiotarsus was present on the medial surface of the fibula. The body of the fibula was a thin rod-like, attached to the proximal half of the lateral surface of the shaft of the tibia (Figure 6 A and B).

Tarsometatarsus

This was a single long bone comprised of fused metatarsal bones II, III, IV that articulated with the distal row of tarsal bones. Metatarsal (I) was undeveloped and united ventromedially with tarsometatarsus via a ligament. The proximal extremities of tarsometatarsus presented the concave articular surfaces divided by a protuberance for articulation with the distal trochlea of tibiotarsus. The shaft of the tarsometatarsus consisted of the dorsal and plantar surfaces. The dorsal surface presented a distinct longitudinal groove, which was deep at the proximal end and became shallow distally. The plantar surface presented three longitudinally directed crests. These crests were the lateral, intermediate and medial crest of the hypotarsus. A groove existed between the medial and intermediate crests. The distal end of tarsometatarsus presented three articular trochleas of metatarsal bones II, III and IV. Lateral and medial intertrochlear notches were present between these trochleas (Figure 7 A and B).

Digits

The Black-crowned Crane had four digits; the first digit was directed medio-plantarly and consisted of two phalanges. The second and third digits presented three and four phalanges, respectively, whereas the fourth digit consisted of five phalanges. The distal phalanx of each digit presented a claw-shaped structure, which formed the bony core of the claw (Figure 7A and B).

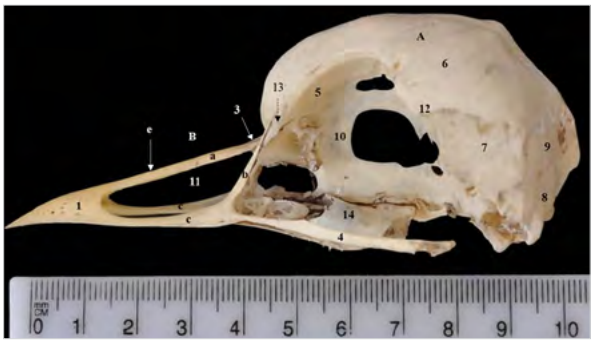

Figure 1A Skeleton of head of Black-crowned Crane (lateral view) showing: A. Neurocranium, B. Splanchnocranium, 1. Premaxillary bone; (e. Frontal process of premaxillary bone, c. Paired maxillary process of premaxillary bone, 3. Nasal bone; (a. Intermaxillary process, b. Maxillary process of nasal bone), 4. Zygomatic bone, 5. Ethmoidal bone, 6. Frontal bone, 7. Squamous portion of temporal bone, 8. Occipital bone, 9. Parietal bone, 10. Interorbital septum, 11. Nasal aperture, 12. Orbital process of temporal bone, 13. Lacrimal bone, 14. Platinum bone

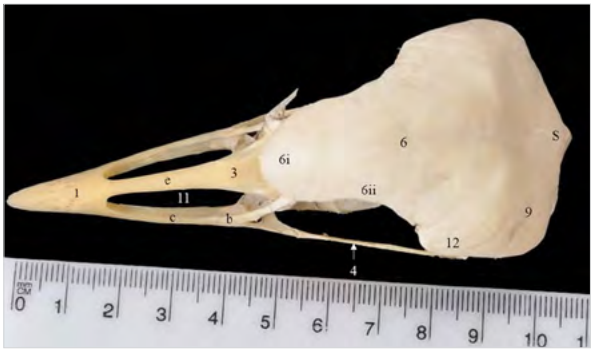

Figure 1B Skeleton of head of Black-crowned Crane (dorsal view) showing: 1. Premaxillary bone, e. Frontal process of premaxillary bone, c. Paired maxillary process of intermaxillary bone, 3. Nasal bone; (b. Maxillary process of nasal bone), 4. Zygomatic bone, 6. Frontal bone (6i. Nasal part, 6ii. Orbital part), 8. Occipital bone, 9. Parietal bone, S. Supraoccipital, 11. Nasal aperture

Figure 1C Skeleton of head of Black-crowned Crane (ventral view) showing: 1. Intermaxillary bone, a. Platinum, b. Vomer, c. Maxillary process, d. Occipital condyle, e. Frontal process of premaxillary bone, f. External acoustic pores, g. Platinum process, h. Basihyoid bone, i. Foramen magnum, j. Zygomatic bone

Figure 1D Mandible of Black-crowned Crane (lateral view) showing: 1. Dental bone, 3. Articular process of angular bone, 4. Supraangular bone, 5. Posterior process of angular bone

Figure 1E Mandible of Black-crowned Crane (dorsal view) showing: 1. Dental bone, 2. Articular bone, 3. Articular process of angular bone, 5. Posterior process of angular bone

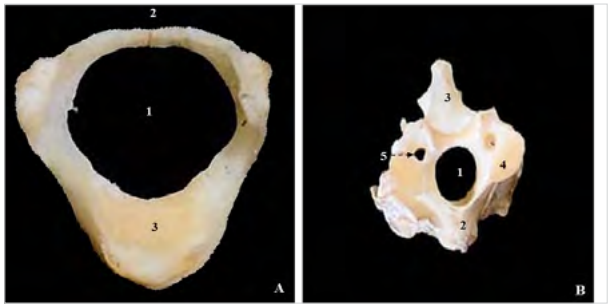

Figure 2A-B A. Atlas of Black-crowned Crane (cranial view) showing: 1. Vertebral foramen, 2. Vertebral arch, 3. Deep articular depression for odontoid process of axis. **B.** Axis of crane (cranial view) showing 1. Vertebral foramen, 2. Odontoid process, 3. Spinous process, 4. Cranial articular fovea, 5. Lateral vertebral foramen

Figure 2C Segment of vertebral column (cervical vertebrae C_3-C_{12}) of Black-crowned Crane (lateral view) showing: Spinous processes (thick arrow), transverse processes (thin arrow). Last two cervical vertebrae were inverted to show the transverse process. White dotted line delineates the two inverted vertebrae

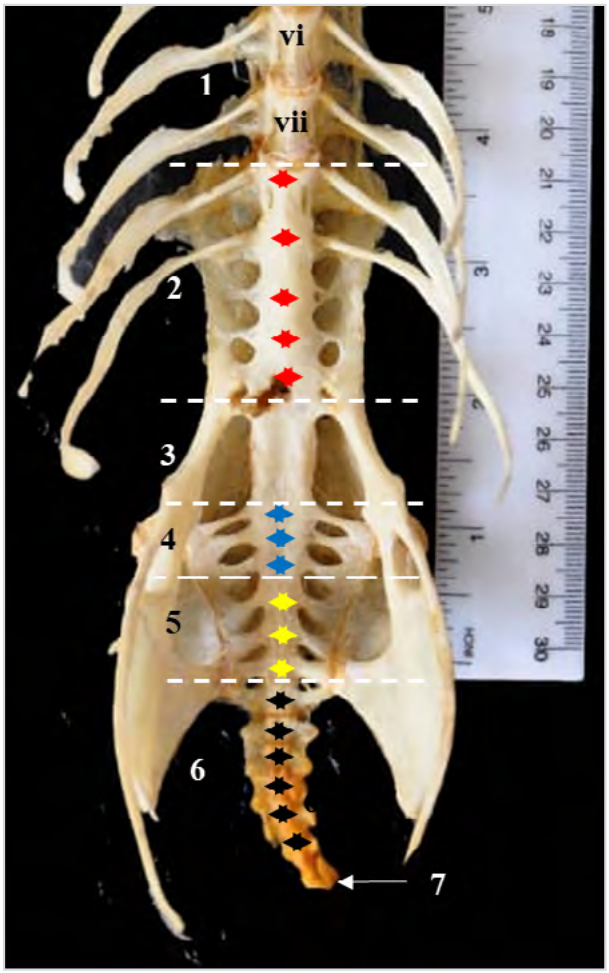

Figure 3 Caudal part of vertebral column of Black-crowned Crane (ventral view) showing: 1. Free 6-7th thoracic vertebrae, 2. Notarium (red dots), 3. Sacrolumbar, 4. Three-fused primary sacral vertebrae (blue dots), 5. Three-fused synsacrocaudal vertebrae (yellow dots), 6. Six-fused free coccygeal vertebrae (black dots), 7. Pygostyle (white arrow). The white dotted line showed boundary between the fused vertebra forming the synsacrum

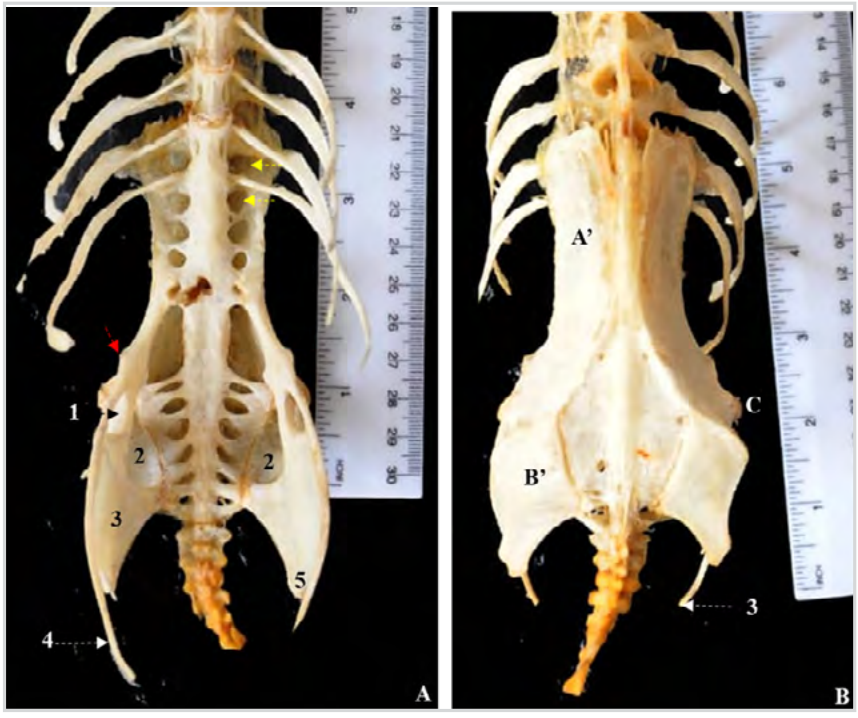

Figure 4 A-B A Pectoral girdle of Black-crowned Crane (ventral and dorsal view) showing: 1. Obturator foramen, 2. Renal fossa, 3. Ischium, 4. Pubis, 5. Angle of ischium; Ilioneural canal (yellow arrows), pectineal process (red arrow); **B.** A' Preacetabular part of ilium, B'. Postacetabular part of ilium, C. Antitrochanter

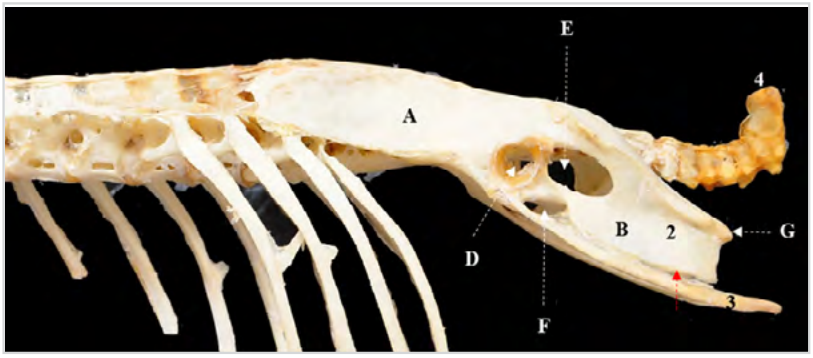

Figure 4C Pectoral girdle of Black-crowned Crane (lateral view) showing: A. Preacetabular part of ilium, B. Postacetabular, D. Acetabulum, E. Ischiatic foramen, F. Obturator foramen, G. Caudal process, 2. Body of ischium, 3. Pubis caudal-slit of pubis (red arrow), 4. Pygostyle

Figure 5 Left and right femur of Black-crowned Crane (anterior view (left) and posterior view right) showing: 1. Head, 2. Trochanter major, 3. Neck, 4. Shaft, 5. Medial trochlear ridge, 6. Lateral trochlear ridge, Trochlea femoris, 8. Condylar fossa, 9. Intratrochlear groove, 10. Muscular line

Figure 6 A-B Left and right tibiotarsus of Black-crowned Crane (anterior view) showing: a. Shaft of tibia, b. Shaft of fibula, c. Lateral condyle, d. Medial condyle, e. Medial crest, f. Groove, g. Bony ridge, h. Lateral trochlea, i. Medial trochlea. **B.** j. Capitulum, k. Lateral condyle, l. Medial condyle, proximal and distal interosseous space (yellow arrow)

Figure 7 Left and right tarsometatarsus of Black-crowned Crane (A. dorsal view and planter view) showing:1**.** Proximal articular facet of tarsometatarsus, articular trochlea (2-3-4) for phalanges of toes (I-IV) ; 5. 1st digit, 6. 2nd digit, 7. 3rd digit, 8. 4th digit, 9. Claw

DISCUSSION AND CONCLUSION

Adult crown-birds have highly apomorphic skulls characterized by a toothless beak, enlarged round orbits, and enlarged and highly pneumatized chondrocranium (Smith-Paredes and Bhullar, 2019). The neurocranium of the Black-crowned Crane was formed by the fusion of 5-6 bones, as observed in cattle egret (Rezk, 2015) and ostrich (Moselhy et al., 2018). Olivia and Christian (2020) stated that the skulls of adult crown- birds were characterized by a high degree of integration due to bone fusion resulting in reduction of the number of bones. Some features of the bones were compared to other species owing to scarcity of literature on basic skull typology in crane bird species. The conspicuous protrusion of the nasal portion of the frontal bone is a striking feature in Black-crowned Crane. It provides for attachment of the modified plumage rostrally to the crown feathers on the head. In guinea fowl, a median ridge composed of spongy bones is present (Nickel et al., 1977). The nasal bone in Black-crowned Crane as in many birds, makes a flexible cartilaginous connection with the frontal bone, which permits the movement of the upper jaw (Dyce et al., 2010).

Hiragi et al. (2014) reported that Red-crowned and Hooded Crane had 17 cervical vertebrae with exceptional cases having 18, fewer than 15 cervical vertebrae we recorded in Black-crowned Crane. There were 7 free thoracic vertebrae in Black-crowned Crane, whereas Red-crowned Cranes had 9-10 or 11 thoracic vertebrae, and Hooded Cranes had 9-10 (Hiragi et al., 2014). The specific number of vertebrae contributing to the formation of the synsacrum was given as 15–16 in Red-crowned Cranes and 14–16 in Hooded cranes (Hiragi et al, 2014). In the present study, the total number of vertebrae forming the synsacrum was 17, excluding the sacrolumbar where gross delineation of the fused vertebral bones was difficult to establish. The synsacrum and the notarium provide rigid support to the dorsal part of the trunk, extending laterally and caudally by the fusion of the synsacrum with the long hip bones (Dyce et al., 2010).

The pelvis of different birds presented marked morphological variations, which indicates differences in specialized locomotor function. In the present study, the preacetabular portion of the ilium is longer than the postacetabular part, as described in domestic fowl (Nickel et al, 1977; Sreeranjini et al., 2011), Crested Serpent-Eeagle and Brown Wood Owl (Choudhary et al., 2020). The puboischiatic incisure presented the oval obturator foramen, caudoventral to the acetabulum

in Black-crowned Crane. The pubis of Crested Serpent-Eagle was completely fused with the ischium (Choudhary et al., 2020), whereas in emu, the pubis was separated from ischium by a large fissure except its cranial portion, which present an incomplete oval to crescent-shaped obturator foramen communicating with the fissure (Kumar and Singh, 2014). The pubis in Black-crowned Crane did not contribute to the formation of the acetabulum, as seen in domestic fowl and duck (Nickel et al., 1977). In emu, all the three primary bones of the pelvic are involved (Kumar and Singh, 2014).

The trochanter major was elevated above the head in Black-crowned Crane similar to findings in Indian Horned- Owl, flamingo and crow (Sridevi et al., 2020),whereas in emu, the trochanter major was flat and did not project above the level of the head (Kumar and Singh, 2014). A pneumatic foramen was present on the anterior surface below the trochanter major in Crested Serpent-Eeagle (Choudhary et al., 2020), andin crow, small numerous pneumatic foramina (Sridevi et al., 2020). The *fovea capitis* was absent in emu (Kumar and Singh, 2014). It was eccentric in our findings, situated posteriorly-medially on the circumference of the head. The shaft is smooth and bears muscular lines on the anterior and posterior surfaces. Most of the observable features of the distal extremity of the Black-crowned Crane were similar to findings in domestic bird. A distinct groove of the caudal surface of the lateral trochlea was also reported in serpent-eagle (Choudhary et al., 2020).

In most avian species, the tibia fuses with tarsal elements, which forms a tibiotarsus that is much longer than the femur and carries the shaft of the feebly developed fibula on its lateral aspect (Dyce et al., 2010). The ratio of length tibiotarsus to femur was 1:2, as reported in emu by Kumar and Singh (2014). A sharp medial bony crest at the proximal end of tibiotarsus observed in the present study, was also reported in peahen (Sreeranjini et al., 2013), cattle egret (Rezk, 2015) and serpent-eagle (Choudhary et al., 2020). However, medial and lateral ridges rather than the crest were observed in emu (Kumar and Singh, 2014). This crest provides for attachment of extensor muscles of the knee joint (McLelland, 1990). A nutrient foramen was reported at the middle of the lateral border of the tibia in emu (Kumar and Singh, 2014), and this has not been observed in the present study. The proximal and distal interosseous spaces in our study have been reported in serpent- eagle (Choudhary et al., 2020). The length of the tarsometatarsus was relatively long in black-crowned. This enables the bird to forage on dry ground short grass typical of Sudano-Sahelian habitat. According to Nickel et al (1977), the length of the metatarsus determines to some extent the ground clearance of the bird in standing position. The deep grooves observed on the dorsal surface of the tarsometatarsus in the present study had earlier been reported in both dorsal and plantar surfaces in emu. In addition, three nutrient foramina towards proximal extremity were seen on the dorsal surface in emu (Kumar and Singh, 2014).

The present study described the gross morphological features of different bony components of axial, pelvis and the limbs of West African Black-Crowned Crane. Observable features were compared to skeleton of *gruidae* birds and other domestic species described in a conventional avian anatomy textbook. The findings will be useful for comparative anatomy and functional aspects of musculoskeletal system in avian.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

CONTRIBUTION

Concept and results interpretation-IA;Sample collection and processing-WA, IS; Literature search/Resources-MK, IS; Manuscript writing and critical review-IA, AM.

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ANATOMIJA AKSIJALNOG I PELVIČNOG SKELETA KOD ZAPADNOAFRIČKOG CRNOKRUNASTOG ŽDRALA (*BALEARICA PAVONINA PAVONINA*)

SAŽETAK

Istraživanje prikazuje makroskopske morfološke karakteristike kostiju aksijalnog i pelvičnog skeleta odraslog crnokrunastog ždrala. Kosti su macerirane standardnom tehnikom, pri čemu su prikazani strukturni detalji. Lubanja se sastoji od splanhnokranijuma i neurokranijuma, međusobno odvojenim velikom koštanom orbitom. Mandibula predstavlja rostralno dentalnu kost koja sadrži sitne otvore i kaudalno supraangularnu kost. Kranijalni segment vertebralne osovine se sastoji od petnaest cervikalnih i sedam slobodnih torakalnih kralježaka. Kaudalni dio vertebralnog stuba je spojen u jedinstveni koštani stub kojeg čine notarijum i lumbalni sinsakrum, tri srasla primarna sakralna kralješka i tri srasla sinsakrokaudalna kralješka. Postoji šest slobodnih kokcigealnih kralježaka od kojih posljednji predstavlja pigostil. Pelvični pojas je nastao koštanom fuzijom ilijuma, ishijuma i pubisa. Maksimalna dužina femura je iznosila 10,5 cm, a tibiotarzusa 24,5 cm. Tarzometatarzus se sastoji od spojenih II, III i IV metatarzalne kosti koje su uzglobljene sa distalnim nizom tarzalnih kostiju. Crnokrunasti ždral posjeduje četiri funkcionalna prsta. Prvi prst se sastoji od dvije falange, drugi i treći prst od tri i četiri falange, a četvrti prst od pet falangi.

Ključne riječi: Aksijalni, crnokrunasti ždral, lubanja, makroskopska anatomija, kosti pelvičnog pojasa

RESEARCH ARTICLE

STEREOMICROSCOPIC AND SCANNING ELECTRON MICROSCOPIC OBSERVATIONS OF THE FORESTOMACH MUCOSA OF AKKARAMAN SHEEP FED WITH STRAW-CONCENTRATE DIET

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ABSTRACT

This research was conducted to observe the mucosal surface of the forestomach components of seven Akkaraman sheep fed with straw-concentrate diet using stereomicroscopy and SEM. The samples were obtained from the animals fed 60% straw and 40% concentrate, and slaughtered in a local slaughterhouse. The results revealed that the ruminal papillae were present on all of the surface of the rumen except the ruminal pila. Their shape varied considerably in shape and size from a short lingual to a long and wide leaf-like forms. Some papilla showed asymmetrical doubled-apices. The cells in the ruminal mucosa were mostly intermediate-type cells. Occasionally, their shapes resembled balloon-type cells. Reticular cristas in the reticulum and omasal lamina in the omasum were also displayed clearly, but certain papilla types and their peculiarities were not eminent at macroscopical level. At microscopical level, there were papilla- type structures present on the omasal lamina. They had smooth surface, mostly possessing one, occasionally double ridges. The epithelial scrap layer formed by the horny cells was eminent, indicating regeneration of the papilla. The results have shown the characteristics of the animals fed with straw-concentrate diet.

Keywords: Intermediate type eaters, sheep, forestomach components, SEM

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INTRODUCTION

Sheep in Türkiye is raised mostly in nonagricultural areas, grasslands, and pastures, contributing to economy through transforming natural vegetation to yields such as meat, milk and wool. Likewise, Akkaraman sheep has an important place within the sheep population in Türkiye, comprising 45.8% of all population (Akçapınar, 2000).

Ruminants digest herbaceous feed through microbial fermentation in their forestomachs. This is augmented by mucosal duplications which differ greatly in shape in accordance with their feeding habits, providing common digestive ability. Several studies have documented gross and ultra structural nature of the mucosa of the forestomachs in ruminants (Loe et al., 1959; Yamamoto, 1998; Steele et al., 2009; Swan and Groenewald, 2000). They have classified them into three categories with regard to the feeding preferences: grazers, concentrate selectors, and intermediate type eaters (Hofmann, 1989). Domesticated ruminants belong either to grazers or to the intermediate type eaters. Grazers digest usually low-quality roughage, particularly different kinds of grass; on the other hand, the intermediate type eaters choose leaves and young plants with high energy content, if present (Hofmann, 1989).

Different feeding habits profoundly affect anatomical peculiarities of the mucosal duplications of the forestomachs, which is related directly to the passage rates of digesta in the gastrointestinal tract, with the longest duration times recorded in the grazers (Hofmann and Schnorr, 1982). Grazers have a larger rumen and a smaller omasal orifice than intermediate types and concentrate selectors (Hofmann, 1989). Animals that belong to the last two groups can send food directly into the abomasum through bypassing forestomach via the reticular groove (Hofmann, 1989). These indicate adaptations of the motility patterns in the forestomachs whith regards to feeding type.

The rumen epithelial barrier is a critical component of the immune system in ruminants (Penner et al., 2011). The destruction of the rumen mucosa, i.e. necrosis, is an important problem in animals fed with concentrated feed (Liu et al., 2013). However, little is known about the true molecular causes underlying this. Changes in ruminal epithelial barrier function, mRNA and tight junction (TJ) proteins in protein expression during concentrated diet feeding have been reported (Liu et al., 2013). In the current intensive ruminant production system, the use of a highly concentrated diet is used to maximize energy intake and improve milk production, or to increase daily weight gain. However, highly fermentable diets put animals at risk. Fast fermentable non-structural carbohydrates increase the rate of fermentation process of acid production. Acid accumulation in the rumen reveals the possibility of impairment in ruminal epithelial barrier function and its exposure to toxins (Beauchemin et al., 2008). The cellular structure of the rumen epithelium is complex. Damages in this section can be viewed histologically and by scanning electron microscopy (Steele et al., 2009).

High grain (HG) feeding shows a strongly disrupted epithelial barrier. Multiple systemic symptoms such as the development of rumenitis and liver abscess caused by the HG diet also show ruminal epithelial cellular damage and changes in TJ protein (Liu et al., 2013). It was reported that the number of papillae increased significantly in the concentrate-fed groups compared to those fed with hay. It was reported that the number of papillae per cm2 was 48 in the group fed with concentrated feed in 4 weeks, and 38 papillae were detected in animals fed with hay. On the other hand, it has been reported that the number of papillae per cm2 of mucosa increases as the duration of concentrate feeding increases among the groups fed concentrate (Gäbel et al., 1987). It has been stated that papillae are under the influence of diet change and feeding time. It has been reported that the shape of the papilla also changes depending on the diet. Small, finger-shaped papillae were observed in the hay-fed group, while large, leaf and tongue-shaped cornified papillae were observed in animals fed with concentrated feed (Ahmed et al., 2013).

Generally, the development and growth of ruminal

papillae has been reported to be highly dietary. Mechanical and chemical stimuli, short-chain fatty acids, the age of the animal and the time of weaning were found to be directly related to the size and shape of the papilla (Anderson et al., 1987; Franco et al., 1992; Zitnan et al., 1999; Swan and Groenewald, 2000; Ahmed et al., 2013). It has been reported that papillae are the largest and most concentrated in the ventral wall of the rumen, in the parts that are most exposed to food. The number of papillae per cm2 of mucosa has been observed to increase significantly in concentrated feeding compared to straw feeding. As the concentrate-feeding time gradually increases, the papillae ruminis take the shape of leaves and elongate (Gäbel et al., 1987; Ahmed et al., 2013). It has also been shown that the development of papillae is dependent on short-chain fatty acids (especially SCFA, mainly butyric and to a lesser extent propionic acids) originating from feed ingredients (Brownlee, 1956).

When the average length and width of papilla ruminis were compared between the concentrate and hay-fed groups, it was observed that the length and width of the papilla ruminis in the concentrate-fed group were strikingly higher (Ahmed et al., 2013). Scanning electron microscope findings; in the images of the rumen papillae, indentations and dead keratinized cells along the surface were more prominent in animals fed with concentrated feed than in hay feeding. Deep cellular damage and parakeratosis were found in rumen papillae surface cells for goats fed with concentrated feed. Rumen papillae sections, nuclei, mitochondria and intercellular connections were normal during hay diet. In goats fed with concentrated feed, cellular necrosis and cellular erosion were seen in all cell layers. The HG diet was reported to cause serious deterioration in the ruminal epithelium during nutrition, especially in subacute ruminal acidosis when cell erosion (parakeratosis) was detected with prominent epithelial cellular damage (Liu et al., 2013). Also, in such cases where the rumen epithelium was disrupted, the layer became permeable to endotoxin and this can cause liver abscesses, laminitis, and inflammatory responses (Steele et al., 2009).

Morphologically, rich deep ridges and indentations were observed in SEM images of rumen papillae from all cattle. Heterogeneous microflora, including abundant bacteria and protozoa, were detected in deep ridges and indentations, especially in the diet based on roughage. At additional magnification, keratinized squamous cells of the stratum corneum layer were evident in the HG diet. Desquamation of dead keratinized cells was evident, while microbial flora decreased (Steele et al., 2009).

Studies have revealed adaptations of the mucosal morphology of the forestomachs in different ruminant species due to both feeding habits and seasonal and regional changes (Flatt et al., 1958), including sheep (Scot and Gardner, 1973) and cattle and goats (Yamamoto et al., 1993). Moreover, mucosal papilla of the forestomachs has been shown to vary greatly, depending on age, feeding type, and region (Habel, 1975; Franco et al., 1992; Banks, 1993; Dyce et al., 2010). Reversable alterations can also be seen in the morphology and function of rumen epithelia within 1-3 weeks of food change (Gäbel et al., 1987; Anderson et al., 2013). In relation with that, this study aimed at revealing the possible morphological relationship between the structures of the mucosal morphology of the forestomachs and feed stuffs in Akkaraman sheep fed with straw-concentrate diet.

MATERIALS AND METHODS

Seven female and adult Akkaraman sheep, a national Turkish breed, were used in the study. The animals were fed 60% straw and 40% concentrate during the period between October and February. The samples taken from the ventral portion of the forestomach for stereomicroscopic and SEM examinations were processed at Gazi University, Ankara, Türkiye. For SEM examination, samples were placed into 3% glutaraldehyde with phosphate buffer (pH: 7.3). After rinsing in buffer, tissues were post-fixed in 1% osmium tetraoxide (OsO4) at 37 °C for 1.5 hours. After post-fixation, tissues were placed in 3N HCl at 60 °C for 20 minutes to remove the extracellular mucus from the surface

of the tissue. Tissue samples were passed alcohol and amyl acetate series and dried with critical-point-dryer. Specimens were coated with gold and observed a Jeol JVM 5000 SEM at 5-15 kv (JSM 6390LV, JEOL, Germany).

RESULTS

The mucosal surface of the forestomach of the Akkaraman sheep was not smooth, expanding into a large area by numerous papillae which varied considerably in shape and size in the various sacs and compartments.

Ruminal papillae were densely distributed eminently all over the ruminal surface except the ruminal pillars and nearby, where they were reduced both in number and length. They were light brown in color, varying profoundly with regard to a location, from a short lingual to long and wide leaf-like forms (Figure 1). Occasionally, some of the papillae showed asymmetrical doubled apices (Figure 1). The short lingual type of the papillae were located mostly dorsally, while long and wide leaf-like papillae were present cranioventrally on the cranial cecal sac. The latter contained two types of grooves in different length and largeness (Figure 2); the primary groove lied centrally throughout the length of the papilla, and the small secondary groove intersecting the primary one (Figure 3).

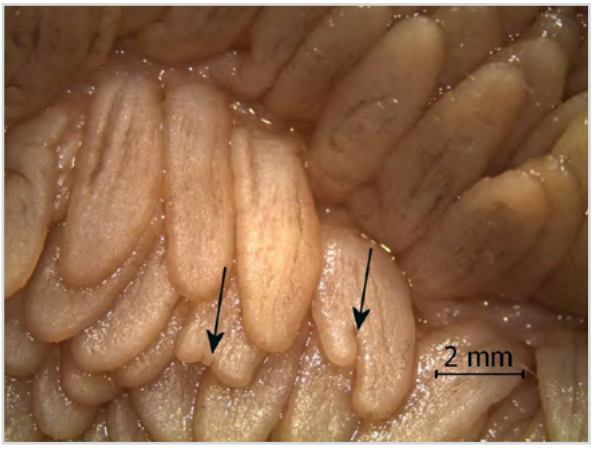

Figure 1 Stereomicroscope view of the rumen papilla in the ventral part of the rumen (Scale 2 mm). Arrows: asymmetrical doubled-apices

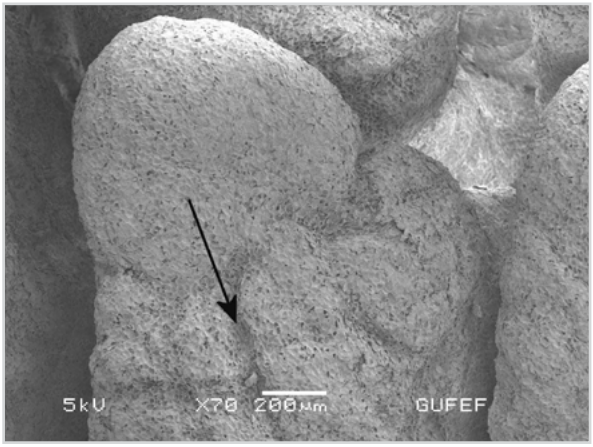

Figure 2 View of ruminal papilla, x35 scale, bar: 200µm. Arrow: primary groove

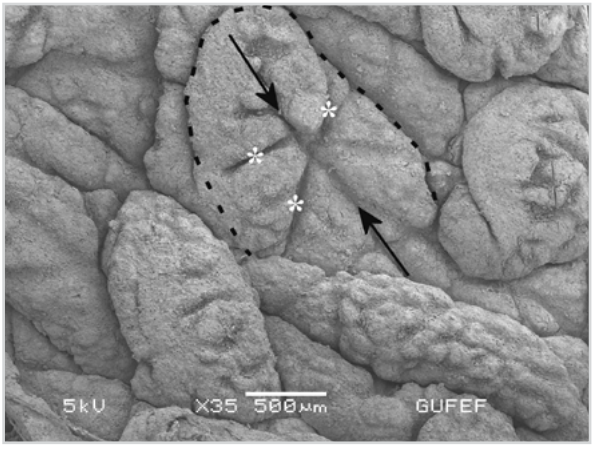

Figure 3 Shallow grooves on the ruminal papilla. x50, scale, bar: 500µm. Arrows: primary groove, asterisk: secondary groove, dotted line: papilla ruminis

Short tongue-shaped papillae were mostly found in the dorsal and lateral wall of the rumen (Figure 4), while long and wide leaf-like papillae were seen in the ventral part (Figure 1). In the samples taken from the lateral wall of the rumen, few

grooves were observed on the rumen papillae. On the other hand, primary very deep grooves and 4-6 secondary grooves were detected on those of the ventral wall. The grooves on the ventral wall of the rumen of the animals fed the intermediate type

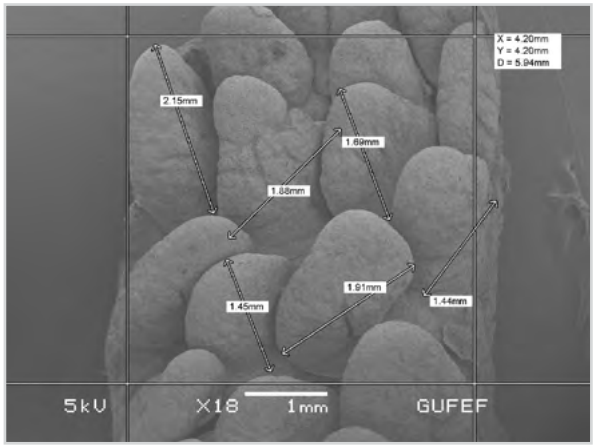

Figure 4 Short tongue-shaped papillae in the dorsal and lateral wall of the rumen

were wrinkled in shape.

Cell debris and necroses were detected in the papillae at 200 magnification in the samples taken from the ventral wall of the rumen. Keratinized squamous cells, very intense in number appeared on the surface and edges of the grooves. Necrotic cell debris was found to be mostly quadrilateral, pentagonal, and hexagonal polygonal. The margins of these squamous cell debris ranged from 18 to 35 micrometers (Figure 5).

At 2500 magnification, a granular view was seen on the ruminal surface (Figure 6). It was due to the presence of the cytoplasmic ridges lying around. Polygonal cells were observed on all the inner surface of the rumen. The ruminal papillae located on the inner dorsal surface of the rumen, were settled sparsely, showing no grooves at all. They, instead, had ridges.

Reticular cristas in the reticulum and omasal lamina in the omasum were shown clearly. They were seen grossly, but some of the papilla types and their

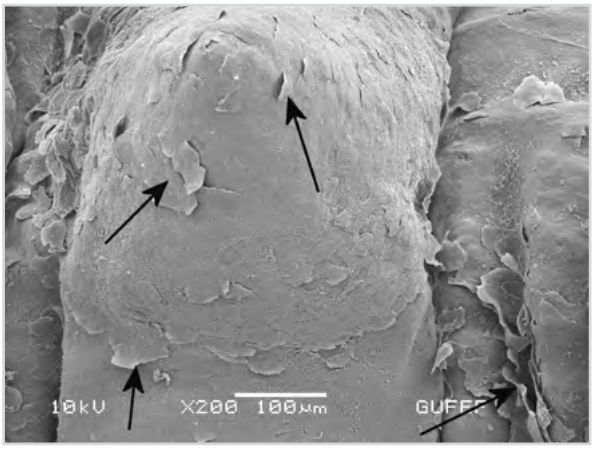

Figure 5 Cell debris and necroses in the ventral wall of the rumen. Arrows: Cell debris and necroses

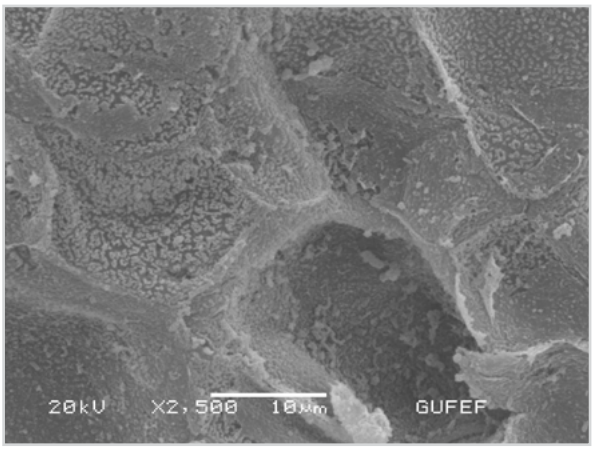

Figure 6 Cytoplasmic ridges of rumen (asterisk), x2500, scale bar: 10µm, arrows: bacterial colony

peculiarities were not eminent at macroscopical level. At microscopical level, there were papilla type structures present on the omasal lamina. They had smooth surface, mostly possessing one (Figure 7), occasionally double ridges (Figure 8). The epithelial scrap layer formed by the horny cells was eminent (Figure 9), indicating regeneration of the papilla.

SEM images showed intermediary balloon-type cells on the rumen surface at 1000 magnification.

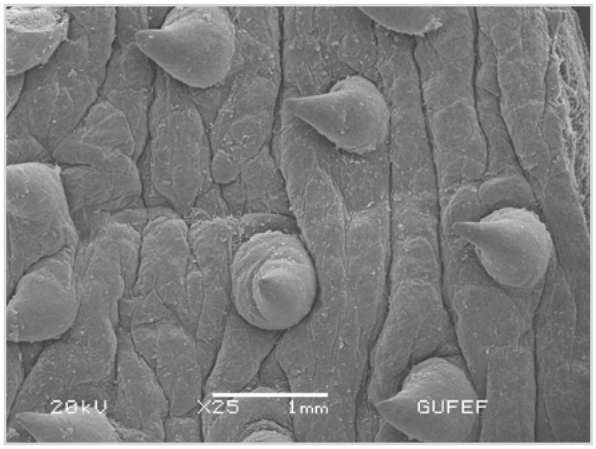

Figure 7 Papilla-type structures of omasal lamina, x25, scale bar: 1mm

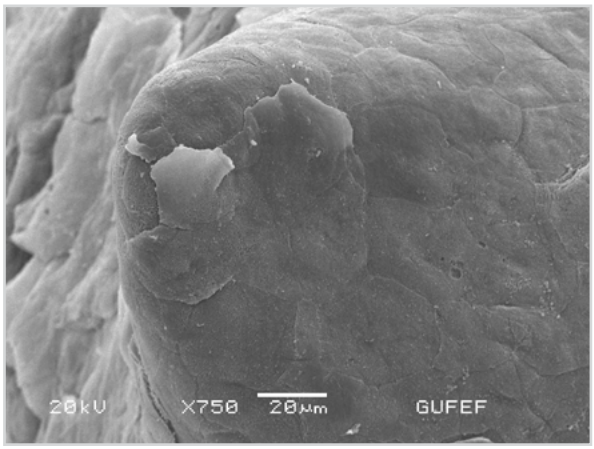

Figure 9 The epithelial scrap layer of omasal lamina. x750, scale bar: 20µm

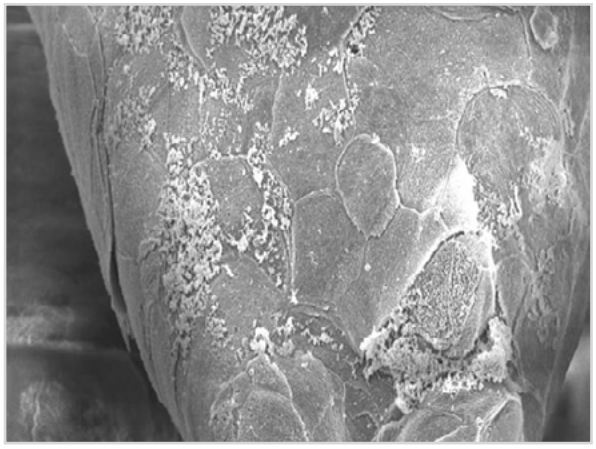

Figure 8 View double ridges of omasal lamina, x1000, scale bar: 10 µm

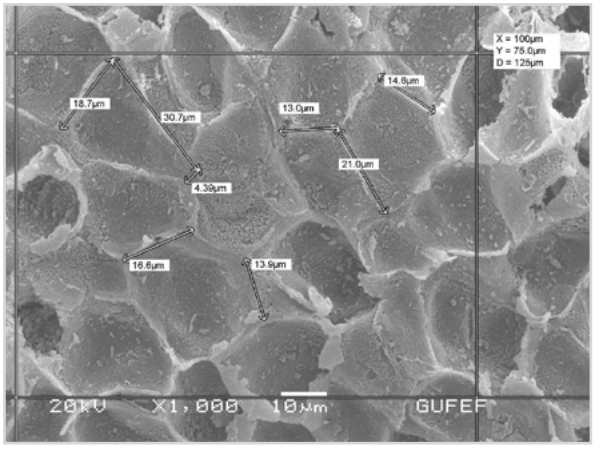

Figure 10 Intermediary baloon-type cells on the ruminal surface x1000, scale bar: 10µm.

Cells were generally observed as pentagonal-hexagonal types. It was determined that each edge ranged from 35 µm to 3.3 µm. The edges were found to be generally unequal in length (Figure 10).

DISCUSSION AND CONCLUSION

At SEM observation, they were determined to function basically as surface enhancement (Tamate et al., 1979; Anderson et al., 1987) to lie in similar fashion, but being various topographically in numbers and shape. These differences on papilla development have been shown partly to be related to a feeding habit (Loe et al., 1959; Sander et al., 1959; Weyrauch and Schnorr, 1979; Dyce et al., 2010; Ahmed et al., 2013), just as found in our results. Lipid acids, particularly butyric and propiyonic acids have been reported to play a very essential role in mucosal development with this variation range (Sander et al., 1959). They have been indicated to exert their role exclusively by both causing structural differences and augmenting absorbtion (Sander et al., 1959; McGilliard et al.,

1965). This study, which was conducted in animals known to have nutritional status, revealed broad variations, particularly in the rumen, as compared to the literature. Especially symmetrical and unsymmetrical bifurcations and characteristic grooves were noted in the end parts of the papilla ruminis.

The results of a study (Scott and Gardner, 1973) that the entire surface of the rumen is covered with polygonal cells, are compatible with our findings, and polygonal cell types - especially hexagonal - were observed in our study. Scott (1973) mentioned that cell borders are seen clearly, and this clarity is present in our study.

Garcia et al. (2012) has reported in their study that the ruminal wall is seen as a smooth surface, indicating no evidence of keratinization or desquamation; this view is not in agreement with our study. This literature has stated that the cells, their outline, and the boundaries between the cells are clear, which is consistent with our study.

Along with our results, most researchers (Penner et al., 2011; Ajmed et al., 2013) have reported that there is cellular necrosis, parakeratosis and cell debris observed in papilla ruminis of animals fed with concentrate feed. Yet, a study (Scott and Gardner, 1973; Penner et al., 2011; Ahmed et al., 2013) has stated that this shedding is a feature of the ruminal epithelium, and that this spill is normal. In our study, and in most literature, necrotic cell debris was observed in the epithelial cells of the papilla ruminis of animals fed with concentrated feed and intermediary type. These results are contrary to those of that literature with the exception of normal cell death. Finally, Steele et al. (2011) (Supplemental Material 1, Supplemental Figure S1, A and B) mentioned that heterogeneous microflora including bacteria and protozoa were seen in SEM images, especially in hay-based nutrition, which was also observed in our study, as displayed in Figure 6.

Hofmann (1989) has categorized ruminants with regard to stomachs' morphology into three types, concentrate-fed (CS), intermediate type eaters (IM), and grazers (GR). This study has focused on the mucosal peculiarities of the IM animals fed concentrates along with straw rich in cellulose. Literature (Hofmann, 1989) has indicated that mucosal changes in animals fed different ratios occur mostly in the rumen and reticulum. Since this study has used IM animals only, no comparison has been done. The ruminal papillae have been observed to scatter evenly in the rumen, just as reported by the literature (Ahmed et al., 2013). Hofmann (1989) has shown that the presence of the ruminal papilla, even on the ruminal surface of the ruminoreticular plica and lacking on the reticular plica, are characteristics of the CS animals. Interestingly, no ruminal papilla was present on the plicas of the IM animals used in this study.

Tamate et al. (1979) have defined primary and secondary grooves on the ruminal papilla in sheep. Yamamoto et al. (1994) and Scott and Gardner (1973) have also mentioned these structures. Our study has likewise determined the primary and secondary grooves while the secondary ones being shallow in shape. These grooves are due likely to the longer stay of the concentrates in this area. Except on the cranial and ventral aspects of the cranial blind sac, the fact that these grooves are shallow and less present (Figure 3) is most probably because of the feeding habit.

Ahmed et al. (2013) reported that the number of grooves on papilla ruminis was one in straw-fed animals, but the number of grooves and depth increased as the animal feed rate increased. The results of our study are compatible with this publication in that that the presence of primary and secondary grooves in the intermediatefeeding habits and the depth of the grooves are moderate. Additionaly, certain papillas have been found to comprise two grooves parallel to each other and equal, without differentiating between primary and secondary. Ahmed et al. (2013) stated that the papilla ruminis form small, flattened tongues in the animals of the same type of ration in their study, and our study shows that the findings are the same as in this study. Mahes et al. (2014) have found smaller pointed and blunt projections like the

secondary papillas between large and small intense papillae on the ventral wall of the rumen in our study. This type of secondary papilla has not been observed in our study. The color of the ruminal papillae was light brown. This study revealed that changes in colour of the papillae were diet-dependent. Ahmed et al. (2013) reported papillae from hay-fed sheep or sheep fed concentrate were light brown in colour. However, papillae with dark brown colour were observed in 6 and 12 weeks concentrate-fed groups. Interestingly, 4 weeks concentrate-fed group showed papillae with both light and dark brown colours. These data are consistent with the results of our study. The scarcely present omasal papilla ending blindly seen on the omasal lamina are usually observed in grass-roughage eaters (GR) animals (Yamamoto et al., 1998). They have also been determined in this study. Subepithelial connective tissue in sheep has been shown to possess several papillar prominences (Tamate et al., 1979; Yamamoto et al., 1993). These structures are less present in Japanese serow (Yamamoto et al., 1998). Presence of these papilla-type structures is also determined in our study (Figure 4). Cytologically, Schnorr and Vollmerhaus (1967) and Hofmann and Schnorr (1982) have defined the keratinized cells as flattened, baloon and intermediate- type cells. The cells seen in our study were mostly intermediate-type. Occasionaly, they were in resemblance with balloon-type cells.

Consequently, this paper observed the mucosal surface of the forestomach components of seven Akkaraman sheep fed on an intermediate- type feeding, using stereomicroscopy and SEM. The ruminal papillae present on all of the surface of the rumen except the ruminal pila, varied considerably in shape and size, some of which possessing asymetrical doubled-apices. The cells in the ruminal mucosa were mostly intermediate-type cells. Certain papilla types on the reticulum and omasum were not eminent at macroscopical level, if present, comprising mostly one, occasionally double ridges. The epithelial scrap layer formed by the horny cells was eminent, indicating regeneration of the papilla. The results have indicated the characteristics of the animals fed on intermediate- type feeding.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for the present study.

CONTRIBUTIONS

Concept – ŞHA; Design – ŞHA; Supervision – ŞHA; Resources - ŞHA; Materials – ŞHA, IK; Data Collection and/or Processing – ŞHA, IK; Analysis and/or Interpretation – ŞHA, IK; Literature Search – ŞHA, IK, LT; Writing Manuscript – ŞHA, IK, LT; Critical Review – ŞHA, IK, LT.

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OPSERVACIJE SLUZNICE PREDŽELUCA KOD AKKARAMAN OVCE PREHRANJIVANE SLAMOM I KONCENTRATOM KORIŠTENJEM STEREOMIKROSKOPIJE I SKENIRAJUĆE ELEKTRONSKE MIKROSKOPIJE

SAŽETAK

Istraživanje je provedeno sa ciljem evaluacije površine sluznice komponenti predželuca korištenjem stereomikroskopije i SEM-a kod sedam ovaca soja Akkaraman hranjenih sa slamom i koncentratom. Uzorci su dobijeni od životinja koje su hranjene sa 60% slame i 40% koncentrata, zaklanih u lokalnoj klaonici. Rezultati su pokazali da su na cijeloj površini buraga bile prisutne ruminalne papile, osim u području pila. Njihov oblik je znatno varirao veličinom i oblikom, od kratkih lingualnih do dugih i širokih u obliku lista. Neke papile su imale asimetrične duple vrhove. Ćelije sluznice buraga su većinom bile intermedijarnog tipa. Mjestimično im je oblik ličio ćelijama balonastog tipa. Jasno su prikazane i retikularne kriste u kapuri i lamina omasuma, s tim da pojedini tipovi papila sa svojim osobitostima nisu bili izraženi na makroskopskoj razini. Na mikroskopskoj razini su u lamini omasuma postojale strukture tipa papila koje su imale glatku površinu i jedan, ponegdje dva grebena. Sloj oljuštenog epitela kojeg čine ćelije u obliku roga je bio upadljiv ukazujući na regeneraciju papila. Rezultati pokazuju karakteristike životinja koje se hrane slamom i koncentratom.

Ključne riječi: Intermedijarni tip jedača, ovce, komponente predželuca, SEM.

SHORT COMMUNICATION

INTRAUTERINE TABLET TREATMENT ACCOMPANIED WITH PARENTERAL APPLICATION OF CEFTIOFUR IN ENHANCING OF REPRODUCTIVE EFFECTIVENESS OF DAIRY COWS IN THE MUNICIPALITY OF SANSKI MOST

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ABSTRACT

Manual assistance during parturition will often contribute to uterine contamination, which may lead to development of uterine infections. About 76% of cows with assistance during parturition, placental retention, or both will develop metritis. Uterine function is often compromised by bacterial contamination during and after parturition, where pathogens persist in the uterine lumen for a long time causing chronical disorders, which decrease fertility. The study have been conducted in the area of municipality Sanski Most, and, in total, 30 cows were included. Reasons for clinical interventions and treatment during the puerperium were mostly dystocia, placental retention and uterine prolapse. According to a type of antibiotic therapy, animals were divided in two groups, the first to receive antibiotics in the form of intrauterine tablets and the second to receive intrauterine tablets together with parenteral administration of ceftiofur. The most common pathological process in both groups was retention of the placenta. Group of animals that had received intrauterine tablets and parenteral ceftiofur had better reproductive parameters and achieved ideal intercalving intervals of 12 months.

Keywords: Dystocia, fertility, metritis, placental retention, therapy

INTRODUCTION

Manual assistance by a farm's personnel or veterinarians during parturition will often contribute to uterine contamination, which may lead to development of uterine infections. Average incidence of dystocia is 2 to 10%, but manual assistance during parturition is common in ≥50% of cases (Kaya et al., 2012). About 76% of cows with assistance during parturition, placental retention, or both will develop metritis (Lewis, 1997), while endometritis is more common in malnourished cows (Heuer et al., 1999).

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Uterine infections are generally classified according to clinical signs and a degree of severity. Metritis indicates that all layers of the uterine wall are inflamed, while endometritis indicates that the endometrium is inflamed, which is considered to be the least severe uterine infection (Lewis, 1997; Sheldon and Owens, 2017). Metritis as inflammation of the uterus caused in most cases by bacteria is characterized by enlargement of the organ and accumulation of the watery red-brown or viscous off-white purulent uterine discharge (Sheldon et al., 2018). The severity of metritis is categorized by the signs of the animal's health, from mild disease to toxaemia. Clinical endometritis is defined as the presence of a purulent discharge from the vagina, while subclinical endometritis, since it is harder to be observed, represents more serious and an emerging issue. Results by Domecq et al. (Domecq et al., 1997) represent the most common health disorders in multiparous cows: metritis (19.9%), placental retention (8.7%) and dystocia (6.5%).

Uterine function is often compromised by bacterial contamination during and after parturition, and pathogens continue to persist in the uterine lumen for a long time causing chronic disorders, which often decrease fertility (Savio et al., 1990). Uterine infections postpartum are results of contamination with G^+ i G^- aerobic and anaerobic bacteria during parturition. Inflammatory processes in the uterus are expected after parturition and usually resolve 14 to 28 days postpartum (Brick, 2011). Cows with a great risk to develop uterine disorders are those with dystocia, parturition assistance, placental retention, stillbirth, twins and milk fever (Lima et al., 2009; Oliviera et al., 2020). Uterine infections like metritis itself may lead to > 150 days of the service period (Britt, 1975). In the first 10 days postpartum, in the uterus lumen *Streptococcus spp*, *Staphylococcus spp* and *Bacillus spp* are isolated without clinically apparent signs of disease, while *Trueperella pyogenes*, *Escherichia coli*, *Fusobacterium necrophorum*, and *Bacteroides spp*. are dominant in clinically affected animals (Földi et al, 2006).

The main goal of chemoprophylactic treatment is to enhance uterine defence and tissue regeneration as well as to remove the inflammatory component (Sheldon et al., 2006). Ideally, such treatment should eliminate pathogens and shorten a withdrawal period for meat and milk (Lewis, 1997), like ceftiofur (Kaya et al., 2012). Intrauterine administration of medications ensures high concentrations of an active substance in the uterine lumen and endometrium, while systemic administration may not result in sufficient and effective concentration of an active substance in the uterine lumen and layers (Földi et al., 2006). In recent years, cephalosporines have proven a good therapeutical effect against G^+ and G^- bacteria recognized as uterine pathogens (Chester et al., 2004), with benefits in the later reproductive performances (Brick, 2011). Ceftiofur from the third generation of cephalosporines has a broad spectrum of effectivness, and, after parenteral administration, minimal inhibitory concentrations in all uterine layers and lochias are quickly achieved ,and metritis incidence in cows with placental retention is reduced (Drillich et al., 2007).

The current study aims to determine the beneficial effects of additional ceftiofur administration in the prevention of early puerperal infections and enhancement of later reproductive performances, compared to traditional/conventional therapy with intrauterine tablets only.

MATERIALS AND METHODS

The study has been conducted in the area of municipality of Sanski Most in 2021. This area has a long tradition in cattle breeding.

Animals

Initially, 40 cows were chosen, but some were excluded due to the additional diagnostics and therapeutic procedures. Hence, 30 cows of different breeds were included in the study (Simmental, Holstein, Oberintal and Montafon). All cows had satisfactory or good zoohygienic conditions, were kept in tie-stalls during the night, while the rest of the day at free-stall system. Machine milking have been performed twice daily. The voluntary

waiting period for the next reproductive protocol was at least 40 days. Cows in the study were 1-6 lactations old. According to clinical signs during examination like difficult birth, increased body temperature, abnormal vaginal discharge, visible placental tissue, etc., the diagnose was formed, while the day of the first therapy, type of therapy and later reproductive performances were taken during the study period.

Groups and therapy

Cows have been divided into two groups considering as much as possible equal representation of different postpartum disorders, severity of clinical signs, age and breed. The control group (n=12) had cows of several breeds like Simmental (n=7), Red Holstein (n=3) and Montafon (n=2), while the experimental group (n=18) had Simmental (n=12), Black Holstein (n=2), Red Holstein (n=2), Montafon (n=1) and Oberinntal/Tyrol Grey (n=1) breed.

The experimental group, after definitive diagnosis, had parenteral administration (intramuscular) of antibiotic ceftiofur hydrochloride (Neoceftiofur HCL 5%, "FM Pharm" d.o.o. Subotica, Serbia, 1ml = 50 mg) in a dose of 1ml/50 kg of body weight in the duration of 5 days. Beside administration of Neoceftiofur HCL, 5% intrauterine tablets were used additionally as the traditional way of treating puerperal uterine disorders. Applied intrauterine tablets consisted of rifaximin (Fatroximin, tablets, 1 tablet = 300 mg of rifaximin) used in a dose of 4 tablets or oxytetracycline chloride (Geomicin F, Genera DD, Rakov Potok, Croatia, 1 tablet = 1g of oxytetracycline chloride) used in a dose of 1-2 tablets. In total, among cows in the experimental group, 8 cows received rifaximin and 10 cows received oxytetracycline tablets besides ceftiofur injections.

The control group was treated traditionally with intrauterine tablets only (Fatroximin in 8 cows and Geomicin F tablets in 4 cows). Intrauterine tablets were used according to instructions of the manufacturer after clinical findings.

In the cases of placental retention, remnants of the placenta were expelled manually prior to the therapy. The same applied to the cases of uterine prolapse/ reposition. In both groups the data about lactation number, body condition score (visually determined), diagnosis and applied therapy had been recorded.

Artificial insemination and pregnancy check

Artificial insemination has been performed after the clinical examination (vulva appearance, presence and characteristics of estral mucus) and with the assistance tool for estrus detection and determination of optimal insemination time - Draminski estrus detector (measure of electric resistance of estral mucus). Diagnosis of pregnancy was established with ultrasonography using a mobile device with a linear endorectal probe (Mindray DP-20, probe 75L50EAV 5-10 MHz) in a period of 35 to 40 days after artificial insemination.

Reproductive parameters and statistical analysis

After the treatment, data for all animals were noted (reproductive results): a period to detection of first estrus postpartum, a period to first postpartum artificial insemination, success of the first and eventually repeated artificial insemination and the length of the service period. Analysis of associated variables (breed, lactation, diagnosis and the beginning of therapy) was done by stratification within groups according to the variable categories and calculation of the average values (median values) and a range of noted values for every group, separately. Results have been presented in the tables to point at the differences between groups according to associated variables (control confounding).

To present initial variables (reproductive results), descriptive statistical analyses were conducted for continued variables presented as calculated median value and standard error of median regarding calculated proportions for nominal initial variables. Comparation of median and proportions between two groups were conducted

using two-way T-test, assuming different variances and Chi-square test for comparation of proportions interpreted for a level of statistical signifficance of 5% (α=0.05).

RESULTS

Determined parameters of general health as well as pathological conditions during parturition and puerperium, separately for the control and the experiment group, were shown in Table 1.

Table 1 Presentation of certain pathological conditions in experimental and control group

Experimental group		Control group	
Diagnosis	Ratio of animals	**Diagnosis**	Ratio of animals
Dystocia	(33.3%)	**Dystocia**	(8.3%)
Retention of placenta (with 1 case of ketosis and milk fever)	(55.5%)	**Retention of placenta**	(75%)
Uterine prolapse	(11.1%)	**Uterine prolapse**	(16.6%)

According to Table 1, the most common pathological process in both groups was retention of the placenta, mainly alone and sometimes accompanied with ketosis or milk fever.

Table 2 Breed, average lactation, postpartum beginning of therapy and body condition status in experimental and control groups

		Control group (n)	Experimental group (n)
Number of animals		12	18
Breed	Simmental	7	12
	Holstein Friesian	3	4
	Other breeds	2	2
Average lactation number		3 (1-6)*	4 (1-5)*
Diagnosis	Dystocia	1	6
	Retention of placenta	9	10
	Uterine prolapse	2	2
Average days of postpartum beginning of therapy		2 (1-2)*	2 (1-5)*
Body condition status		3.3	3.2

* median and range

Table 3 Incidence of placental retention among breeds

Breed	Experimental group	Control group
Simmental	38.8% (n=7)	50% (n=6)
Holstein Friesian	16.6% (n=3)	5.5% (n=1)
Montafon		11% (n=2)

Table 4 Reproductive results achieved after therapy in control and experimental group (median and standard error of the mean) as well as values of statistical testing and statistical differences (p<0,05). In control group, where only intrauterine tablets were used as a routine way of treatment, many reproductive parameters are lower comparing to experimental group, where ceftiofur was applied together with intrauterine tablets

	Days PP* until observed estrus	Days PP* until AI**	Pregnancy after first AI** (%)	Pregnancy after second AI** (%)	Total pregnancies (%)	Service period
Control group (n=12)	91.42 (7.51)	96.92 (8.83)	41.7%	85.7%	91.6%	114.17 (9.67)
Experimental group (n=18)	**66.5 (6.57) *****	76.61 (7.19)	61.1%	85.7%	94.4%	**85.67 (7.55) *****

Bolded numbers represent comparisons found to be significantly different.
* Postpartum
** Artificial insemination
***Level of significance 5%

According to comparisons of averages by t- test, statistically significant difference between the experimental and control group is confirmed for difference in days PP until observed estrus (p = 0.019) and difference in duration of the service period (p=0.029). Other comparisons by t-test (difference in days PP until AI) and by chi-square test (proportion of pregnancy after AI) were not found significantly different at set level of statistical significance (p<0.05).

DISCUSSION AND CONCLUSIONS

Numerous professional literature reports about the negative effects of uterine infections to reproductive performances (Schultz-Rajala and Gröhn, 2000) and delay of estrus and ovulation (Benmrad and Stevenson, 1986) exist. This delay of first observed estrus and artificial insemination is very visible in the control group, which had a lot of retained placentas. This condition is related to developing of metritis, which is identified as a main reason for decreased fertility in cows (Drillich et al., 2007) visible in the control group, which had negative statistical significance in a time interval to observed estrus and the service period.

Cows with a high risk of uterine disorders are those with dystocia, parturition assistance, retention of the placenta (Dubuc et al., 2011; Lewis, 1997) as well as stressful conditions and poor body condition which decrease immunity (Brick 2011). According to Sheldon (Sheldon et al., 2006), metritis and endometritis are common in dairy cows and, in order to prevent them, active control of the puerperium has to be undertaken. Application of intrauterine tablets gives high concentrations of an active substance in the uterine lumen and endometrium, and little is absorbed in the systemic blood stream, while parenteral administration often does not give enough of inhibitory concentrations in the uterus (Földi et al., 2006). Meanwhile, in Europe, it is still a common practice to deal with retention of the placenta manually and use intrauterine tablets. However, in recent years, many reports suggest that this way of treatment decreases uterine defense mechanisms, disturbs later fertility and decreases milk production (Drillich et al., 2007). Because of the above mentioned, recommended treatment is parenteral administration of therapeutics like the third generation of caphalosporines, ampicillin, penicillin, oxytetracycline, cloxacillin and sulphonamids (Espadamala et al., 2018). Our

experimental group was administered intrauterine tablets and ceftiofur parenterally. Ceftiofur was used for 5 days, and tablets are effective only one day, which can explain more positive effect against microorganisms and better reproductive parameters.

Puerperal metritis usually occurs within the first two weeks postpartum (Brick, 2011; Földi et al., 2006) as a result of extensive bacterial contamination (usually 5-10 days), caused by microbes like *Escherichia coli*, *Fusobacterium necrophorum*, *Bacteroides spp*. and *Trueperella pyogenes*. Because of mixed infection, this disorder is treated with medicaments effective against G^- and G^+ bacteria. Frequency of metritis in dairy cows is in the range 10 to 36% (average 23%), mostly in the first 10 days postpartum (McLaughlin et al., 2012). However, most of our therapies began on the first (dystocia) or second day (retention of placenta, uterine prolapse) postpartum, which probably helped for contaminants in the uterine lumen to be eliminated earlier, while the obtained reproductive parameters were desirable.

Metritis is common after dystocia and retained placenta, when it often spreads into endometritis, causing protracted consequences to fertility (Feu et al., 2009; Reist et al., 2003) due to chronic inflammation of the endometrium. Cows with retained placenta or metritis have double chances to get subclinical endometritis after 30 days postpartum (Galvão et al., 2009). Usually, clinical endometritis is present in 14 - 40%, while *Escherichia coli* and *Trueperella pyogenes* are common isolates. Used antibiotic protocol has to be effective against these bacteria in order to reduce clinical endometritis and enhance reproductive performances (Kasimanickam et al., 2016).

Timely diagnostics and therapy of cows with retention of the placenta and metritis are very important, because later pregnancy rates are similar to healthy cows (Risco et al., 2007). Based on a short time period to observed first estrus postpartum, high pregnancy rates and the short service period in the experimental group suggested that the combination of parenteral administration of ceftiofur and intrauterine tablets gave the same results as in animals without disturbed function of genital organs during puerperium. Many intrauterine tablets as an active substance have oxytetracyclines, but several studies report about increased resistance of uterine-isolated *T. pyogenes* to oxytetracyclines (Ziv et al., 1995), and there is also an irritating effect to the endometrium. Parenteral administration of oxytetracyclines usually doesn't achieve minimal inhibitory concentrations in the uterine layers and lumen. Because of mentioned facts, more and more often in therapy of uterine infections parenteral administration of antibiotics is used. Time required for achieving minimal inhibitory concentrations of ceftiofur in serum, endometrium and lochia is 1.2 - 1.5 hours (Wang et al., 2018). Intrauterine tablets as a traditional way of treatment probably still have positive effects against most microorganisms responsible for metritis or endometritis, but their negative effect inside the uterine lumen lasts longer, while estrus detection, pregnancies after first AI and the service period are lower in comparison to the experimental group that used ceftiofur as additional help.

During the last several years, in many countries ceftiofur has been used in the treatment of retained placenta, and many studies report about clinical recovery, but not improvement in reproductive performances (Haimerl and Heuwieser, 2014). This was not the case in our experimental group, where almost all animals (94.4%) become pregnant during ideal three months of the service period. Piccardi et al., (2016) reported that after ceftiofur administration in cases of retained placenta, metritis incidence was reduced and clinical cure rate improved, probably because of decreased number of microorganisms in the uterus. However, Oliviera (Oliviera et al., 2020) also reported about later detrimental effects to fertility, milk production and culling despite the therapy. Our results do not agree with Oliviera et al. (2020) in a part of a detrimental effect to fertility and culling, especially in the experimental group. One of the explanations could be the breed of the cows, where Oliviera et al. had in their study Holstein

cows, while in our study, most of the cows were Simmental breed.

Giuliodori et al. (2013) had also treated a group of Holstein-Friesian cows affected by puerperal and clinical metritis with ceftiofur, which have resulted in the service period of 129 and 111 days, while their control group of healthy cows had 109 days. Their control group have similar results to our control group, but cows treated with ceftiofur had a significantly shorter service period, which could be also related to breed, as previously mentioned by Oliviera et al. (2020).

Many authors report that systemic administration of ceftiofur during 3 consecutive days in cows with placental retention is successful at achieving minimal inhibitory concentrations against pathogens like *E. coli* and *T. pyogenes* (Drillich et al., 2006; Krueger et al., 2013), and this effect could be extended with additional administration for one or two days, like it was in our experimental group. Other authors (Lima et al., 2019; Piccardi et al., 2016) report that the clinical rate of recovery from metritis should be 75-80% after administration of ceftiofur for 3-5 days, which was probably the case in the experimental group having statistically significant shorter period to first observed estrus and service period.

Mellado et al. (2017) report that the treatment with ceftiofur in duration of three days did not give desirable results in prevention of infection, and they suggest treatment for 5 or 6 days. Something similar is reported by Kaya et al. (2012), where treated animals had a significantly shorter service period and the good pregnancy rate. Results of those authors coincide with our results as well.

Our study indicates that the most common cause for veterinary interventions in early puerperium was placental retention. Treatment of cows by utilization of intrauterine tablets alone or in combinations with ceftiofur has given a very high rate of pregnant cows in both groups. However, additional administration of ceftiofur had better success of first insemination postpartum and had statistically significant better results in shortening days to first observed estrus and duration of the service period. This additional utilization of ceftiofur has enabled ideal intercalving interval of 12 months to be achieved in the experimental group.

Additional application of ceftiofur had a good effect in dealing with prevention of uterine infections during the early postpartum period, especially in Simmental breed, and could probably be used as the only medicament.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHOR'S CONTRIBUTIONS

BČ and SB conceived and designed the study, carried out the experimental work, made substantial contributions to acquisition, analysis and interpretation of data and participated in manuscript writing.SŠH performed the statistical analysis and made a substantial contribution to interpretation of data and manuscript writing. AĆ, LV, AM, PB, AHP AŠR made substantial contributions to writing the manuscript, critically revised the manuscript and approved its submission.

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INTRAUTERINO LIJEČENJE UZ PARENTERALNU APLIKACIJU CEFTIOFURA U POBOLJŠANJU REPRODUKTIVNE EFIKASNOSTI MLIJEČNIH KRAVA U OPĆINI SANSKI MOST

SAŽETAK

Manuelna pomoć tokom poroda doprinosi kontaminaciji uterusa i postaje važan faktor u razvoju infekcija uterusa. Otprilike 76% krava koje su imale asistencije prilikom poroda, zaostajanju posteljice ili oboje će razviti metritis. Funkcija uterusa je često narušena bakterijskom kontaminacijom u toku i nakon poroda, gde ti patogeni perzistiraju u lumenu uterusa duži vremenski period, uzrokujući hronične poremećaje koji smanjuju plodnost. Istraživanje je sprovedeno u općini Sanski Most i uključivalo je 30 krava. Razlozi za kliničke intervencije i terapiju su uglavnom bili distocia, zaostajanje posteljice i prolaps uterusa. Prema vrsti antibiotske terapije životinje su se podijelile u dvije grupe, prvu koja je dobivala samo intrauterine tablete i drugu koja je primala intrauterine tablete i parenteralno ceftiofur. Najčešći patološki proces u obje grupe je bila zaostala posteljica. Grupa životinja koja je dobivala ceftiofur je imala bolje reproduktivne parametre i dostigla je idealan međutelidbeni period od 12 meseci.

Ključne reči: Distocia, mjeseci, plodnost, terapija, zaostala posteljica

CASE REPORT

ASSESSMENT OF THE HYGIENIC CORRECTNESS OF DRINKING WATER ON THE FARM OF SOUTH AMERICAN CHINCHILLA BEFORE AND AFTER "SHOCK" TREATMENT WITH STABLE LIQUID CHLORINE DIOXIDE: A CASE REPORT

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ABSTRACT

Chinchillas consume low daily amounts of water, and due to their sensitivity and susceptibility to digestive tract diseases, they require extremely high-quality water. For the conventionally used disinfectants it is difficult or almost impossible to reach the standards of cleanliness of water distribution systems. Stable liquid chlorine dioxide is a disinfectant, which successfully and provenly removes most microorganisms and has no negative effect on the equipment and animal health.

The aim of the study was to investigate a desinfectant potential in establishing the microbiological purity of the water tank at critical points before and after treatment with chlorine dioxide. The results show that microbiological contamination at both critical points is higher before than after treatment.

In conclusion, more frequent control of water quality is needed as well as more frequent disinfection of the water distribution system on the farm. Also, stable liquid chlorine dioxide can be a desinfectant of choice in order to meet the criteria prescribed by the Rulebook on the Healthiness of Drinking Water.

Key words: Stable liquide chlorine dioxide (Cl_2O), disinfection, water hygiene

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INTRODUCTION

Chinchilla originates from South America, more specifically from central Chile, Bolivia and Peru. It is an animal from the order Rodentia (rodents) (Quesenberry, 2015), and there are several types of chinchilla: *Chinchila chinchilla* (large or royal chinchilla), *Chinchila brevi caudata* (small short-tailed chinchilla) and *Chinchila lanigera* (small long-tailed chinchilla). The last two mentioned chinchilla species exist in nature (wilderness of the Andes), but their population is rare today, because the local population, in addition to their fur, also hunts them for their meat (Katica and Crnkić, 1999; Spotorno et al., 2004).

Chinchila chinchilla (large or royal chinchilla) and *Chinchila lanigera* (small long-tailed chinchilla) are listed as critically endangered species by the International Union for Conservation of Nature due to drastic population decline (IUCN, 2013).

The beginning of chinchilla farming dates back to 1923, with the arrival of 13 individuals from Chile to the United States. In this way, the number of individuals on the first established farm multiplied (Katica and Crnkić, 1999; Richardson, 2003; Hsu et al., 2015). The trend of farming chinchillas was actualized in Europe in the middle of the last century. Thanks to farm breeding, this rodent species has been saved from extinction, since in South American countries it is exploited for human consumption and mass exploitation of its high-quality fur (Katica and Crnkić, 1999; Katica and Delibegović, 2019).

Access to quality, fresh hay must be provided at all times with fresh water to maintain dental and digestive health (Donnelly and Schaeffer, 1997). Daily water intake is 2-12ml/100g of body weight at room temperature. Chinchillas drink much more when fed exclusively with hay than with mixed or pelleted food (Katica and Delibegović, 2019; Wolf et al., 2020). Although drinking tap water is adequate, excessive chlorine can adversely affect general health (Hoefer and Crossley, 2001).

Also, in addition to this, for the successful intensive farming of chinchillas, adequate accommodation and necessary hygiene of equipment and inventory inside the cages with which the animals are in contact is important.

Opportunistic bacterial infections in chinchillas can cause disease, either localized to one organ or as septicemia. Animals that become ill are usually immunocompromised due to, for example, age, malnutrition or factors related to breeding (poor hygiene, poor ventilation, contaminated food and water). *Enterobacteriaceae* and *Pseudomonas aeruginosa* are associated with significant morbidity and mortality in chinchillas. However, these agents can also be isolated from clinically healthy animals, so most of these agents are not considered primary pathogens (MSD MANUAL, 2023). Stress or contaminated drinking water are predisposing factors for the development of infection with *Pseudomonas aeruginosa*.

In the context of maintaining the necessary inventory hygiene and drinking water sanitation inside the premises of the chinchilla farm, numerous disinfectants are used in practice. One of these is stable liquid chlorine dioxide, which is increasingly used for these purposes (Ališah, 2020). In a stable liquid form, chlorine dioxide is very easy to use in all types of water sanitation, both for drinking and for all other categories (swimming pools, waste water, etc.). It is characterized by stability at a wide range of temperatures (10 ^{0}C to 40 ^{0}C), as well as pH values (from 1.5 to 10) (Ališah, 2020).

Stabilized liquid chlorine dioxide is a yellow liquid with a chlorine smell. It does not contain aldehydes and does not foam, and works very efficiently even at low concentrations. It has a wide range of effects on all types of microorganisms, so it can be said that it is bactericidal, including sporogenous bacteria, virucidal, fungicidal and algicidal. After application on work surfaces, it is not necessary to rinse since, apart from negligible amounts of chloride, it leaves no residues or odors (Gagić et al., 2013).

We present a case report from one of the farms of South American chinchillas in the Canton of Sarajevo, that is, an assessment of the hygienic

Picture 1 Aspect of the chinchilla farm cage

correctness of drinking water before and after the "shock" treatment with stable liquid chlorine dioxide in the water distribution system of the farm.

Case description

The farm of South American chinchillas is located on the outskirts of Sarajevo Canton. The farm premises where the mentioned animals (*Chinchilla lanigera* - small long-tailed chinchilla) are grown meet all the standards of good production practice and have built-in equipment for temperature and air humidity regulation (temp. 23.5°C, relative humidity about 60%), which follows daily.

The animals are housed individually in cages, and 60 cages belong to one so-called battery (Picture 1). The dimensions of the cage are: width 40 x length 50 x height 35 cm. Each cage has a feeder as well as drinking bowls that end in the form of a nipple in the cage.

Picture 2a Critical point I – water tank

Picture 2b Critical point II - nipple system

Chinchillas are fed with conventional pelleted food, which consists of pressed grains with appropriate vitamin and mineral supplements. The interior of the cage is organized in such a way that the animals are kept on plastic trays lined with spruce and pine sawdust, and the mat is changed weekly and more often, if necessary. All animals in the cages appeared clinically healthy. We have identified critical points based on the results of our previous research, which prove that biofilm formations which are created in the interior of closed water distribution systems, are one of the leading both health and hygiene problems on animal farms (Ališah, 2020; Jović, 2020).

After defining the critical points and marking them, we started with the sampling of the water consumed by the animals on the farm. The first critical point is the water tank from which the water is supplied to the chinchillas through the hoses (Picture 2a), while the second critical point is the end of the hose-nipple system in the cages (Picture 2b).

The water tank has a volume of 10 liters and is washed and disinfected daily. Water from the city's water supply is poured into it daily, and if necessary, more often, which is under the regular hygiene and sanitary supervision of the Institute for Public Health of the Canton of Sarajevo. From the water tank, water is distributed evenly through a rubber hose with a diameter of 0.5 cm, free-falling, to each cage (Picture 2a), which ends with a nipple (Picture 2b). Water samples were taken on two occasions. On the first, two samples were taken, one from the vessel marked "Inlet-before-treatment vessel", which is located on top of the battery (Picture 2a). We poured the water from the water tank directly into a sterile bottle. After that, another sample was taken from the water distribution system, that is, from the nipple from one of the cages at the bottom of the battery in such a way that the water was allowed to flow directly into the sterile bottle.

We chose the position of the bottom of the battery because we wanted to be sure that the sample we collected was water that had passed through the entire water distribution system. We labeled this sample "Last point of sampling - before treatment".

The samples taken were distributed to the laboratory for sanitary microbiology of the Public Institution - Institute of Public Health of the Sarajevo Canton for microbiological testing. The microbiological analysis of the water was performed according to the standard methodology, which is in accordance with the regulations of the Rulebook on Health Suitability of Drinking Water (Official Gazette of BiH, 2017).

Furthermore, we approached the so-called "shock" water treatment with a disinfectant based on stable liquid chlorine dioxide. Since it was a concentrated agent, it was necessary to make a solution. According to Ališah (2020), the appropriate dose of the mentioned disinfectant was determined as 40 ml/10L of water added to the water tank, previously emptied and refilled with tap water.

After that, we put the water tank back on top of the battery and connected it to the water distribution hose. To stop animals to drink this water, we separated all the hoses from the cage. In order to make sure that the solution entered the entire system evenly, we let the water from the pipe flow freely, until we smelled residual chlorine dioxide at all hose outlets. This was a sign that the entire system was filled with the used solution. Then, the entire system was closed and the solution was left to act for 60 minutes.

At the end of the scheduled time, the water with the solution was allowed to flow out of the entire system, after which we rinsed it with "new" water from the tap, in such a way that we filled the water tank and let the entire amount of new tap water pass through the system. We repeated the entire procedure of taking samples from the same defined critical points: "Inlet water tank - after treatment" and "Last point of sampling - after treatment".

Table 1 lists the results of the microbiological examination of the first critical point, the drinking water from the water tank before treatment and after it had previously been adequately treated with the "shock" treatment with stable liquid chlorine dioxide.

Table 1 Comparison of results before and after "shock" treatment of the critical point "water tank"

Test parameter	Unit of measure	Test result BEFORE	Test result AFTER TREATMENT	Reference value	Analytical method
Escherichia coli on 36°C±2°C;21h±3h	Cfu/100ml	<1 cfu	<1 cfu	0	MF BAS EN ISO 9308-1:205
Enterococcus spp. na 36°C±2°C;44h±4h.	**Cfu/100ml**	**50 cfu**	**<1 cfu**	**0**	**MF TM203:2021**
The number of coliform germs on 36°C±2°C;21h±3h	Cfu/100ml	<1 cfu	<1 cfu	0	MF BAS EN ISO 9308-1:205
Total number of live germs, 22°C±2°C;68h±4h	Cfu/ml	<1 cfu	<1 cfu	100	BAS EN ISO 6222:2003
Total number of live germs, 36°C±2°C;44h	**Cfu/ml**	**280 cfu**	**<1 cfu**	**20**	**BAS EN ISO 6222:2003**
Pseudomonas aeruginosa on 36°C±2°C;44h±4h.	**Cfu/100ml**	**80 cfu**	**<1 cfu**	**/**	**MF BAS EN ISO 16266:2009**
Sulfite-reducing anaerobes (Clostridia) on 37°C±1°C; 20h±4h ili 44h±4h.	Cfu/50ml	<1 cfu	<1 cfu	/	MF BAS EN 26461-2003
Staphylococcus aureus on 36h±2h; 21h±3h	Cfu/100ml	<1 cfu	<1 cfu	/	Membrane filtration method

Table 2 lists the results of the microbiological examination of the second critical point, the drinking water from the end of the hose-nipple before treatment and after it had previously been adequately treated with the "shock" treatment with stable liquid chlorine dioxide.

Table 2 Comparison of the obtained test results of water samples from the hose before and after the "shock" treatment

Test parameter	Unit of measure	Test res ult BEFORE TREATMENT	Test result AFTER TREATMENT	Reference value	Analytical method
Escherichia coli on 36°C±2°C;21h±3h	**Cfu/100ml**	**90 cfu**	**<1 cfu**	**0**	**MF BAS EN ISO 9308-1:205**
Enterococcus spp. on 36°C±2°C;44h±4h.	**Cfu/100ml**	**60 cfu**	**<1 cfu**	**0**	**MF TM203:2021**
The number of coliform germs on 36°C±2°C;21h±3h	**Cfu/100ml**	**160 cfu**	**<1 cfu**	**0**	**MF BAS EN ISO 9308-1:205**

Test parameter	Unit of measure	Test res ult BEFORE TREATMENT	Test result AFTER TREATMENT	Reference value	Analytical method
Total number of live germs, 22°C±2°C;68h±4h	Cfu/ml	<1 cfu	<1 cfu	100	BAS EN ISO 6222:2003
Total number of live germs, 36°C±2°C;44h	**Cfu/ml**	**160 cfu**	**<1 cfu**	**20**	**BAS EN ISO 6222:2003**
Pseudomonas aeruginosa on 36°C±2°C;44h±4h.	**Cfu/100ml**	**120 cfu**	**<1 cfu**	/	**MF BAS EN ISO 16266:2009**
Sulfite-reducing anaerobes (Clostridia) on 37°C±1°C;20h±4h or 44h±4h.	Cfu/50ml	<1 cfu	<1 cfu	/	MF BAS EN 26461-2003
Staphylococcus aureus on 36h±2h; 21h±3h	Cfu/100ml	<1 cfu	<1 cfu	/	Membrane filtration method

DISCUSSION AND CONCLUSIONS

The fact is that without ensuring a minimum of basic zoohygienic and ethological conditions, there is no successful breeding of South American chinchillas in farm conditions. We started the research at the personal request of the owner of the chinchilla farm, for whom the well-being of the animals is important, in addition to economic profit. Although in the literature and good production practice the emphasis is primarily on housing conditions, microenvironment, qualitative-quantitative food, regular veterinary supervision and preventive therapy, very often, if not always, the importance of drinking water is forgotten, which is evident when trying to find literature data on this topic.

In most farm animals, water represents two-thirds of the daily ration, and in addition to quantity, it must also meet strict quality standards. This means that no matter what is given to animals, it must have the same microbiological composition as for human consumption. Looking back at the rights of animals, which were proposed as early as 1965 and revised and adopted in 1979, it is clearly visible that food and water were put first, among other things, in the context of "freedom from hunger and thirst" (Vučinić et al., 2023).

When talking about the quality of water for chinchillas, there is a deficit in the available literature, so that bottled water is preferred for chinchillas as pets (Anonymous, 2022), and for farmed chinchillas, water from urban water distribution is used (Hagen et al., 2014).

Based on the successful application of the disinfectant based on stable liquid chlorine dioxide in poultry farms of broiler chickens (Ališah, 2020) in various aspects of disinfection, we decided to use this preparation in our study.

Our findings prove the presence of conditionally pathogenic bacteria *Escherichia coli, Enterococcus spp*, the number of coliform germs, the total number of live germs, which, according to the Rulebook on microbiological testing of drinking water: with the addition of the parameter *Pseudomonas aeruginosa*, should not be found in drinking water (Bach, 2018).

From the submitted test results, it is clearly visible that the water taken from the sites defined as critical points and before the "shock" treatment does not meet the criteria prescribed by the Rulebook on the Healthiness of Drinking Water (Official Gazette of BIH, 2017). The water from the position marked - "Water tank-before treatment" does not meet the

criteria regarding the detection of enterococcal bacteria and the total number of live germs in the submitted sample. Water from the position marked "Last point of sampling - before treatment" by far exceeds the number of allowed tested bacteria, and as such is extremely health-hazardous for consumption.

The results of analysis of the samples from Table 1 and Table 2 clearly and unequivocally indicate the effectiveness of the "shock" treatment with the solution of stable liquid chlorine dioxide and its successful elimination of the mentioned bacteria in drinking water.

Since there are evident large gaps in the literature related to analysis of the hygienic correctness of drinking water as well as the use of disinfectants for the disinfection of water distribution systems on farms, we could not adequately compare the results of our study.

When taking anamnestic data from the owner of the farm as well as through our inspection, we found that the animals looked clinically healthy. However, they consumed water that was obviously hygienically incorrect. Looking for a rational explanation of a possibility for the animals to be clinically healthy despite it, the possible answer is that they are not immunocompromised, and that they consumed a sufficient amount of quality, balanced food with satisfactory other zoohygiene aspects. Furthermore, the chinchillas on the examined farm are young adults and their immune system successfully coped with conditionally pathogenic bacteria, *Escherichia coli, Enterococcus spp,* coliform germs, and live germs, as well as *Pseudomonas aeruginosa* (MSD MANUAL, 2023).

It is a wrong assumption that if the water is clean at the source, it is of the same quality at the place where the animal drinks from, which was also proven by this assessment of the hygienic correctness of drinking water. Our experiences with stable liquid chlorine dioxide have proven to be effective. It is significant to scientifically present the importance of hygiene in water distribution systems, because they are the transporters of drinking water.

The obtained results are alarming and indicate the necessity of periodic analysis of the hygienic correctness of drinking water as well as the necessity of disinfection of the water distribution system on the farm.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

CONTRIBUTIONS

AA: 1,2, 5, 6, 7, 8, 9; BA: 3, 6, 7, 8, 9; NK: 7, 8, 9, 10; AH: 4, 5, 6, 10; GA: 3, 5, 6, 8, 10

(1) Conception; **(2)** Design; **(3)** Supervision; **(4)** Fundings; **(5)** Materials; **(6)** Data Collection and/or Processing; **(7)** Analysis and/or Interpretation of the Data; **(8)** Literature Review; **(9)** Writing; **(10)** Critical Review

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PROCJENA HIGIJENSKE ISPRAVNOSTI VODE ZA PIĆE NA FARMI JUŽNOAMERIČKE ČINČILE PRIJE I POSLIJE "ŠOK" TERAPIJE STABILNIM TEČNIM HLORDIOKSIDOM: PRIKAZ SLUČAJA

SAŽETAK

Činčile konzumiraju male dnevne količine vode, ali zbog osjetljivosti i podložnosti obolijevanja digestivnog trakta, zahtjevaju izuzetno kvalitetnu vodu. Konvencionalna dezinfekciona sredstva teško ili skoro nikako ne mogu da zadovolje standarde čistoće vododistributivnih sistema. Stabilni tečni hlor dioksid je dezinfekciono sredstvo, koje uspješno i dokazano odstranjuje mikroorganizme i nema negativno djelovanje na opremu niti na zdravlje životinja.

Cilj rada je bio istražiti dezinfekcioni potencijal u utvrđivanju mikrobiološke čistoće spremnika za vodu, na određenim kritičnim tačkama, prije i nakon tretmana sa stabilnim tečnim hlor dioksidom. Rezultati su pokazali da mikrobiološka kontaminacija, na obje kritične tačke je veća prije, u odnosu na rezultate nakon tretmana.

Kao zaključak se izvodi da je neophodna mnogo češća kvalitativna kontrola vode, kao i redovnija dezinfekcija vododistributivnih sistema farme, a stabilni tečni hlor dioksid je dezinfekciono sredstvo koje, u pogledu efikasnosti, zadovoljava kriterije Pravilnika o zdravstvenoj ispravnosti vode za piće.

Ključne riječi: Stabilni tečni hlordioksid (Cl_2O), dezinfekcija, higijenska ispravnost vode

PROFESSIONAL PAPER

UTJECAJ RAZLIČITIH POSTUPAKA KONZERVIRANJA I TEHNOLOŠKOG PROCESA PRERADE NA KVALITET, HIGIJENSKU ISPRAVNOST I ODRŽIVOST BRČANSKOG PRŠUTA

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SAŽETAK

Goveđi pršut je veoma cijenjen suhomesnati proizvod na teritoriji cijele Bosne i Hercegovine (BiH), koji se proizvodi upotrebom različitih tehnoloških varijanti. Evidentna su istraživanja koja unapređuju tehnološki proces prerade pršuta koji pozitivno utječu na njegov kvalitet, higijensku ispravnost i održivost.

Specifičnost „Brčanskog pršuta" je njegov proces konzerviranja, koji podrazumijeva samo dodatke soli, šećera i vode, bez nitrita, nitrata ili drugih aditiva. Rezultat su poželjne organoleptičke i mikrobiološke karakteristike, koje u isto vrijeme zadovoljavaju zahtjeve potrošača i tako opravdava svoje ime kao prirodna i zdrava hrana.

Istraživanjem su obuhvaćene dvije varijante pršuta; suho soljeni „Brčanski pršut" u trajanju pojedinih faza (10 dana soljenje, 10 dana dimljenje i 10 dana zrenje) i vlažno soljeni „Brčanski pršut" (8 dana soljenje, 10 dana dimljenje i 10 dana zrenje) te skladištenje gotovih proizvoda na dva temperaturna režima (na 7^0C i 15^0C).

Ključne riječi: Brčko, dimljena govedina, pršut

UVOD

U BiH goveđi pršut spada u veoma cijenjene i tražene suhomesnate proizvode. Proizvodi se uz korištenje različitih tehnoloških varijanti. Uslovi proizvodnje i tradicionalne metode konzerviranja mesa tj. soljenje i salamurenje se razlikuju u pojedinim krajevina BiH pa je razumljivo da se po kvalitetu gotovi proizvodi razlikuju jedni od drugih.

Soljenje i salamurenje mesa spada u hemijske metode konzerviranja mesa. Salamurenjem i soljenjem postiže se duža održivost mesa, ali ovim postupkom konzerviranja meso gubi u hranljivoj i biološkoj vrijednosti. Uz pojedine aditive salamureno meso je sočnije, ima bolju

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aromu te mu se na taj način povećava kulinarska vrijednost. Nadalje, oko 70% proizvedenog mesa se potroši kao salamureno meso i to kao vrijedni proizvodi: polukonzerve, konzerve, suhomesnati i kobasičarski proizvodi itd. (Honikel, 2008).

Danas se u svijetu sve više upotrebljavaju gotova jela gdje je meso dobrim dijelom salamureno. Konzerviranje mesa samo kuhinjskom soli obično se zove soljenje mesa, a kod salamurenja mesa upotrebljavaju se pored soli još i nitrati, nitriti, šećer, askorbinska kiselina, polifosfati i drugi aditivi. Svrha soljenja mesa je prvenstveno konzervirajući efekat, a kod salamurenja mesa, osim konzervirajućeg efekta pojačava, odnosno ističu se i poželjna organoleptička svojstva (boja, ukus, konzistencija itd.) (Desmond, 2006).

Dejstvo kuhinjske soli na meso je višestruko; prvenstveno se sastoji u oduzimanju vode, odnosno so smanjuje sposobnost mesa da veže vodu te time mijenja osmotski pritisak mišićnih bjelančevina, zatim usporava rast i razmnožavanje gnjiležnih bakterija i uklanja strani miris mesa. Od nepoželjnih efekata to je razaranje mioglobina što nepovoljno djeluje na boju koja postaje tamno siva, mrka. Povećana koncentracija kuhinjske soli dovodi do pada akitvnosti vode koja je jedan od bitnih faktora za razvoj i opstanak mikroorganizama, inhibira rast većine gnjiležnih bakterija (koncetracija kuhinjske soli od 10 do 15%) (Jo et al., 2020). Halofilne bakterije podnose koncentraciju NaCl od 15 do 20%, a neke i do 25 %, dok najveću otpornost prema NaCl pokazuju halotolerantne bakterije iz roda Leuconostoc, Micrococcus, Sarcina, Pseudomonas i drugi, zatim plijesni i Torula kvasci. Kuhinjska so povećava osmotski pritisak te uzrokuje dehidrataciju bakterijskih ćelija. Štetno djelovanje na bakterije ispoljava se još i preko jona hlora, u inhibiranju proteolitičkih fermenata, smanjenoj topivosti kiseonika i senzibilizaciji prema ugljen dioksidu (Feng et al., 2016).

Na pH mesa kuhinjska so djeluje tako da koči pad pH mesa, ako je prije soljenja bio veći od 6,0, odnosno izaziva porast pH, ako je soljenje izvršeno u fazi kada je pH mesa bio manji od 6,0 (Stankov, 2019). Pored kuhinjske soli od sastojaka salamure značaj imaju i nitriti, nitrati, šećeri, askorbinska kiselina i polifosfati. Od svih pomenutih sredstava pored kuhinjske soli jedino što se koristi je šećer u procesu salamurenju mesa, jer poboljšava ukus i konzistenciju mesa te stvaraju uslove za fiksiranje boje, najčešće glukoze (Berardo et al., 2016).

Šećeri služe jednim dijelom kao izvor energije korisnoj denitrificirajućoj mikroflori u salamuri (bakterije iz roda Vibrio, Micrococcus, Alcaligens, kvascima, itd.), a drugim dijelom u ispoljavanju reduktivnih osobina. Razgradnjom šećera, snižava se pH salamure, čime dolazi do usporavanja razvoja gnjiležnih bakterija (kojima kiselkasta sredina ne odgovara). Snižavanje pH salamure povoljno utječe na postizanje poželjne konzinstencije mesa, a potpomaže i redukciju nitrata. Kao i ostali ingredijenti i šećeri mogu biti kontaminirani nekim vrstama mikroorganizama, a jačina kontaminacije zavisi o sirovini, načinu proizvodnje i o sadržaju vode (Thomas et al., 2013, Sebranek and Bacus, 2007).

Cilj rada je istraživanje koje obuhvata dvije varijante pršuta; suho soljeni "Brčanski pršut" u trajanju pojedinih faza (10 dana soljenje, 10 dana dimljenje i 10 dana zrenja) i vlažno soljeni "Brčanski pršut" (8 dana soljenje, 10 dana dimljenje i 10 dana zrenja) te procjena utjecaja skladištenje pomenutih proizvoda na različitim temperaturnim ambijentima.

MATERIJAL I METODE

Korištene životinje **u istraživanju**

Upotrebljena su goveda, križanci, starije dobi od 10 do 12 godina, ženskog spola. Zaklane su u komunalnoj klaonici u Gornjem Rahiću, Brčko, BiH. Klaonica je udaljena od pogona za proizvodnju suhomesnatih proizvoda 6 kilometara. Nakon primarne obrade mesa, isto je skladišteno na temperaturnom ambijentu od +4^{O}C. Narednog dana butna muskulatura je iskoštena i oblikovana u komade odgovarajuće veličine i tako je pripremljeno sirovo meso za istraživanje proizvodnje "Brčanskog pršuta".

Tehnološki proces proizvodnje "Brčanskog pršuta"

U toku tehnološkog procesa proizvodnje "Brčanskog pršuta" provjerena je opravdanost različitih postupaka konzerviranja natrijevim hloridom (suhi i vlažni postupak) i utjecaj na kvalitet, higijensku ispravnost i održivost finalnog proizvoda. Istraživanjima su obuhvaćene sljedeće grupe uzoraka:

- sirovo oblikovano meso – inicijalni uzorak,
- uzorci mesa konzervirani suhim postupkom do 10 dana,
- uzorci mesa konzervirani vlažnim postupkom do 10 dana,
- uzorci mesa nakon spiranja i cijeđenja,
- uzorci mesa nakon dimljenja do 10 dana,
- uzorci "Brčanskog pršuta" nakon zrenja do 10 dana,
- uzorci "Brčanskog pršuta" suho soljenog nakon skladištenja do 185 dana na različitim temperaturnim ambijentima (+7^{O}C i +15^{O}C),
- uzorci "Brčanskog pršuta" vlažno soljenog nakon skladištenja do 185 dana na različitim temperaturnim režimima (+7^{O}C i +15^{O}C).

Kod navedenih grupa uzoraka sirovog oblikovanog mesa i uzoraka u toku tehnološkog procesa proizvodnje, kao i finalnih proizvoda "Brčanskog pršuta" nakon proizvodnje i skladištenja na različitim temperaturnim režimima, obavljene su sljedeće pretrage:

1. organoleptička pretraga sirovog oblikovanog mesa i "Brčanskog pršuta" u toku proizvodnje, zrenja (fermentacije) i skladištenja,
2. mikrobiološka pretraga sirovog oblikovanog mesa i "Brčanskog pršuta" u toku proizvodnje, zrenja (fermentacije) i skladištenja,
3. fizikalno-hemijska pretraga sirovog obikovanog mesa i "Brčanskog pršuta" u toku proizvodnje, zrenja (fermentacije) i skladištenja,
4. iskorištenost (randman) polaznog (inicijalnog) sirovog mesa, kao i kaliranje proizvoda u različitim tehnološkim fazama proizvodnje i različitim režimima skladištenja.

Dobrobit životinja

Tokom istraživanja, prema korištenim životinjama smo se striktno pridržavali Zakona o dobrobiti životinja Bosni i Herceegovine (Sl. glasnik BiH, br. 25/2009 i 9/2018).

REZULTATI

Rezultati istraživanja prezentirani su Tabelama od 1 do 11.

Srednje vrijednosti mase uzoraka mesa prije soljenja (suho i vlažno) su bile 1014.00 g odnosno 1132.50 g, a nakon završetka tehnološkog procesa (zrenja) iznosile su 604.44 g, odnosno 645.07 g. Kalo za suho, odnosno vlažno soljene uzorke "Brčanskog pršuta" u toku tehnološkog procesa prerade iznosio je 40.39% odnosno 43.04%. Randman za suho soljeni proizvod iznosio je 59.61%, a za vlažno soljeni 56.59% (Tabela 1).

Tabela 1 Kaliranje suho, odnosno vlažno soljenog mesa i po fazama tehnološkog procesa prerade
Table 1 Normative losses in dry- and wet- salted meat per technological process during processing

Faze tehnološkog procesa prerade	Broj uzoraka	Masa u g (srednja vrijednost)			
		Suho soljeno	Kalo %	Vlažno soljeno	Kalo %
Inicijalna vrijednost	10	1014.00	-	1132.50	-
Masa nakon soljenja	10	1025.00	+1.06	1240.00	+9.49

Faze tehnološkog procesa prerade	Broj uzoraka	Masa u g (srednja vrijednost)			
		Suho soljeno	Kalo %	Vlažno soljeno	Kalo %
Masa nakon cijeđenja	10	1023.529	0+.93	1223.52	+8.03
Masa nakon dimljenja	10	622.90	38.57	652.88	42.35
Masa nakon zrenja	10	604.44	40.39	645.07	43.04
Randman		59.61%		56.59%	

U suho soljenim uzorcima mesa, odmah nakon faze soljenja srednja vrijednost sadržaja vode je bila 68.44%, NaCl 3.60%, ukupnog šećera 0.21%, a pH 5.52, odnosno u vlažno soljenim uzorcima vode 73.76%, NaCl 3.10%, ukupnog šećera 0.22%, a pH 5.53. Nakon faze zrenja u suho, odnosno vlažno soljenim uzorcima sadržaj vode se smanjio i srednja vrijednost je bila 50.19%, odnosno 56.84%, a NaCl-a se povećao na 5.52%, odnosno 4.43%, dok je sadržaj ukupnih šećera zadržao istu vrijednost kod oba uzorka, odnosno 0.20%, a pH se u oba slučaja neznatno smanjio na 5.36, odnosno 5.31 (Tabela 2).

Tabela 2 Fizikalno-hemijski parametri u mesu i pršutu tokom tehnološkog procesa prerade
Table 2 Physico-chemical parameters of meat and prosciutto per technological process during processing

Faze tehnološkog procesa prerade	Način soljenja	Broj uzoraka	Voda %	NaCl %	Ukupni šećer %	pH
Inicijalna vrijednost	-	10	74.01	-	-	5.56
Faza soljenja	Suho	5	68.44	3.60	0.21	5.52
	Vlažno	5	73.76	3.10	0.22	5.53
Faza cijeđenja	Suho	5	65.42	4.33	0.20	5.48
	Vlažno	5	72.28	3.92	0.22	5.47
Faza dimljenja	Suho	5	51.15	5.10	0.19	5.42
	Vlažno	5	57.85	4.20	0.19	5.40
Faza zrenja	Suho	5	50.19	5.52	0.20	5.36
	Vlažno	5	56.84	4.43	0.20	5.31

U sirovom mesu inicijalne srednje vrijednosti aerobnih mezofilnih bekterija su utvrđene u broju $5.0x10^3$/g, psihrofilne $0.3x10^2$/g, halofilne bakterije $0.5x10^2$/g, enterobakterije $0.6x10^2$/g i mikrokoke $0.2x10^2$/g. Najveća srednja vrijednost aerobnih mezofilnih bakterija u uzorcima suho, odnosno vlažno soljenog mesa je utvrđena nakon cijeđenja i kretala se od 7.1 $x10^3$/g, odnosno 7.5 $x10^3$/g, a u

manjem broju utvrđene su u finalnom proizvodu – nakon zrenja i kretale su se 5.3 x10^3/g, odnosno 5.5 x10^3/g. Najveća srednja vrijednost psihrofilnih bakterija utvrđena je u finalnom proizvodu i kretala se od 1.8 x10^2/g do 2.0 x10^2/g (suho, odnosno vlažno soljeni proizvod), a najmanji broj bakterija u mesu nakon soljenja i kretao se od 0.5 x10^2/g do 0.7 x10^2/g. Halofilne bakterije u najvećem broju su utvrđene u finalnom proizvodu – nakon zrenja i kretale su se od 5.9 x10^2/g do 6.1 x10^2/g (suho, odnosno vlažno soljeni proizvod). Najmanji broj halofilnih bakterija utvrđen je u mesu nakon soljenja i srednja vrijednost se kretala od 1.0 x10^2/g do 1.2 x10^2/g. Enterobakterije i mikrokoke nakon soljenja i u daljnjem toku prerade nisu izolovane (Tabela 3).

Tabela 3 Dinamika mikroorganizama u mesu tokom tehnološkog procesa proizvodnje do finalnog proizvoda
Table 3 Dynamics of microorganisms in meat during technological process of production to final product

Vrsta uzorka	Broj uzoraka	Način soljenja	Aerobne mezofilne bakterije u 1 g (x10^3)	Psihrofilne bakterije u 1 g (x10^2)	Halofilne bakterije u 1 g (x10^2)	Entero- bakterija u 1g (x10^2)	Mikrokoke u 1g (x10^2)
Sirovo meso	10	-	5.0	0.3	0.5	0.6	0.2
Soljeno meso	10	Suho	6.0	0.7	1.2	negativne	negativne
	10	Vlažno	6.5	0.5	10	negativne	negativne
Meso nakon cijeđenja	10	Suho	7.1	1.1	33	negativne	negativne
	10	Vlažno	7.5	1.0	30	negativne	negativne
Meso nakon dimljenja	10	Suho	5.8	1.7	42	negativne	negativne
	10	Vlažno	6.3	1.6	40	negativne	negativne
Meso nakon zrenja	10	Suho	5.3	1.8	59	negativne	negativne
	10	Vlažno	5.5	2.0	61	negativne	negativne

Napomena: salmonele, koagulaza pozitivne stafilokoke, sulfitreducirajuće klostridije, *E. coli* te proteus vrste nisu izolirane u ispitivanim uzorcima.

Kaliranje izraženo u srednjoj vrijednosti, suho, odnosno vlažno soljenih uzoraka "Brčanskog pršuta" skladištenih na 7^{O}C iznosio je za suho soljene proizvode 10.78% tek nakon 75 dana (jer je do tada proizvod dobijao na težini, 25-i dan +1.92%, 40-i dan +1.55%, a 60-i dan 1.46%) i dalje se povećavao tako da je 185-i dan iznosio 19.88%. Za vlažno soljene uzorke kalo je nakon 25 dana 3.22%, 75-i dan 22.99% i dalje se povećavao tako da je 185-i dan iznosio 33.33% (Tabela 4).

Tabela 4 Kaliranje suho, odnosno vlažno soljenog "Brčanskog pršuta" tokom skladištenja na 7^{0}C do 185 dana

Table 4 Nominal losses in dry- and wet- salted *Brčko prosciutto* during storage at 7^{0}C for up to 185 days

Dužina skladištenja (dani)	Broj uzoraka	Masa u g (srednja vrijednost)			
		Suho soljeno	Kalo %	Vlažno soljeno	Kalo %
1	10	706.66	-	731,11	-
25	10	719.44	+1.92	707.55	3.22
40	10	709.37	+1.55	701.90	3.99
60	10	712.85	+1.46	666.70	8.81
75	10	630.40	10.78	563.00	22.99
90	10	604.05	14.52	548.10	25.03
110	10	583.40	17.43	501.90	31.35
125	10	579.60	17.98	496.40	32.10
185	10	566.10	19.88	487.40	33.33

Kaliranje suho, odnosno vlažno soljenih uzoraka "Brčanskog pršuta" skladištenih na 15^{O}C iznosio je 40-i dan 2.14%, odnosno 2.85% i dalje se povećavao te je 75-i dan iznosio 21.25%, odnosno 31.25%, da bi 185-i dan iznosio 23.99%, odnosno 35.50% (Tabela 5).

Tabela 5 Kaliranje suho, odnosno vlažno soljenog "Brčanskog pršuta" tokom skladištenja na 15^0 C do 185 dana

Table 5 Nominal losses in dry- and wet- salted *Brčko prosciutto* during storage at 15^{0}C for up to 185 days

Dužina skladištenja (dani)	Broj uzoraka	Masa u g (srednja vrijednost)			
		Suho soljeno	Kalo %	Vlažno soljeno	Kalo %
1	10	742.22	-	690,50	-
25	10	742.00	-	690.50	-
40	10	726.34	2.14	670,80	2.85
60	10	708.25	2.49	597.00	13.55
75	10	590.00	21.25	474.70	31.25
90	10	560.40	21.30	462.60	33.00
110	10	557.80	22.24	456.30	33.92
125	10	540.00	23.75	450.75	34.72
185	10	528.10	23.99	445.40	35.50

Uporedo sa kaliranjem "Brčanskog pršuta" skladištenog na 7, odnosno 15^{0}C do 185 dana, dolazilo je do promjena u fizikalno-hemijskim parametrima (Tabela 6 i 7).

Srednja vrijednost sadržaja vode za suho, odnosno vlažno soljene uzorke se od inicijalnih 50.19%, odnosno 56.84% konstantno smanjivala, tako da je 185-i dan iznosila 35.46%, odnosno 27.98%. Inicijalna vrijednost NaCl-a se od 5.52, odnosno 4.43% konstantno postepeno povećavao za suho, odnosno vlažno soljene uzorke skladištene na istom temperaturnom režimu te je 90-i dan srednja vrijednost iznosila 7.60%, odnosno 6.97%, do maksimalne vrijednosti 185-og dana kada je iznosila 11.91, odnosno 13.81%. Tokom skladištenja proizvoda utvrđeno je i neznatno povećanje šećera, tako da je za suho, odnosno vlažno soljene uzorke te je 90-i dan imao srednje vrijednosti od 0.22%, odnosno 0.26%, a 185-i dan 0.29%, odnosno 0.37%. pH je maksimalnu srednju vrijednost za suho, odnosno vlažno soljene uzorke imao 60-i dan i iznosio je 6.42, odnosno 6.40, da bi 185-i dan imao vrijednost 5.50, odnosno 5.36 (Tabela 6).

Tabela 6 Fizikalno-hemijski parametri u suho, odnosno vlažno soljenom uzorku "Brčanskog pršuta" tokom skladištenja na 7^0 C do 185 dana

Table 6 Physico-chemical parameters of dry- and wet- salted samples of *Brčko prosciutto* during storage at 7^0 C for up to 185 days

Dužina skladištenja (dani)	Broj uzoraka	Vrsta soljenja	Fizikalno – hemijski parametri			
			Voda %	NaCl %	Ukupni šećer %	pH
1	5	suho	50.19	5.52	0.20	5.36
	5	vlažno	56.84	4.43	0.20	5.31
25	5	suho	51.23	5.61	0.20	5.44
	5	vlažno	55.50	4.59	0.21	5.52
40	5	suho	52.64	5.90	0.20	5.92
	5	vlažno	55.40	5.11	0.23	5.80
60	5	suho	54.40	6.10	0.20	6.42
	5	vlažno	55.00	5.41	0.24	6.40
75	5	suho	49.01	6.97	0.21	6.40
	5	vlažno	53.36	5.56	0.24	6.12
90	5	suho	44.24	7.60	0.22	6.10
	5	vlažno	48.26	6.97	0.26	5.92
110	5	suho	37.72	8.57	0.22	6.00
	5	vlažno	46.33	8.05	0.26	5.81
125	5	Suho	36,21	9,30	0,22	5,80
	5	vlažno	30.84	9.57	0.27	5.65
185	5	suho	35.46	11.91	0.29	5.50
	5	vlažno	27.98	13.81	0.37	5.35

Kod suho, odnosno vlažno soljenih uzoraka "Brčanskog pršuta" skladištenih na 15^OC voda se od inicijalnih vrijednosti 50.19, odnosno 56.84% progresivno smanjivala te je 90-i dan iznosila

37.23%, odnosno 34.48%, da bi 185-i dan iznosila 27.98%, odnosno 20.15%. NaCl se od inicijalnih vrijednosti 5.52, odnosno 4.43% postepeno povećavao za suho, odnosno vlažno soljene uzorke skladištene na istom temperaturnom režimu te je 90-i dan iznosio 9.18%, odnosno 9.13%, a 185-i dan 13.28%, odnosno 16.05%. I u ovom slučaju vrijednosti sadržaja vode i NaCl-a su obrnuto proporcionalne. Blagi porast registrovan je i kod šećera za suho, odnosno vlažno soljene uzorke te je 90-i dan imao vrijednosti od 0.24%, odnosno 0.28%, a 185-i dan 0.36%, odnosno 0.9%. pH je maksimalnu vrijednost za suho, odnosno vlažno soljene uzorke imao 60-i dan i iznosio je 6.51, odnosno 6.48, da bi 185-i dan imao vrijednost 5.45, odnosno 5.38 (Tabela 7).

Tabela 7 Fizikalno-hemijski parametri u suho, odnosno vlažno soljenom uzorku "Brčanskog pršuta" tokom skladištenja na 15^0 C do 185 dana
Table 7 Physico-chemical parameters of dry- and wet- salted samples of *Brčko prosciutto* during storage at 15^0 C for up to 185 days

Dužina skladištenja (dani)	Broj uzoraka	Vrsta soljenja	Fizikalno – hemijski parametri			
			Voda %	NaCl %	Ukupni šećer %	pH
1	5	suho	50.19	5.52	0.20	5.36
	5	vlažno	56.84	4.43	0.20	5.31
25	5	suho	50.07	5.71	0.21	5.48
	5	vlažno	57.03	4.90	0.23	5.62
40	5	suho	49.82	6.05	0.21	6.00
	5	vlažno	48.47	5.32	0.25	5.99
60	5	suho	48.57	6.49	0.21	6.51
	5	vlažno	44.18	7.22	0.25	6.48
75	5	suho	41.21	7.14	0.22	6.39
	5	vlažno	40.32	7.78	0.26	6.25
90	5	suho	37.23	9.18	0.24	6.11
	5	vlažno	36.48	9,13	0,28	6,05
110	5	suho	33.26	9,49	0,28	5,90
	5	vlažno	31.01	9.48	0.29	5.79
125	5	suho	31.04	12.06	0.30	5.77
	5	vlažno	28.00	10.92	0.30	5.61
185	5	suho	27.98	13.28	0.36	5.49
	5	vlažno	20.15	16.05	0.49	5.38

Mikrobiološkim ispitivanjima obuhvaćeni su uzorci suho, odnosno vlažno soljenog "Brčanskog pršuta" skladišteni na 7 i $15\,^0$C kroz period do 185 dana (Tabele 8 i 9).

Aerobne mezofilne bakterije minimalnu srednju vrijednost su imale prvog dana skladištenja i iznosile su za suho, odnosno vlažno soljene uzorke "Brčanskog pršuta" 5.3, odnosno 5.5 $x10^3$/g, a maksimalnu vrijednost su imale 185-i dan i to

7.5, odnosno 7.9 x10^3/g. Psihrofilne bakterije su minimalnu srednju vrijednost imale prvog dana skladištenja i to 1.8, odnosno 2.0 x10^2/g, dok su maksimalnu vrijednost imale 185-i dan i to 7.0, odnosno 7.5 x10^2/g. Halofilne bakterije su, također, inicijalno imale minimalne srednje vrijednosti od 5.9, odnosno 6.1 x10^2/g, a maksimalne vrijednosti su imale 185-i dan i to 9.5, odnosno 9.9 x10^2/g. Salmonele, koagulaza pozitivne stafilokoke, sulfitreducirajuće klostridije, *E. coli* te proteus vrste nisu izolirane u ispitivanim uzorcima (Tabela 8).

Tabela 8 Zastupljenost mikroorganizama u suho, odnosno vlažno soljenom uzorku "Brčanskog pršuta" skladištenog na 7^0C do 185 dana
Table 8 Incidence of microorganisms in dry- and wet- salted sample of *Brčko prosciutto* stored at 7^0C for up to 185 days

Dužina skladištenja (dani)	Broj uzoraka	Vrsta soljenja	Aerobne	Psihrofilne	Halofilne
			mezofilne bakterije u 1g (x10^3)	bakterije u 1g (x10^2)	bakterije u 1g (x10^2)
1	5	suho	5.3	1.8	59
	5	vlažno	5.5	2.0	61
25	5	suho	5.7	2.6	62
	5	vlažno	5.6	2.,8	69
40	5	suho	6.0	3.3	67
	5	vlažno	5.9	3.7	72
60	5	suho	6.2	4.1	69
	5	vlažno	6.1	4.6	7.5
75	5	suho	6.3	5.0	7.3
	5	vlažno	6.3	5.2	7.9
90	5	suho	6.7	5.5	7.7
	5	vlažno	6.4	5.,9	8.1
110	5	suho	6.9	5.8	8.2
	5	vlažno	6.8	6.7	8.8
125	5	suho	7.3	6.3	9.1
	5	vlažno	7.2	7.1	9.3
185	5	suho	7.5	7.0	9.5
	5	vlažno	7.9	7.5	9.9

Aerobne mezofilne bakterije kod uzorka "Brčanskog pršuta" proizvedenog od suho, odnosno vlažno soljenog mesa skladištenih na 15^0C, prvi dan skladištenja utvrđene su u srednjoj vrijednosti od 5.3, odnosno 5.5x10^3/g i postepeno se povećavao taj broj, 60-i dan skladištenja 6.5, odnosno 6.6x10^3/g, a maksimalnu vrijednost su imale 185-i dan i to 9.5, odnosno 9.8 x10^3/g. Psihrofilne bakterije su minimalnu srednju vrijednost imale prvog dana skladištenja i to 1.8, odnosno 2.0 x10^2/g, 60-i dan skladištenja 3.6, odnosno 3.9x10^2/g, dok su maksimalnu vrijednost

imale 185-i dan i to 6.5, odnosno 6.7 x10^2/g. Halofilne bakterije su, također, inicijalno imale minimalne srednje vrijednosti od 5.9, odnosno 6.1x10^2/g, 60-i dan skladištenja 6.5, odnosno 6.6x10^2/g, a maksimalne vrijednosti su imale 185-i dan i to 7.7, odnosno 7.9 x10^2/g (Tabela 9).

Tabela 9 Zastupljenost mikroorganizama u suho, odnosno vlažno soljenom uzorku "Brčanskog pršuta" skladišten na 15^{0}C do 185 dana
Table 9 Incidence of microorganisms in dry- and wet- salted sample of *Brčko prosciutto* stored at 15^{0}C for up to 185 days

Dužina skladištenja (dani)	Broj uzoraka	Vrsta soljenja	Aerobne mezofilne bakterije u 1g (x10^3)	Psihrofilne bakterije u 1g (x10^2)	Halofilne bakterije u 1g (x10^2)
1	5	suho	5.3	1.8	5.9
	5	vlažno	5.5	2.0	6.1
25	5	suho	5.9	2.3	6.2
	5	vlažno	5.9	2.6	6.3
40	5	suho	6.2	2.9	6.2
	5	vlažno	6.3	3.1	6.4
60	5	suho	6.5	3.6	6.5
	5	vlažno	6.6	3.9	6.6
75	5	suho	6.9	4.1	6.6
	5	vlažno	7.1	4.5	6.9
90	5	suho	7.1	4.7	6,8
	5	vlažno	7.7	5.1	7.0
110	5	suho	7.9	5.3	7.1
	5	vlažno	8.5	5.9	7.3
125	5	suho	8.4	5.8	7.5
	5	vlažno	9.1	6.2	7.7
185	5	suho	9.5	6.5	7.7
	5	vlažno	9.8	6.7	7.9

"Brčanski pršut" proizveden od suho soljenog mesa organoleptičkom ocjenom poentiran je sa 20.00 bodova (srednja vrijednost) i svrstan u ekstra klasu, a od vlažno soljenog mesa poentiran je sa 19.83 boda (srednja vrijednost) i svrstan je u I klasu. Skladištenjem pršuta na 7^{0}C mijenjala su se i organoleptička svojstva tako da je proizvod od suho soljenog mesa 75-i dan poentiran sa 18.74 boda i svrstan u I klasu, a od vlažno soljenog mesa poentiran sa 16.00 bodova i svrstan u II klasu. Pršut od vlažno soljenog mesa skladišten na 7^{0}C je 125-i dan imao znatno izmijenjena svojstva te je poentiran sa 12.32 boda i svrstan u van klasu, a pršut od suho soljenog mesa na istom temperaturnom režimu je tek 185-i dan bitno promjenio organoleptička svojstva te je poentiran sa 11.08 bodova i svrstan u van klasu (Tabela 10).

Tabela 10 Organoleptička ocjena suho, odnosno vlažno soljenog "Brčanskog pršuta" skladištenog na 7^0 C do 185 dana

Table 10 Organoleptic assessment of dry- and wet- salted *Brčko prosciutto* stored at 7^0 C for up to 185 days

Dužina skladištenja (dani)	Broj uzoraka	Vrsta soljenja	Organoleptička ocjena						Poeni	Klasa
			I	II	III	IV	V	VI		
		suho	3.00	6.00	2.00	3.00	4.00	2.00	20.00	Extra
1	10	vlažno	2.92	5.96	1.97	2.96	3.99	1.97	19.83	I
		suho	2.98	5.93	1.99	2.97	3.99	1.96	19.82	I
25	10	vlažno	2.94	5,95	1.92	2.93	3.97	1.95	19.64	I
		suho	2.95	5.87	1.96	2.91	3.94	1.92	19.55	I
40	10	vlažno	2.91	5.82	1.87	2.76	3.75	1.88	19.99	I
		suho	2.91	5.71	1,84	2.80	3.79	1.81	18.92	I
60	10	vlažno	2.61	5.69	1.62	2.60	3.71	1.81	18.04	I
		suho	2.84	5.69	1.81	2.78	3.77	1.85	18.74	I
75	10	vlažno	2.27	5.41	1.09	2.28	3.32	1.63	16.00	II
		suho	2.13	5.42	1.56	2.61	3.68	1.80	17.20	II
90	10	vlažno	1.96	5.13	0.99	1.98	3.00	0.98	14.04	III
		suho	2.05	4.98	1.27	2.41	3.59	1.73	16.03	II
110	10	vlažno	1.67	5.00	0.80	1.93	2.86	0.86	12.32	van k.
		suho	1.85	4.83	1.09	2.17	3.09	1.62	15.01	III
125	10	vlažno	1.61	4.83	0.69	0.98	2.58	0.59	11.28	van k.
		suho	1.03	4.25	0.92	1.52	2.29	1.07	11.08	van k.
185	10	vlažno	1.03	4.04	0.59	0.29	1.92	0.,39	8.26	van k.

Legenda:

I - Spoljni izgled	bodovanje od 0-3	**Ekstra klasa 20 bodova**
II - Sastav i izgled presjeka	bodovanje od 0-6	**I klasa od 18.00 – 19.99**
III - Konzistencija	bodovanje od 0-2	**II klasa od 16.00 – 17.99**
IV - Boja	bodovanje od 0-3	**III klasa od 14.00-15.9**
V - Ukus i miris	bodovanje od 0-4	**Van klase manje od 14.00 bodova**
VI - Prihvatljivost proizvoda	bodovanje od 0-2	

"Brčanski pršut" pripremljen za skladištenja na 15^0C, proizveden od suho soljenog mesa organoleptičkom ocjenom poentiran je sa 20.00 bodova (srednja vrijednost) i svrstan u extra klasu, a od vlažno soljenog mesa poentiran je sa 19.63 boda (srednja vrijednost) i svrstan je u I klasu. Skladištenjem pršuta na 15^0C mijenjala su se i organoleptička svojstva, tako da je proizvod 60-i dan kod suho soljenog mesa poentiran sa 18.26 boda i svrstan u I klasu, a od vlažno soljenog mesa poentiran sa 17.16 bodova i svrstan u II klasu. Pršut od suho soljenog mesa je 90-i dan poentiran sa 14.99 boda i svrstan u III klasu, a od vlažno soljenog mesa poentiran je 13.31 bodova i svrstan u van klasu, dok je suho soljeni proizvod tek 185-i dan bitno promjenio organoleptička svojstva te je poentiran sa 12.46 bodova i svrstan u u van klasu (Tabela 11).

Tabela 11 Organoleptička ocjena suho, odnosno vlažno soljenog "Brčanskog pršuta" skladištenog na 15^0 C do 185 dana

Table 11 Organoleptic assessment of dry- and wet- salted *Brčko prosciutto* stored at 15^0 C for up to 185 days

Dužina skladišten-ja- dani	Broj uzora-ka	Vrsta soljenja	Organoleptička ocjena						Poeni	Klasa
			I	II	III	IV	V	VI		
1	10	suho	3.00	6.00	2.00	3.00	4.00	2.00	20.00	Extra
		vlažno	2.89	5.92	1.97	2.95	3.96	1.93	19.63	I
25	10	suho	2.91	5.90	1.97	2.92	3.89	1.91	19.50	I
		vlažno	2.82	5.80	1.81	2.80	3.81	1.81	18.85	I
40	10	suho	2.87	5.81	1.86	2.71	3.65	1.97	18.69	I
		vlažno	2.76	5.72	1.64	2.70	3.69	1.71	18.22	I
60	10	suho	2.71	5.68	1.79	2.69	3.59	1.75	18.21	I
		vlažno	2.48	5.59	1.29	2.59	3.61	1.60	17.16	II
75	10	suho	2.27	5.13	1.36	2.34	3.8	1.69	16.27	II
		vlažno	2.02	5.31	0.98	1.98	3.01	0.99	14.29	III
90	10	suho	2.08	5.00	1.07	2.14	3.29	1.41	14.99	III
		vlažno	1.81	5.02	0.95	1.83	2.79	0.91	13.31	van kl.
110	10	suho	2.01	4.87	0.92	1.99	3.02	1.22	14.03	III
		vlažno	1.56	4.66	0.78	1.02	2.56	0.81	11.39	van kl.
125	10	suho	1.84	4.62	0.81	1.67	2.47	1.05	12.46	van kl.
		vlažno	1.40	4.12	0.61	0.69	2.12	0.47	9.41	van kl.
185	10	suho	0.75	3.37	0.64	1.06	1.92	0.74	8.48	van kl.
		vlažno	1.00	3.62	0.50	0.49	1.63	0.31	7.55	van kl.

Legenda:

I - Spoljni izgled bodovanje od 0-3
II - Sastav i izgled presjeka bodovanje od 0-6
III - Konzistencija bodovanje od 0-2
IV - Boja bodovanje od 0-3
V - Ukus i miris bodovanje od 0-4
VI - Prihvatljivost proizvoda bodovanje od 0-2

Ekstra klasa 20 bodova
I klasa od 18.00 – 19.99
II klasa od 16.00 – 17.99
III klasa od 14.00-15.9
Van klase manje od 14.00 bodova

DISKUSIJA I ZAKLJUČAK

Proizvodnja mesa u našim krajevima ima dugu tradiciju, kao i tendenciju uvođenja novih proizvoda. Na domaćem tržištu se susreću različiti suhomesnati proizvodi koji se najčešće proizvode po tradicionalnoj tehnologiji, a koju karakterizira neujednačen kvalitet i organoleptička svojstva. Kako proizvodnja suhomesnatih proizvoda ovisi o mikrobiološkim i fizikalno – hemijskim svojstvima koji imaju odlučujuću ulogu u postizanju poželjnih organoleptičkih svojstava, odgovarajućeg kvaliteta, higijenske ispravnosti i održivosti, tako su i naša istraživanja počela od inicijalnog supstrata za proizvodnju finalnog proizvoda. Značajno je napomenuti da se radi o proizvodu sa dugom tradicijom za čiju se proizvodnju koristi isključivo meso butne i leđne muskulature starijih krava u dobi između 10 i 12 godina.

Promjena mase suho, odnosno vlažno soljenih uzoraka mesa po fazama tehnološkog procesa prerade imala je očekivani trend. Kod suho, odnosno vlažno soljenih uzoraka mesa nakon faze soljenja došlo je do povećanja mase za 1.06, odnosno 9.49%, nakon faze cijeđenja povećanje je bilo 0,93, odnosno 8,03%, a da bi nakon faze dimljenja kalo iznosio 38.57, odnosno 42.35%. Sa završenom fazom zrenja kalo je iznosio 40,39 (suhi postupak), odnosno 43.04% (vlažni postupak). Što će reći, da je randman za suho, odnosno vlažno soljene uzorke iznosio 59.61, odnosno 56.59%. Navedene vrijednosti kala korespondiraju sa navedenim vrijednostima drugih autora (Pegg and Honikel, 2014; Vidal et al., 2021). Razlike u kaliranju između suho i vlažno soljenih uzoraka mesa i finalnih proizvoda dovode se u vezu sa različitim osmotskim pritiskom, a samim tim i različitom otpuštanju vode iz mesnog supstrata.

U proizvodnji suhomesnatih proizvoda posebna pažnja se poklanja inicijalnoj mikroflori sirovina, jer se u toku tehnoloških faza prerade pod raznim uvjetima razmnožavaju preživjeli mikroorganizmi (Krvavica. et al., 2012). Srednja vrijednost ukupnog broja mikroorganizamakod sirovog mesa kretala se od $0.2x10^2$/g za mikrokoke, $0.3 x10^2$/g za psihrofilne bakterije, $0,5x10^2$/g za halofilne bakterije, $0,6x10^2$/g za enterobakterije, do $5.0 x10^3$/g za aerobne mezofilne bakterije. Utvrđene skupine bakterija su u tolerantnim vrijednostima, tako da je sirovo meso sa mikrobiološkog aspekta predstavljalo povoljnu sirovinu za tehnološki proces proizvodnje "Brčanskog pršuta".

Nakon soljenja (suho i vlažno) 10-i dan u ispitivanim uzorcima mikroorganizmi su pokazivali neznatno povećanje, tako da je srednja vrijednost halofilnih bakterija povećana na 1.0, odnosno $1.2x10^2$/g, psihrofilnih bakterija na 0.7, odnosno $0.5 x10^2$/g, a aerobnih mezofilnih bakterija na 6,0, odnosno $6.5x10^3$/g. Najveća srednja vrijednost aerobnih mezofilnih bakterija u uzorcima suho, odnosno vlažno soljenog mesa je utvrđena nakon cijeđenja i iznosila je 7.1, odnosno $7.5 x10^3$/g.

Po fazama tehnološkog procesa proizvodnje suho, odnosno vlažno soljenog mesa i finalnog proizvoda, aerobne mezofilne bakterije su utvrđene u najvećem broju u mesu nakon cijeđenja od 7.1, odnosno $7.5x10^3$/g, a u manjem broju su utvrđene u finalnom proizvodu nakon zrenja i srednja vrijednost se kretala od 5.3, odnosno $5.5 x10^3$/g. Najveći broj psihrofilnih bakterija utvrđen je u finalnom proizvodu i kretao se od 1.8, odnosno $2.0 x10^2$/g, a najmanji broj u mesu nakon soljenja i iznosio je 0.5, odnosno $0.7 x10^2$/g. Halofilne bakterije u najvećem broju su utvrđene u finalnom proizvodu nakon zrenja i kretale su se od 5.9, odnosno $6.1 x10^2$/g, a najmanji broj u mesu nakon soljenja i kretao se od 1.0, odnosno $1.2 x10^2$/g. Enterobakterije i mikrokoke nakon soljenja i u daljem toku prerade nisu izolirane.

Inicijalni broj mikroorganizama (aerobne mezofilne bakterije, psihrofilne i halofilne) utvrđen u sirovom mesu, zadržao se tokom tehnoloških faza prerade na nivou iste logaritamske potencije, što se može dovesti u vezu sa bakteriostatskim efektima NaCl-a i pojedinih sastojaka dima (fenoli, formaldehidi i sl.), odnosno baktericidnim na enterobakterije i mikrokoke. Navedene bakterijske skupine su utvrđene jedino u sirovom mesu u broju 0.2, odnosno $0.6x10^2$/g, što je znatno niže od vrijednosti koje su utvrdili drugi autori (Savić et al. 1991). U ispitivanim uzorcima tokom

tehnoloških faza prerade, kao i finalnom proizvodu nisu utvrđene salmonela, koagulaza pozitivne stafilokoke, sulfitreducirajuće klostridije, *E. coli,* kao ni proteus vrste, što se sa mikrobiološkog aspekta ocjenjuje veoma povoljno, a finalni proizvod proglašava mikrobiološki ispravnim.

Mase suho, odnosno vlažno soljenih uzoraka "Brčanskog pršuta" tokom skladištenja na 7 i 15^{0}C do 185 dana su imale očekivani trend koji se reperkusirao na fizikalno-hemijske promjene u smislu smanjenja sadržaja postotka vode, rasta postotka NaCl-a i ukupnih šećera.

Masa suho soljenih uzoraka tokom skladištenja na 7^{0}C do 185 dana se u prvih 60 dana povećavala, a maksimalnu vrijednosti je imala 25-og dana. To se dovodi u vezu sa povećanjem higroskopičnosti proizvoda koja se zadržava na 7^{0}C do 60 dana. Smanjenje mase suho soljenih uzoraka započelo je 75-og dana skladištenja i kalo je iznosio 10.78% i zatim je konstantno rastao do maksimalne vrijendosti 19.88% sa 185 dana skladištenja. Masa vlažno soljenih uzorka skladištenih na 7^{0}C do 185 dana se od početka smanjivala tako da je 25-i dan kalo iznosio 3.22%, a 185-i dan 33.33%.

Masa suho, odnosno vlažno soljenih uzoraka "Brčanskog pršuta" tokom skladištenja na 15^{0}C do 185 dana znatno se razlikovala u odnosu na skladištenja proizvoda na 7^{0}C što se dovodi u vezu sa temperaturom skladištenja koja je utjecala na brže i izdašnije otpuštanje vode iz proizvoda, odnosno kaliranje.

Uporedo sa kaliranjem suho, odnosno vlažno soljenih uzoraka "Brčanskog pršuta" dolazilo je i do promjena u fizikalno-hemijskim parametrima. U toku skladištenja suhomesnatih proizvoda registrirano je postepeno smanjenje količine vode, a povećanje količine NaCl-a što ukazuje da su navedene promjene u direktnoj ovisnosti jedna od druge. Vrijednosti pH mesa u toku prerade kao i skladištenja zadržale su uobičajene vrijednosti na koje je blago utjecalo konzerviranje, dimljenje i temperatura skladištenja (Durack, 2008; Toldra, 2002).

Na održivost suhomesnatih proizvoda pored inicijalne mikroflore utječe i temperatura i dužina skladištenja, kao i relativna vlažnost mikroambijenta (Krvavica et al., 2013; Incze, 2004). U uzorcima "Brčanskog pršuta" skladištenih na 7^{0}C do 185 dana registrirano je blago povećanje inicijalne mikroflore (aerobne mezofilne, psihrofilne i halofilne bakterije).

Neznatna razlika u zastupljenosti ukupnog broja mikroorganizama u suho, odnosno vlažno soljenim uzorcima "Brčanskog pršuta" dovodi se u vezu sa načinom konzerviranja, odnosno neznatno većim sadržajem vode u vlažno soljenim uzorcima "Brčanskog pršuta". Tokom 185 dana skladištenja "Brčanskog pršuta" na 7^{0}C nisu izolirane salmonela, koagulaza pozitivne stafilokoke, sulfitreducirajuće klostridije, *E. coli* kao ni proteus vrste.

Organoleptička ocjena "Brčanskog pršuta" proizvedenog od suho, odnosno vlažno soljenog mesa vršena je metodom poentiranja sa maksimalnih 20 bodova. Površina uzorka je bila suha, čista i tamnosmeđe boje. Uredno je obrezanih rubova, bez zasjeka, a na prerezu mišično tkivo je smeđe boje, a ka sredini prereza crvenkasto-smeđe sa tačkicama masnog tkiva (marmoriranost) krem boje. Konzistencija je čvrsto elastična. Miris prijatan, prihvatljiv, blago aromatičan, kao i ukus (punoća ukusa). Skladištenjem pršuta na 7^{0}C mijenjala su se i organoleptička svojstva tako da je proizvod od suho soljenog mesa 75-i dan poentiran sa 18.74 boda i svrstan u I klasu, a od vlažno soljenog mesa poentiran sa 16.00 bodova i svrstan u II klasu. Pršut od vlažno soljenog mesa skladišten na 7^{0}C je 125-i dan imao znatno izmijenjena svojstva te je poentiran sa 12.32 boda i svrstan u van klasu, a pršut od suho soljenog mesa na istom temperaturnom režimu je tek 185-i dan bitno promjenio organoleptička svojstva te je poentiran sa 11.08 bodova i svrstan u van klasu (Tabela 7). Uzorci van klase imali su površinu tamnosmeđe boje, uredno obrezanih rubova, bez zasjeka. Mišično tkivo je tamnosmeđe boje, a masno blijedo-krem boje. Konzistencija tvrda, miris svojstven, a ukus izrazito slan. Pošto su zadovoljeni kriteriji zdravstvene ispravnosti

(mikrobiološke i fizikalno hemijske karakteristike) preporučeno je da proizvod može ići za potrošnju u javnim kuhinjama, odnosno za kuhanje. Bolja, odnosno trajnija organoleptička svojstva utvrđena su kod "Brčanskog pršuta" proizvedenog od suho soljenog mesa što se može dovesti u vezu sa postupkom konzerviranja i stabilnijim ponašanjem takvih proizvoda (Rivera et al., 2019).

Suho soljeni "Brčanski pršut" tokom skladištenja na 7^0C do 185 dana uspio je u pogledu mikrobiološke, fizikalno-hemijske organoleptičke ocjene zadržati poželjna svojstva do 75-og dana, da bi nakon toga tj. sa 90 dana zadovoljio mikrobiološke norme, ali zbog smanjenja sadržaja vode dobija tvrđu konzistenciju, a time se procenat soli povećava.

Suho soljeni "Brčanski pršut" tokom skladištenja na 15^0C do 185 dana zadovoljio je u pogledu navedenih svojstava tražene norme do 60-og dana, da bi sa 75 dana zadovoljio mikrobiološke norme, ali zbog smanjenog sadržaja vode dobija tvrđu konzistenciju, a što ima za posljedicu da se procenat soli povećavao.

Vlažno soljeni "Brčanski pršut" tokom skladištenja na 7^0C do 185 dana uspio je u pogledu navedenih svojstava zadovoljiti norme do 60-og dana kada je kalo iznosio 8.81% čime je dobio nešto tvrđu konzistenciju, a od 75-og dana izrazito tvrdu konzistenciju čime nije zadovoljio navedena svojstva.

Vlažno soljeni "Brčanski pršut" tokom skladištenja na 15^0C do 185 dana zadovoljio je propisane norme do 60-og dana kada je kalo iznosio 13.55% čime je proizvod dobio tvrđu konzistenciju, a sa 75 dana izrazito tvrdu uz 7.78% soli (povećan salinitet).

Zbog specifičnih organoleptičkih i fizikalno-hemijskih svojstava, kao i izrazitom prepoznatljivošću na tržištu odnosno tradicionalnu udomaćenost u regionu Posavine, odnosno sjeveroistočne BiH, prednost je dobila suho soljena forma "Brčanskog pršuta" (koja je i dobila veći broj bodova u odnosu na vlažnu formu) te ga kao takvog i kandidovati kao reprezenta posavskog kraja.

U tom smislu bi trebalo izvršiti doedukacije postojećih individualnih proizvođača u smislu sinhronizacije tehnološkog procesa proizvodnje, što bi doprinijelo podizanju cjelokupnog kvaliteta i dalo pečat prepoznatljivom originalnom proizvodu posavskog kraja.

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INFLUENCE OF DIFFERENT PROCEDURES FOR CONSERVATION AND TECHNOLOGICAL PROCESSING ON QUALITY, FOOD SAFETY AND SUSTAINABILITY OF BRČKO PROSCIUTTO

ABSTRACT

Beef prosciutto is a highly valued cured meat product throughout the territory of Bosnia and Herzegovina, produced using various technological variants. In line with this, there have been numerous studies on how the improvement of the prosciutto processing technology affects its quality, food safety, and sustainability.

The specificity of Brčko prosciutto lies in its preservation process, using only salt, sugar, and water, without nitrites, nitrates, or other additives. The result is specific and desirable organoleptic and microbiological characteristics, while meeting all consumers' demands, justifying its name as a natural and healthy food.

Two variants have been studied: the first being dry-salted Brčko prosciutto with shorter duration of each phase (10 days of salting, 10 days of smoking, and 10 days of aging). The second variant is wet-salted Brčko prosciutto (8 days of salting, 10 days of smoking, and 10 days of aging), along with the storage at two temperature regimes (at 7°C and 15°C).

Keywords: Brčko, smoked beef, prosciutto

PROFESSIONAL PAPER

ZNAČAJ HEMATOLOŠKIH PARAMETARA KOD PSA TORNJAKA U KLINIČKOJ PRAKSI

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SAŽETAK

Bosansko-hercegovačka pasmina tornjak je autohtona pasmina, poznata po svojoj snažnoj fizičkoj konstituciji i izdržljivosti. Tornjak je samouvjeren pastirski pas, odličan čuvar na prostoru koji mu je povjeren, ali istovremeno blag i nježan za svoje ljude, bliske prijatelje i djecu. Cilj rada je istražiti i analizirati hematološke parametre kod pasmine tornjak, te utvrditi njihov značaj u kliničkoj praksi radi boljeg razumijevanja zdravlja i liječenja ove pasmine. Dosadašnje dostupne hematološke studije kod pasa tornjaka su vrijedan doprinos u rasvjetljavanju ove nepoznanice, ali su, nažalost, limitirane nedostatnim brojem istraživanih uzoraka.

Ključne riječi: Hematologija, pas, tornjak

UVOD

Stočari nomadi su tornjaka doveli na prostor Bosne i Hercegovine. Prikaz pasa se nalazi i na stećcima, npr. stećak u okolini Gvoznog (Salkić i sar., 2006; Katica i sar., 2004).

FCI (Federation Cynologique International) je priznao tornjaka kao novu bosansko – hercegovačko – hrvatsku pasminu pasa. Tornjak je svrstan u II grupu FCI sistematike u sekciju 2.2. planinski tip. Pripada molosoidnim psima ili LGD (livestock guardian dogs) i ubraja se u pastirske pse.

Nastanjen je u planinsko – brdskim predjelima Bosne i Hercegovine. To je pas prijatnog, raskošnog izgleda zbog dvobojne i dvobojne boje sa paležnim oznakama, te duge dlake sa karakterističnim nošenim kitnjastim repom. Plemenit, snažan, čvrst, skladan izdašnog vrlo dostojanstvenog hoda, dobre naravi, hrabar, nepokolebljiv i nepodmitljiv, vrlo oštar, ali ne i podmukao pas. Samouvjeren pastirski pas, odličan čuvar na prostoru koji mu je povjeren, ali istovremeno blag i nježan za svoje ljude, bliske prijatelje i djecu (Softić i sar., 2006).

Danas svaka pasmina pasa predstavlja individue s visokim nivoom fenotipske homogenosti, smanjenom genetskom raznolikošću unutar pasmina i većom genetskom divergencijom između pasmina (Shearin i Ostrander, 2010).

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Faktori koji imaju utjecaj na hematološke i biohemijske parametre su okruženje, godišnja doba i način života, poznati kao spoljašnji faktori i unutrašnji faktori, kao što su starost, spol i status sterilizacije/kastracije (Connolly i sar., 2020; Lee i sar., 2020; Chang i sar., 2016).

Rezultati hematoloških parametara mogu pružiti objektivnu informaciju o stanju životinje u vrijeme uzorkovanja i pružiti informacije o nutritivnom statusu, statusu bolesti ili stresu kojem je podvrgnuta (Perez i sar., 2003). Za svaku životinjsku vrstu treba koristiti specifične referentne intervale kako bi se uporedile hematološke vrijednosti i osiguralo da se rezultati pravilno interpretiraju (Meyer i Harvey, 2004).

Cilj rada je istražiti i analizirati hematološke parametre kod pasmine tornjaka, te utvrditi njihov značaj u kliničkoj praksi radi boljeg razumijevanja zdravlja i liječenja ove pasmine.

Klinički značaj hematoloških parametara kod pasa

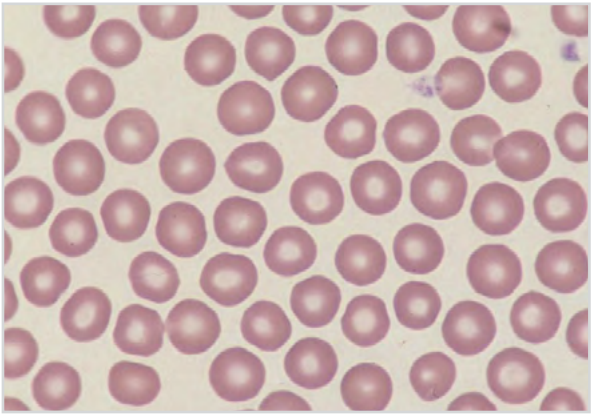
Sve krvne ćelije imaju ograničen životni vijek, ali kod zdravih životinja, broj ćelija u cirkulaciji se održava na prilično konstantnom nivou. Da bi se to postiglo, ćelije se u cirkulaciji moraju konstantno obnavljati, što se događa stvaranjem i oslobađanjem ćelija iz koštane srži, a takva mjesta se nazivaju medularna mjesta. U slučajevima povećane potražnje, proizvodnja se događa i izvan koštane srži u tzv. ekstramedularnim mjestima kao što su slezena, jetra i limfni čvorovi. Samim time, hematopoeza, proizvodnja krvnih ćelija predstavlja složen i visoko regulisan proces (Reagan i sar., 2019).

Nakon rođenja, sve krvne ćelije su nastale iz pluripotentnih matičnih ćelija prisutnih u koštanoj srži i krvi (Latimer, 1995). Pluripotentne matične ćelije se diferenciraju na limfoidne i mijeloidne matične ćelije. Limfoidne ćelije se diferenciraju u B i T limfocite, a mijeloidne ćelije čine preteču ćelija koje uključuju eritroidnu lozu (koje se formiraju u eritroidne ćelijske kolonije), granulocitno – monocitne ćelijske kolonije, megakarioblastne ćelijske kolonije, eozinofilne ćelijske kolonije i bazofilne ćelijske kolonije (Duncan i sar., 2003; Latimer, 1995).

Crvena krvna zrnca/eritrociti

Eritrociti se formiraju od nediferenciranih matičnih ćelija unutar koštane srži procesom eritropoeze (Aspinall i sar., 2020). Eritropoeza je proces u kojem se hematopoetski nezrele ćelije razvijaju u retikulocite i eritrocite, a regulisana je faktorima rasta – eritropoetin, interleukin-6 (IL-6, IL-3, IL-11), inhibitornih faktora i faktora translacije (Car, 2000). Proizvodnja eritrocita se kontroliše hormonom eritropoetinom, čije glavno mjesto sinteze su bubrezi, a jetra može proizvesti 10-15% eritropoetina plazme (Aspinall i sar., 2020; Car, 2000). Kod većine sisara, bubreg je i senzorni organ i glavno mjesto proizvodnje eritropoetina, pa je hronično zatajenje bubrega povezano s anemijom. Faktori koji utječu na eritropoezu i koji ovaj proces mogu potisnuti su: opskrba hranjivim materijama (željezo, folat ili vitamin B_{12}), interakcija ćelija – ćelija između eritroidnih prekursora, limfoidnih ćelija i drugih komponenti hematopoetskog mikrookruženja, kao i hronične iscrpljujuće bolesti i endokrini poremećaji, npr. hipotireoza ili hiperestrogenizam (Cotter, 2022).

Morfološke karakteristike zrelih crvenih krvnih zrnaca većine sisara su općenito vrlo slične po tome što im svima nedostaje jezgro, obojena su crvenkasto do crvenkasto – narandžastom bojom i općenito su bikonkavne ćelije diskoidnog oblika (Slika 1) (Reagan i sar., 2019). Oni sadrže crveni pigment – hemoglobin, složeni protein koji sadrži željezo. Njihov oblik i tanka ćelijska membrana im daju veliku površinu za razmjenu gasova i omogućavaju kiseoniku da difundira u ćeliju, gdje se spaja sa hemoglobinom i formira oksihemoglobin. Također, pošto su jedine ćelije koje ne sadrže jezgro, to im omogućava da se veća količina hemoglobina spakuje u relativno malu ćeliju. Njihova glavna funkcija je transport kiseonika i malog udjela ugljičnog dioksida oko tijela (Aspinall i sar., 2020). Prosječan životni vijek eritrocita kod zdravog psa je 110 dana (Duncan i sar. 2003).



Slika 1 Eritrociti psa. Većina ćelija je slične veličine i imaju izraženo središnje bljedilo. 100x objektiv (Reagan i sar., 2019)

Figure 1 Erythrocytes in dog. Most cells are similar in size with a prominent central pallor. 100x magnification (Reagan et al., 2019)

Faktori koji utječu na broj eritrocita, koncentraciju hemoglobina i vrijednost hematokrita su: spol, starost, način ishrane, fizička aktivnost, estrusni ciklus, bremenitost, laktacija. Jedinke muškog spola imaju veći broj eritrocita u jedinici zapremine u odnosu na ženski spol, što se može dovesti u vezu sa stimulatornim efektom testosterona na produkciju eritrocita (Božić i Zvekić, 2017).

Iako je rijetko oboljenje, kod pasa se javlja *polycythemia rubra vera* (eritrocitoza) koja predstavlja hroničnu mijeloproliferativnu bolest u kojoj postoji hiperprodukcija eritrocita, leukocita i trombocita (Božić, 2012).

Smanjena proizvodnja eritrocita može biti rezultat primarnih bolesti srži (npr. aplastična anemija, hematopoetski malignitet ili mijelofibroza) ili drugih uzroka (npr. zatajenje bubrega, lijekovi, toksini ili antitijela usmjerena protiv prekursora eritrocita) (Cotter, 2022).

Bijela krvna zrnca/leukociti

Mijeloidne matične ćelije stvaraju prekursorske ćelije, uključujući granulocitno – monocitne formirajuće kolonije, megakariocitne kolonije, eozinofilne kolonije i bazofilne kolonije (Duncan i sar., 2003). Ove jedinice kolonizacije se nadalje diferenciraju u prekursorske ćelije i na kraju u zrele ćelije (Duncan i sar., 2003). Što označava da se pojedinačne loze leukocita mogu samostalno razvijati, izuzev neutrofila i monocita koji potječu iz bipotentne prekursorske ćelije (Latimer i Rakich, 1989).

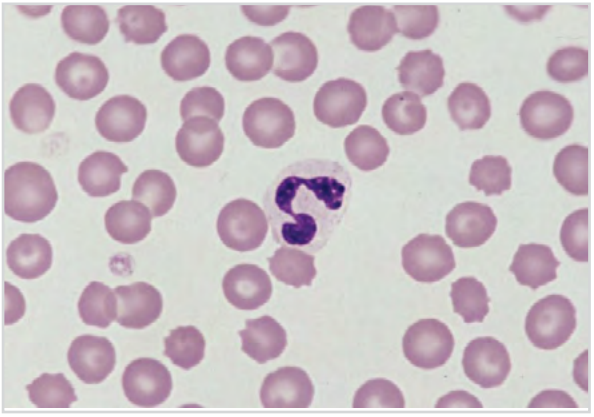
Leukociti za razliku od eritrocita su manje brojni i sadrže jezgro. Mogu se klasificirati kao granulociti ili agranulociti u zavisnosti da li sadrže vidljive granule u njihovoj citoplazmi kada se oboje i uoče pod mikroskopom. Njihova funkcija je odbrana organizma od infekcije (Aspinall i sar., 2020).

Granulociti

Granulociti se proizvode u koštanoj srži i čine oko 70% svih leukocita. Unutar citoplazme se nalaze granule i segmentirano ili režanjsko jezgro koje može da varira u obliku (Aspinall i sar., 2020). Postoje tri vrste granulocita:

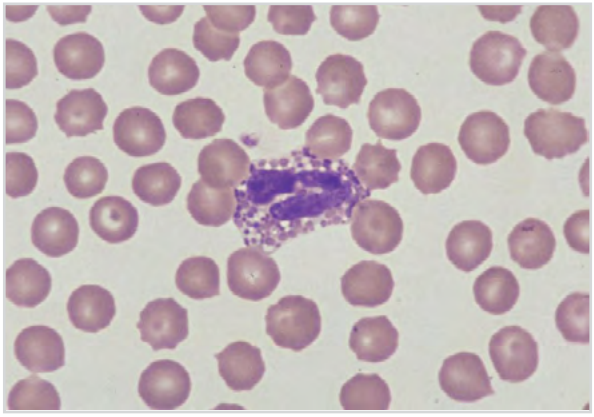
Neutrofili su najbrojniji leukociti kod zdravog psa i promjene u broju neutrofila će znatno utjecati na ukupni broj bijelih krvnih ćelija (Latimer i Rakich, 1989). Oni preuzimaju neutralne boje i granule se boje ljubičasto (Slika 2). Primarna funkcija neutrofila je fagocitoza bakterija, ali i gljivice, alge, paraziti i virusi mogu biti uništeni (Latimer, 1995; Smith, 2000). Oni također mogu izazvati antitijelima – ovisnu ćelijsku citotoksičnost i uništiti zaražene ili oštećene ćelije (Latimer i Rakich, 1989; Smith 2000).

Uobičajeni uzroci neutrofilije uključuju infektivnu ili neinfektivnu upalu (trauma, operacija, opekotine i sl.), egzogene ili endogene kortikosteroide i epinefrin. Imunoposredovane bolesti, poput imunoposredovane hemolitičke anemije su povezane sa neutrofilijom zbog djelovanja upalnih citokina (Individual WBC | eClinpath.), dok se neutropenija može javiti usljed adhezije leukocita za zidove krvnih sudova, uništavanja neutrofila ili smanjene formacije u koštanoj srži, kao i kod bakterijskih infekcija. Također, neželjene reakcije na lijekove mogu dovesti do neutropenije ili čak pancitopenije (smanjen broj eritrocita, leukocita i trombocita) (Wood, 2022).



Slika 2 Segmentirani neutrofil psa. 100x objektiv (Reagan i sar., 2019)

Figure 2 Segmented neutrophils in dog. 100x magnification (Reagan et al., 2019)



Slika 3 Eozinofil psa. 100x objektiv (Reagan i sar., 2019)

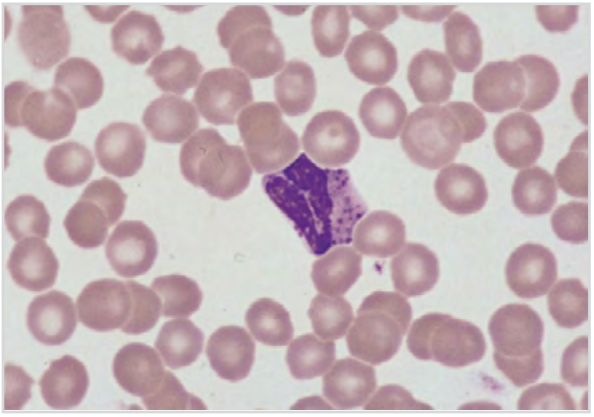
Figure 3– Eosinophil in dog. 100x magnification (Reagan et al., 2019)

Eozinofili su odsutni ili su prisutni u vrlo malom broju kod zdravih životinja. Ove ćelije su obično slične veličine kao neutrofili, ali su često nešto veće. Jezgro je vrlo slično neutrofilima, po tome što su segmentirana, ali segmenti često nisu tako dobro izraženi. Citoplazma je tamnoplava i ima višestruke crvenkaste do crvenkasto – narandžaste granule (Slika 3). Kod pasa, eozinofilne granule su okrugle i prilično varijabilne veličine i broja. Pored jezgre i granula, u citoplazmi se često nalaze i višestruke vakuole promjenjive veličine (Reagan i sar., 2019). Eozinofili su smješteni uglavnom u labavom vezivnom tkivu organa koji služe kao ulazne tačke za strane tvari, kao što je koža, respiratorni i digestivni sistem (Young, 2000).

Eozinofilija se javlja kod parazitarnih infekcija (npr. *Dirofilaria immitis*), ali važno je napomenuti da nisu sve parazitarne infekcije povezane sa povećanim brojem eozinofila (npr. mikoplazme). Prisutna je i kod alergijskih stanja, enteritisa, nefritisa, pneumonija, dermatitisa, zatim kod pojedinih tumora (tumor mastocita, limfom, tumor skvamoznih ćelija i sl.), kod hipoadrenokorticizma (Addisonova bolest), idiopatskog hipereozinofilnog sindroma (kod rotvajlera) i hronične eozinofilne leukemije (Individual WBC | eClinpath; Wood, 2022; Božić i Zvekić, 2017; McEwan i sar., 1985), dok se smanjen broj eozinofila ili eozinopenija javlja kao reakcija na stres i usljed terapije kortikosteroidima (Wood, 2022).

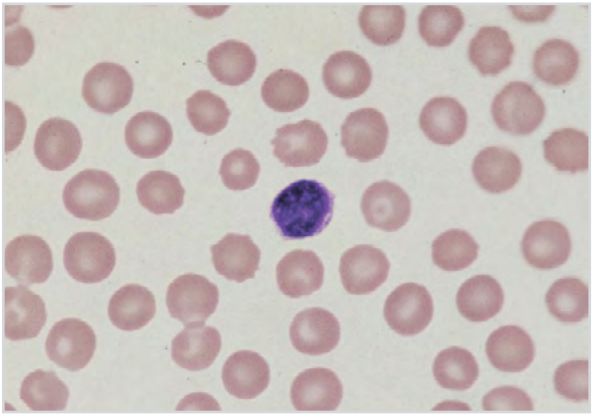
Eozinofili igraju glavnu ulogu u kontroli parazitarne infestacije, te kao odgovor na navedeno javlja se eozinofilija ili povećan broj eozinofila. Također, učestvuju u regulaciji alergijskih i upalnih procesa, te luče enzime koji inaktiviraju histamin (Aspinall i sar., 2020).

Bazofili se rijetko uočavaju u perifernoj krvi svih uobičajenih domaćih vrsta (Reagan i sar., 2019). Najmanje ih ima krvi pasa (Latimer, 1995). Slične su veličine ili nešto veći od neutrofila, citoplazma je svijetloljubičasta i jezgro segmentirano (Slika 4). Mali broj sitnih, okruglih, ljubičastih citoplazmatskih granula ponekad može biti prisutan u bazofilima pasa (Reagan i sar., 2019). Bazofili luče histamin, koji pojačava upalu i heparin, koji je prirodni antikoagulans koji spriječava stvaranje nepotrebnih krvnih ugrušaka (Aspinall i sar., 2020). Njihova funkcija podrazumijeva učestvovanje u imunološki posredovanim upalnim procesima i tumornoj toksičnosti, a njihova aktivnost je uveliko posredovana T limfocitima. Bazofilija ili povećan broj bazofila se obično javlja zajedno s eozinofilijom, a infekcija srčanim crvom je najčešći uzrok bazofilije kod pasa (Latimer, 1995).



Slika 4 Bazofil psa. 100x objektiv (Reagan i sar., 2019)

Figure 4 Basophil in dog. 100x magnification (Reagan et al., 2019)



Slika 5 Limfocit psa. 100x objektiv (Reagan i sar., 2019)

Figure 5 Lymphocyte in dog. 100x magnification (Reagan et al., 2019)

Budući da većina zdravih životinja ne posjeduje bazofile u krvi, referentni interval prikazuje nula bazofila, zbog čega se ne može smatrati relativnim nalazom (Individual WBC | eClinpath).

Agranulociti

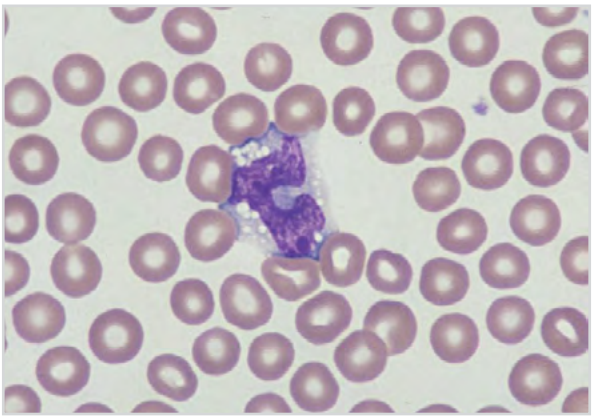
U agranulocite spadaju leukociti koji u svojoj citoplazmi ne sadrže granule. Postoje dvije vrste:

Limfociti koji su drugi najbrojniji elementi leukocita u krvi pasa i najčešći tip bijelih krvnih zrnaca, čineći 80% svih agranulocita (Aspinall i sar., 2020; Latimer, 1995). Obično su okruglog oblika, nešto manji od neutrofila i imaju okrugla do ovalna i ponekad blago uvučena jezgra (Slika 5). Hromatinski uzorak se sastoji od glatkih, staklastih područja pomiješanih sa područjima koja su više zgrudana ili razmotana. Prisutna je mala količina svijetloplave citoplazme (Reagan i sar., 2019). Limfociti su glavni tip ćelija imunog sistema i formiraju se u limfoidnom tkivu, iako potječu od matičnih ćelija u koštanoj srži. Odgovorni su za specifični imuni odgovor, a postoje dva različita tipa: B limfociti, koji proizvode antitijela i uključeni su u humoralni imunitet i T limfociti, koji su uključeni u ćelijski imunološki odgovor (Aspinall i sar., 2020).

Najčešći uzroci limfocitoze su fiziološka ili limfocitoza povezana sa starenjem. Fiziološki posredovana epinefrinom limfocitoza je dosta rijetka pojava kod pasa, dok fiziološka limfocitoza je izrazito česta kod mladih životinja. Limfocitoza može biti uzrokovana određenim hormonima, stimulacijom imunološkog sistema infekcijom, hroničnim oboljenjima (npr. artritis i leukemija), nedostatkom kortizola. Javlja se kod hipoadrenokorticizma, antigenske stimulacije (npr. infektivni agensi *Ehrlichia canis*), nelimfoidne neoplazije i hronične limfocitne leukemije (Wood, 2022; Individual WBC | eClinpath).

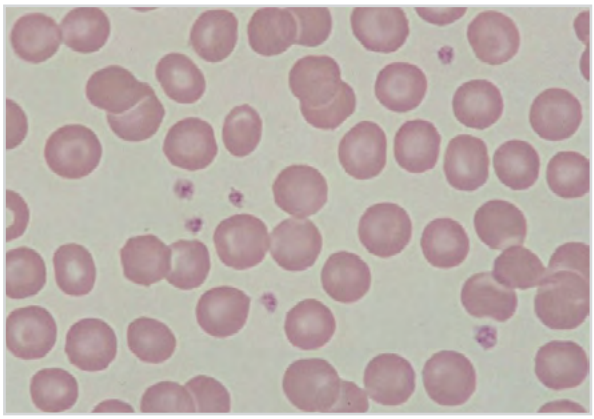
Suprotna pojava, leukopenija je uzrokovana endogenim ili egzogenim kortikosteroidima (stres leukogram), smanjenom proizvodnjom ili gubitkom limfocita, određenim virusnim infekcijama, nasljednim bolestima i primarnim ili sekundarnim imunodeficijencijama (Wood, 2022; Individual WBC | eClinpath).

Monociti su odsutni ili su prisutni u malom broju u perifernoj krvi i izgledaju vrlo slično kod svih uobičajenih domaćih vrsta i laboratorijskih životinjskih vrsta. Jezgro im može biti različitog oblika, poput ovalnog ili u obliku zrna graha ili imati više udubljenja i režnjeva. Nuklearni hromatin je fino granuliran sa nekoliko područja



Slika 6 Monocit psa. 100x objektiv (Reagan i sar., 2019)

Figure 6 Monocyte in dog. 100x magnification (Reagan et al., 2019)



Slika 7 Trombociti psa. 100x objektiv (Reagan i sar., 2019)

Figure 7 Thrombocyte in dog. 100x magnification (Reagan et al., 2019)

kondenzacije (Slika 6). Umjerena količina citoplazme je tipično plavo – siva i može imati više diskretnih vakuola promjenjive veličine (Reagan i sar., 2019). Monociti su fagocitne ćelije koje migracijom u tkiva sazrijevaju i postaju makrofazi (Aspinall i sar., 2020).

Uobičajeni uzroci monocitoze kod pasa su najčešće odgovor na stres kod infektivnih ili neinfektivnih upala. Može se javiti i nakon primjene hematoterapijskih agenasa, kod različitih tipova tumora (npr. limfom) i akutne ili hronične mijeloične leukemije. Monocitopenija se može teže dokumentovati jer se referentni intervali monocita često spuštaju na nulu i nemaju klinički značaj (Individual WBC | eClinpath).

Krvne pločice/trombociti

Trombociti su ćelijski fragmenti formirani u koštanoj srži od velikih ćelija koje se nazivaju megakariociti. To su mali diskovi bez jezgri i prisutni su u krvi u velikim količinama (Aspinall i sar., 2020). Plave su boje i mogu imati višestruke, fine, ružičaste do ljubičaste citoplazmatske granule (Slika 7) (Reagar i sar., 2019). Njihova primarna uloga je u hemostazi, tako što se adheriraju na subendotelium pri čemu olakšavaju lokalizovano formiranje trombina i fibrina čime smanjuju gubitak krvi (Gentry, 2000). Također, igraju važnu ulogu u upalnim procesima i zarastanju rana putem direktne interakcije ćelije – ćelije i otpuštanjem medijatora iz aktiviranih trombocita (Gentry, 2000).

Trombocitoza je rijetka i često idiopatska. Može biti povezana s primarnim oboljenjem srži kao što je megakariocitna leukemija. Često se povezuje s hroničnim gubitkom krvi i nedostatkom željeza zbog povećane proizvodnje trombocita u srži koja reaguje na kontinuiranu potrošnju i gubitak. Jedan od najčešćih poremećaja krvarenja kod životinja predstavlja trombocitopenija. Smanjena proizvodnja trombocita u srži može biti uzrokovana lijekovima, toksinima ili primarnim poremećajima srži kao što su aplazija, fibroza ili malignitet hematopoeze. Kod primarnih poremećaja koštane srži, često je smanjeno više od jedne ćelijske linije hematopoeze, čemu je rezultat pancitopenija (Cotter, 2022).

Dosadašnja dostupna istraživanja

Studiju Alilović i sar. (2022) ćemo predstaviti unutar Tabele 1. Ista je vrijedan znanstveni doprinos u rasvjetljavanju nepoznanica u vezi sa hematološkim parametrima psa tornjaka, ali je limitovana zbog nedostatnog broja uzoraka.

Tabela 1 – Prikaz hematoloških parametara pasmina: tornjaka, turskog kangala i uličnih pasa (Alilović i sar., 2022; Stjepanović, 2014; Kockaya i sar., 2021)

Table 2 – Overview of hematological parameters in different breeds: Tornjak, Turskish Kangala and stray dogs (Alilović et al., 2022; Stjepanović, 2014; Kockaya et al., 2021)

Parametar	**Tornjak**			**Kangal**		**Ulični psi**		
	Max ± Min	Mean	RI	Mean ± S.E.M		Max ± Min	Mean	RI
				Odrasla ženka	Odrasli mužjak			
RBC (x10^{12}/L)	9.58±4.48	7.35	5.50-8.50	8.14±0.35	8.00±0.21	7.25±3.57	6.17±1.10	5.50-8.50
HCT (%)	62.00±28.60	47.03	37.00-55.00	67.42±2.35	65.57±1.23	0.55±0.28	0.45±0.07	0.370-0.550
HGB (g/dL)	21.40±8.90	16.39	12.00-18.00	18.80±0.70	18.77±0.69	174.00±75.00	135.60±31.43	120-180
MCV (fL)	70.30±53.60	64.03	60.00-77.00	83.08±1.81	82.02±0.95	78.30±69.30	73.57±2.58	60.0-77.0
MCH (pg)	24.10±17.10	22.28	21.20-25.90	23.07±0.16	23.40±0.39	24.70±12.80	21.85±3.38	18.5-30.0
MCHC (g/dL)	38.10±30.30	34.80	32.00-36.00	27.85±0.58	28.55±0.61	326.00±296.00	309.90±7.97	300-375
RDW (%)	28.50±14.20	18.45	13.60-21.70	11.85±0.39	12.03±0.35	X	X	X
RETIC (%)	6.90±0.20	1.00	0.00-1.50	X	X	X	X	X
RETIC-HGB (pg)	25.70±15.10	21.93		X	X	X	X	X
WBC (x10^{9}/L)	20.97±7.57	13.00	6.00-17.00	12.77±1.72	12.95±1.27	X	X	X
NEU (x10^{9}/L)	14.48±3.57	7.50	3.00-11.50	X	X	22.88±3.64	9.10±6.27	2.00-12.0
LYM (x10^{9}/L)	5.91±1.15	3.25	1.00-4.80	X	X	5.61±0.67	2.53±1.37	0.50-4.90
MONO (x10^{9}/L)	2.10±0.39	0.86	0.15-1.35	0.57±0.06	0.60±0.07	2.68±1.36	1.88±0.44	0.30-2.00
EOS (x10^{9}/L)	7.13±0.03	1.36	0.10-1.25	X	X	2.08±0.24	0.66±0.54	0.10-1.49
BASO (x10^{9}/L)	0.20±0.01	0.04	Rare	X	X	0.09±0.02	0.05±0.02	0.00-0.10
PLT (K/µL)	462.00±55.00	251.70	200.00-500.00	343.25±48.80	276.50±48.31	592.00±114.00	347.80±165.100	175-500
MPV (fL)	17.80±8.40	11.41	8.70-13.20	9.10±0.31	9.05±0.18	X	X	X
PDW (fL)	16.80±8.60	12.13	9.10-19.40	15.92±0.08	16.37±0.16	X	X	X
PCT (%)	0.57±0.06	0.28	0.14-0.46	0.31±0.04	0.25±0.05	X	X	X

Max – maksimum; Min – minimum; Mean – Srednja vrijednost; RI – referentni interval; X – nedostatak podataka. Tornjak (Alilović i sar, 2022); Turski kangal (Kockaya, 2021); Ulični psi (Stjepanović, 2014)

Max – maximum; Min – minimum; Mean – Mean value RI – reference interval; X – no data available. Tornjak (Alilović et al, 2022); Turkish Kangal (Kockaya, 2021); Stray dogs (Stjepanović, 2014)

Psi lutalice nemaju opterećenja naslijeđa, kao ni odgovarajuću zdravstvenu brigu ni njegu, ali nisu istraživane ni druge starosne kategorije značajne u kliničkoj praksi. U skladu sa navedenim ne možemo sa sigurnošću utvrditi eventualne značajne razlike između hematoloških parametara pasmine tornjak i drugih rasa – turski kangal. Kockaya i sar. (2021) su kod pasmine kangal utvrdili da efekti starosti i spola nisu bili značajni za hematološke parametre i sušta je suprotnost sa dosadašnjim poznatim istraživanjima i novost je za kliničku praksu. Stjepanović (2014) je kod uličnih pasa uočio povećan broj eritrocita, leukocita (osim limfocita) i trombocita. Stres može utjecati na krvnu sliku proučavanih pasa. Eritrocitni parametri pasa lutalica pokazuju visoku varijabilnost vrijednosti za retikulocite, što bi moglo ukazivati na razvoj pojedinih hematoloških oboljenja kod pojedinih pasa, kao što je anemija. Široke disperzije vrijednosti leukocitnih parametara, mogu se povezati sa starosnom dobi i spolu životinja. Starosna dob može imati učinak na ukupne vrijednosti parametara leukocita, gdje mladi psi imaju nešto više vrijednosti u odnosu na starije pse (Stjepanović, 2014).

Kod pasa lutalica široka disperzija vrijednosti leukocita i trombocita se može pripisati različitosti pasmina, dobi i spolu psa, stresa okoline, potencijalnim oboljenjima, neuhranjenosti i generalno lošim zdravstvenim stanjem. Naime, pored navedenog kod trombocita, značajan učinak može imati i fizički napor (Stjepanović, 2014).

Kod pasmina istarskog gonič̆a, Labura i sar. (2011) u svom istraživanju su naveli da vrijednosti eritrocita, hemoglobina, hematokrita, MCV-a i sedimentacije su bile zanemarivo iznad gornje fiziološke granice, dok je broj leukocita bio unutar fizioloških granica. Oni smatraju da povišenja ovih parametara mogu biti posljedica dehidracije.

Novaković i sar. (2011) su istraživali hematološke parametre u laktaciji kod pasmine tornjak te prikazuju da je većina njihovih parametara bila u fiziološkim granicama, osim limfocita koji su bili nešto viši u odnosu na fiziološku granicu, a što ukazuje na povećanu odbrambenu sposobnost organizma u periodu laktacije. Blago su bile povišene i vrijednosti hemoglobina, što se objašnjava većim potrebama za kiseonikom tokom laktacije. Također, navedeni autori navode i slične vrijednosti za eritrocite dobivene u istraživanju Bauera i Tadića (1983) ispitivane kod šarplaninca.

ZAKLJUČAK

Analiza hematoloških parametara predstavlja značajan aspekt zdravstvene brige, te su ključne za dijagnozu, monitoring i terapiju hematoloških bolesti. Nužne su dodatne hematološke studije sa značajno većim brojem uzoraka da bi se dobiveni hematološki nalazi u kliničkoj praksi mogli sa većim procentom sigurnosti porediti sa različitim dobnim skupinama kod psa tornjaka.

Definitivno, nepoznanica kod tornjaka je „biološka karta“, a između ostalog i hematološki parametri.

Pored dodatnih istraživanja, jednako je važna i edukacija kako bi se podigla svijest o potrebama ove pasmine među doktorima veterinarske medicine i široj javnosti, a osigurala bi bolju njegu o ovim psima i doprinijelo njihovom očuvanju u budućnosti.

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IMPORTANCE OF HEMATOLOGICAL PARAMETERS IN TORNJAK SHEPHERD DOG IN CLINICAL PRACTICE

ABSTRACT

Bosnian and Herzegovinian Tornjak is an indigeneous dog breed well-known for its strong constitution and endurance. Tornjak is a confident shepherd dog, who excels in guarding a designated territory, but, at the same time has a tender disposition toward his owners, close friends and children. Aim of our study was to research and analyze hematological parameters in Tornjak breed and establish their importance in clinical practice in order to achieve better understanding of health and treatment of the breed. Hematological studies available in the literature on Tornjak have significantly contributed to clarification of the issue with the limitation of the small samples.

Key words: Dog, hematology, Tornjak

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