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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Development Status of The Hindlimb Extremity Bones of The Watchdog Hybrid Fetus (40 Days Old)

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Abstract: Hindlimb extremity bones of 40-day-old watchdog hybrid fetuses were examined. Knowing the normal formation of the extremities is important for understanding possible disorders and their treatment. The developmental processes of the movement system do not occur as sequentially as expected. Alizarin red and alcian according to Inouye technique bones of 40-day-old animals were stained with alcian blue. Dissections of bones preserved in appropriate solutions were performed. Stereomicroscopic and normal photographs were taken with a digital camera. Measurements were taken from the dissected legs with a 150 mm Mitutoyo brand caliper. Hindlimb bones of 40-day-old guard dog hybrid fetuses were observed to have primary ossification centers, while some bones had no ossification centers. A primary ossification center was observed in the corpus of the femur, tibia, fibula, and metatarsus bones of the hind limb, while no ossification center was observed in the proximal and distal ends. Also, patella, ossa tarsi, and ossa digitorum pedis no ossification centers were observed.

Keywords: *Bone, Fetus, Hind limb, Watchdog hybrid.*

Bekçi Köpeği Melezi Fetüsünün (40 Günlük) Arka Ekstremite Kemiklerinin Gelişim Durumu

Özet: 40 günlük bekçi köpeği melezi fetüslerinin arka bacak ekstremite kemikleri incelendi. Ekstremitelerin normal oluşumunu bilmek, olası bozuklukları ve tedavilerini anlamak için önemlidir. Hareket sisteminin gelişim süreçleri beklendiği gibi sırayla gerçekleşmez. Inouye tekniğine göre 40 günlük hayvanların kemikleri alizarin red ve alcian blue ile boyandı. Uygun solüsyonlarda saklanan kemiklerin diseksiyonları yapıldı. Stereomikroskop ve dijital kamera ile fotoğrafları çekildi. Diseke edilen bacaklardan 150 mm'lik Mitutoyo marka kumpas ile ölçümler alındı. 40 günlük bekçi köpeği melez fetüslerinin arka bacak kemiklerinde birincil kemikleşme merkezleri gözlenirken, bazı kemiklerde hiç kemikleşme merkezi olmadığı görüldü. Arka ekstremite kemiklerinden femur, tibia, fibula ve metatarsus kemiklerinin gövdelerinde birer tane primer ossifikasyon merkezi gözlenirken, proksimal ve distal uçlarda ossifikasyon merkezi gözlenmedi. Ayrıca, patella, ossa tarsi ve ossa digitorum pedis'te ossifikasyon merkezi gözlenmedi.

Anahtar Kelimeler: *Arka ekstremite, Fetus, Kemik, Bekçi köpeği melezi.*

Introduction

Understanding the typical development of the foot is crucial for identifying potential disorders and their treatment. The development of the movement system doesn't follow a strictly sequential pattern as one might expect. That's why it should be investigated. Most of the mammalian skeleton, except the skull bones, is formed by endochondral ossification, which is the replacement of cartilage by bone tissue (Wang et al., 2023; Zhang et al., 2011). The vertebral column, pelvis, and extremity bones are formed by endochondral ossification. In this type of bone formation, cartilage tissue forms first and is eventually replaced by bone tissue (Ko and Sumner, 2021; Serra-Vinardell et al., 2020). The first sign of endochondral is a local expansion of bone formation with hypertrophy of the chondrocyte in the middle (diaphysis) of the cartilage model in the long bone (Kume et al., 2012). A miniature model is formed from hyaline cartilage, followed by bone tissue replacing the cartilage model. Until the bones take their final shape, new bone is made on one hand, and a part of the bone is destroyed (remodeling). On the other hand, in the cartilage model the diaphyseal region, the mesenchyme cells in the inner layer of the perichondrium covering the cartilage divide and differentiate into osteoprogenitor cells, which in turn differentiate into osteoblasts (Stevens, 2008). A cylindrical bone cuff is formed in the diaphysis of the cartilage model by intramembranous ossification. The membrane surrounding the bone cuff is now called periosteum (Topaloğlu et al., 2017). Ossification begins in the embryonic stage and continues throughout postnatal life. While primary ossification centers (POC), which form the diaphysis of the bone, first appear during the pregnancy phase, secondary ossification centers (SOC), which may be one or more, appear in the epiphysis of long bones after birth (Getty, 1975; Song, 2022). Ossification in long bones begins towards the end of the embryonal period. First, diaphyses are formed from the POC. In humans, the first foci of ossification of all bones become visible in the 12th week. Ossification is observed in the clavicle before all bones in the mammals' body, followed by the femur (Arıncı and Elhan, 2001; Williams and Dyson, 1989).

The part that ossifies from the second ossification center is the epiphysis of the bone. The femur from the second ossification center tibia with its distal the proximal part forms in the 9th month of intrauterine life in humans. Although most of the second ossification centers are formed after birth, the first ossification center is present at birth (Arıncı and Elhan, 2001).

The diaphysis formed from the first ossification center does not immediately fuse with the epiphysis formed from the second ossification center. This union does not occur until the normal bone dimensions of an adult person are reached. During bone growth, there is a cartilage growth plate between diaphysis and epiphysis. (Doğuer and Erençin, 1962; Williams and Dyson, 1989).

It is important to know the normal development and ossification stages of bones for the diagnosis and treatment of intrauterine anomalies, developmental disorders, and genetic bone tissue diseases (Barone, 1986; Dyce et al., 1987; Atalgın and Çakır, 2006). The formation of the lower extremities begins with bud-like formations of the lower lateral coccyx wall in the late embryonic stages (Gardner et al., 1959). These regions also depend on the variation of elongation and adjustment of the spatial position of the bones, and they do not show a constant growth mode. The relationships between specific foot regions also change considerably throughout prenatal life. Unlike many other body regions, the human foot must undergo several important changes during the fetal period due to its limited and highly specialized functions in postpartum life (Debrunner and Jacob, 1998; Pisani, 1998). The medial foot shows a size maximum relative to other foot regions and the medial foot grows faster than the lateral one (Gruber et al., 2001).

Gruber et al. (2001) state that the human fetal foot has an irregular growth mode and that growth priorities within the foot skeleton vary with age and region. Although the growth of the fetal foot skeleton is irregular, it is not unconnected. The result of this peculiar growth mode is to form the foot and is therefore functionally directed toward its specific purposes. In postnatal life, research has shown that various aspects of the human lower limb undergo irregular changes, influenced by specific growth-priority areas that correspond to different age groups (Schilling, 1985). Bone is very sensitive to external stimuli (Rogers et al., 2021). Increasing knowledge about bone development and bone repair has important therapeutic implications for the treatment of bone disease and aging-associated degeneration (Salhotra et al., 2020).

Nowadays, various techniques are applied to visualize the ossification stages, including single and double staining techniques, radiography, ultrasonography, MRI, and various histological staining methods. Especially double staining techniques give successful results in experimental studies on bone development of animals (Atalgın and Çakır, 2006; Atalgın et al., 2007; Atalgın and Kürtül, 2009). Therefore, we aimed to apply the alizarin red and alcian blue double staining technique to reveal the ossification stages by imaging the hind limb ossification centers of fetuses of an approximately 40-day-old watchdog hybrid.

Materials and Methods

In this study, the hind limbs of 40-day-old watchdog hybrid fetuses were examined. Four dead fetuses were used as research material. Their ages were confirmed using crown rump length (CRL) measurements suggested in the work of Evans and Sack (1973). It was found to be approximately 40 days old and 100 mm long. Samples obtained from the watchdog were kept in 10% formaldehyde solution and washed with distilled water. They were then stored in containers filled with 95% ethanol.

To observe the mineralization stages, the materials were stained in a final solution containing alcian blue (300 mg alcian blue and 100 ml 70% ethanol) and alizarin red (100

mg alizarin red and 100 ml 95% ethanol). This solution was prepared by adding 100 ml glacial acetic acid and 1700 ml 70% ethanol. The hind limbs were placed in mixed staining solution in an etuve at 40 °C for four days and then washed under running water for 2 hours. After washing, they were stored in a container containing 2% KOH. Additionally, the materials were cleaned with 20% glycerin and 1% KOH and stored in 50% and 80% glycerin for 7 days. Finally, they were preserved in 100% glycerin solution. A digital caliper was used to measure the cartilaginous outlines and ossified parts of the bones. Since dead material is used, ethics committee approval is not required.

Results

Femur: The total length of the femur was measured as 12 mm, its body was mostly ossified, and primary ossification centers (POC) were observed in the diaphyseal region. The center of primary ossification measured 5,8 mm. However, no secondary ossification center (SOC) was observed in the epiphyseal region (Figure 1.).

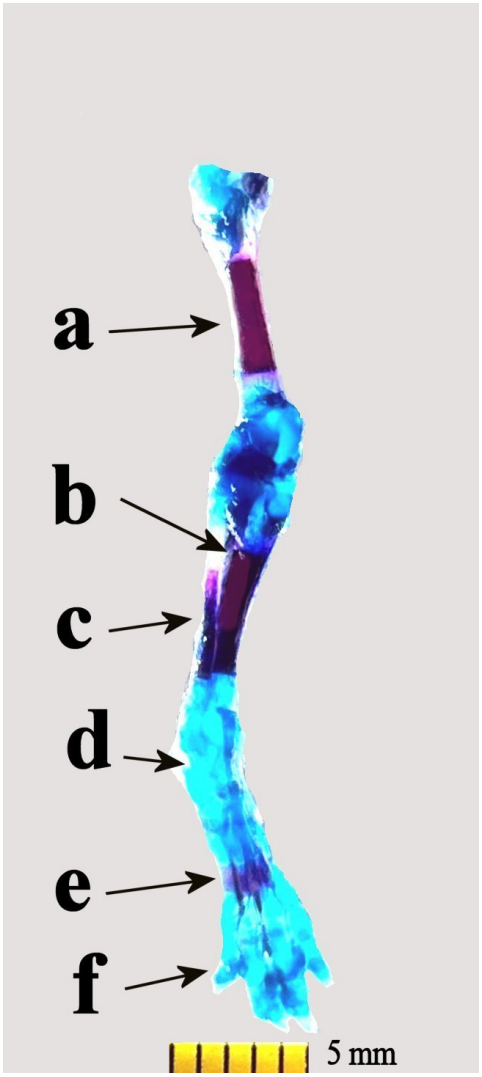

Figure 1. Appearance of the hind limb bones of a 40-day-old watchdog stained according to the Inouye technique. a) Femur b) Tibia c) Fibula d) Ossa tarsi e) Metatarsus f) Phalanx.

Patella: The bone model is entirely cartilaginous (Figure 1.).

Ossa cruris: The total length of the tibia was calculated to be 9,3 mm. The fibula, which is also close in size, measured 12 mm, and while the diaphyseal part of both bones had primary ossification centers, no secondary ossification centers were observed in the epiphyseal parts. While the POC in the fibula had a length of 5 mm, the POC in the tibia was found to be 5.8 mm long (Figure 1.).

Ossa tarsi: The total length of the ossa tarsi was measured as 5 mm. Os tarsi fibulare, os tarsi tibiale (talus) and os tarsale no POC or SOC were found in the quartum bones. The bone model is entirely cartilaginous (Figure 1.).

Os metatarsale: Ossa the total length of the metatarsale was measured 5 mm, and the POC was calculated to be 1 mm long in total. Neither primary nor secondary ossification centers were found in the bone models (Figure 1. and Figure 2.).

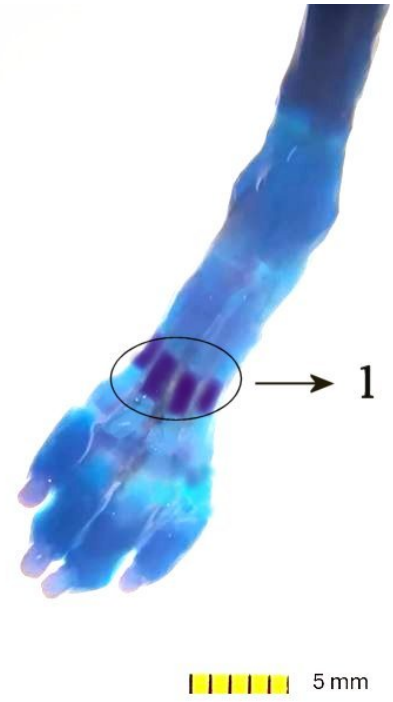

Figure 2. Appearance of the metatarsus of a 40-day-old watchdog stained according to the Inouye technique. 1)Metatarsus

Phalanx: the phalanx was determined 4 mm. Phalanx proximalis, medialis and distalis no POC or SOC was found in the medialis (Figure 1.).

Discussion and Conclusion

Generally, in domestic mammals, the femur consists of one primer ossification center and four secondary ossification centers. The first ossification in the femur occurs in the corpus in intrauterine life. It has been reported that ossification at the distal end begins earlier than the proximal end (Arıncı and Elhan, 2001; Barone, 1986; Willams and Dyson, 1989).

The earliest of these centers is diaphysis; It is observed that it is observed on the 60th or 70th days of intrauterine

life. Generally, our findings in 40-day-old fetuses are consistent with this study.

The femur in dogs develops from five ossification centers (Hare, 1961). The distal end of the femur develops from a single center. According to literature corpus ossis femoris, is formed at birth, the ossification center of the caput ossis femoris was observed in the 1st, 2nd or 3rd weeks after birth (Chapman, 1965). Our findings in 40-day-old fetuses are consistent with this study.

In dogs, the ossification centers united at the proximal end merge with the corpus of the femur at 30-36 months after birth. He reported that the ossification center at the distal end merges with the corpus in the 30th to 37th weeks (Chapman, 1965). In the study, no secondary ossification center was observed in 40-day-old fetuses.

In dogs, the ossification centers joined at the proximal end merge with the body at 30-36 months after birth. He reported that the ossification center at the distal end merges with the corpus of the femur in the 30th to 37th weeks (Chapman, 1965). The observation of only the POC in 40-day-old fetuses is compatible with these data.

Atalgın and Çakır (2006) reported that in newborn rabbits, the patella was observed to be oval and had a cartilage outline without an ossification center. In the study, it was observed in the form of cartilage in the patella too, and no growth centers were observed.

The tibia generally develops from 4 ossification centers (Getty, 1975), one of these centers forms the diaphysis, the other two form the proximal end, and the other forms the distal end (Barone, 1986). In the same literature, it was reported that this center was seen in the 2nd month after birth in carnivora (Barone, 1986). He stated that ossification at the distal end occurred on the 25th day in dogs (Chapman, 1965). But Hare (1961) reported that the tibia in dogs ossifies in 5 centers. In the same literature, it is stated that the diaphysis is well-developed at birth (Hare, 1961).

Ossification at the distal end is observed a little later than the proximal end, at the 1st month in carnivora. This center is seen at the earliest in the 2nd month of carnivora. According to Barone (1986), the closure of the growth cartilage occurs at the distal end towards the 10th month in dogs (Barone, 1986). Generally, the growth cartilage closes around the 8th month. At the proximal end, the closure of the growth cartilage occurs a little later.

The study conducted is in accordance with (Baron, 1986; Chapman, 1965). In this study, while primary ossification centers were observed in the diaphysis region of the tibia, no growth centers were found in the proximal and distal epiphyseal regions.

The fibula in domestic mammals develops from three centers (Barone, 1986; Chapman, 1965). When the ossification center of the tibia is seen, the corpus fibula is observed at approximately the same time (Barone,1986). Being the same size in the same period in our study shows that it is compatible with our data.

In dogs, the ossification center of diaphysis is well-developed at birth. In the same literature, it was reported that the ossification center at the proximal end was observed in the 2nd and 3rd months, while the centers at the distal end were observed in the 4th, 5th, 6th and 7th weeks. It was stated by (Barone, 1986; Chapman, 1965) that in carnivora, the distal epiphysis closes at the end of the 2nd month, while the proximal epiphysis closes later than this. The study conducted is in accordance with Chapman (1965), and Baron (1986).

In dogs, tarsus bones develop from a single ossification center, except for the calcaneus. One of these centers forms the body of the calcaneus, the other the tuber calcanei. He reported that the tarsal bones except the calcaneus showed the POC in the 2nd, 3rd, 4th and 5th weeks, and that the ossification center of all tarsal bones was seen at the end of 2 months. The study was not in accordance with Hare (1961). The reason for this situation is that the dog fetus used in the study was approximately 5.5 weeks old and no growth center was detected in the tarsal bones.

It has been stated that there are two ossification centers in dogs; one of these centers forms the corpus metatarsale, while the other forms the distal end (Chapman, 1965; Hare, 1961).

The fact that the dog fetus in the study was approximately 5.5 weeks old and no growth centers were detected in the tarsal bones contradicts Hare's findings, which are thought to be due to environmental effects, nutrition, and genetic factors.

Phalanxes develop from two ossification centers each. One of them is at the diaphysis and the other is at the distal end. The ossification center in diaphysis is present at birth in dogs (Hare, 1961). Secondary ossification centers at the proximal end are seen on the 30th day in dogs (Chapman, 1965; Hare, 1961). Only primary ossification centers were observed in the study.

These areas are also influenced by the differences in bone elongation and the adjustment of their spatial positioning, and they do not follow a consistent growth pattern. The connections between specific regions of the foot also undergo significant changes throughout prenatal development. The fact that the dog fetus in the study was approximately 5.5 weeks old and no growth centers were detected in the tarsal bones contradicts Hare's (1961) findings, which is thought to be due to environmental effects, nutrition and genetic factors.

As a result, a POC was formed and measured in the hind limb bones of the fetuses of the 40-day-old watchdog hybrid. No SOC has been formed. The data obtained were compared with existing literature data and contributed to the lack of literature on the subject. To date, the causes of many skeletal abnormalities remain unknown, leading to largely empirical approaches in the treatment of foot deformities. The goal of our study is to create a foundation for a morphologically accurate and standardized treatment approach.

Ethical Approval

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees". (In this case, the "Ethics Statement Form" or

"Informed Consent Form" must be filled in, signed by all authors and uploaded to the system.)

(Note: If animals were used in the study, the research should be approved by the ethics committee and the relevant document should be uploaded to the system. For studies that do not use animals, but that were carried out by collecting data, the permission document obtained from the relevant institutions and organizations, indicating that they obtained the data, information and documents within the framework of academic and ethical rules, "Informed Consent Form". or "Ethics Declaration Form" must be filled in and uploaded to the system.)

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Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

Author Contributions

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Design: ŞHA, MK
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Data Collection and/or Processing: ŞHA, MK, KC
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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

The Relationship Among the Hygiene Score, Bedding Type and the California Mastitis Test Score in Family-Type Dairy Farming

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Abstract: It is known that subclinical mastitis has a significant effect on milk yield losses. The hygiene of both the barn and the cow plays an important role in the etiology of subclinical mastitis. In cases where the hygiene score (HS) worsens, the incidence of subclinical mastitis increases. The presented study aimed to determine the relationship between the diagnosis of subclinical mastitis with the California Mastitis Test (CMT) and the Hygiene score (HS). The study was conducted on 80 dairy cows of different breeds from small family-type dairy farms (n=10). Hygiene scores (HS) were recorded in different parts of the body of each animal and the incidence of subclinical mastitis was investigated with CMT in each mammary lobe. When the bedding materials in the barn were compared with the hygiene score (HS); a significant relationship was found between the bedding material of the legs (P=0,037) and belly (P=0,025), respectively. However, no significant relationship was found between the CMT test and the legs and belly (P>0,05). It has been determined that the positivity rate in CMT test results increases in animals with increased hygiene scores (HS). As a result, the relationship between the CMT test and subclinical mastitis and hygiene scores (HS) is thought to be insufficient. No significant relationship was found between the hygiene score (HS) of the rear and animals with positive CMT test results in any mammary lobe (P>0.05). It has been determined that the positivity rate in CMT test results increases in animals with increased hygiene scores (HS). As a result, the relationship between CMT test and subclinical mastitis and hygiene scores (HS) is thought to be insufficient. It would be more effective if the relationship between subclinical mastitis and hygiene score (HS) is supported by Somatic Cell Count (SCC) determination in addition to the CMT test.

Keywords: *CMT test, Dairy farm, Hygiene score, Subclinical mastitis.*

Aile Tipi İşletmelerde Hijyen Skoru, Yataklık Tipi ile California Mastitis Test Skoru Arasındaki İlişki

Özet: Subklinik mastitisin süt verim kayıplarında önemli düzeyde etkili olduğu bilinmektedir. Subklinik mastitisin etiyolojisinde hem ahırın hemde ineğin hijyeni önemli bir rol oynar. Hijyen skorunun kötüleştiği durumlarda subklinik mastitis insidası artar. Sunulan bu çalışmada Kaliforniya Mastitis Testi (CMT) ile subklinik mastitisin belirlenmesiyle hijyen skoru (HS) arasında ilişkinin belirlenmesi amaçlandı. Küçük aile tipi işletmelerde (n=10) bulunan farklı ırklara ait 80 adet sağmal ineklerde araştırma gerçekleştirildi. Her bir hayvanda vücudun farklı bölgelerinde hijyen skoru (HS) kaydedilerek her bir meme lobunda CMT ile subklinik mastitis insidansı araştırıldı. Barınak içerisindeki altlık materyalleri hijyen skoru (HS) ile karşılaştırıldığında; sırasıyla ayakların (P=0,037) ve karın bölgesinin (P=0,025) altlık materyali ile aralarında anlamlı bir ilişki olduğu bulunmuştur. Ancak CMT testi ile bacaklar ve karın bölgesi ile aralarında anlamlı bir ilişki bulunmamıştır (P>0,05). Herhangi bir meme lobunda pozitif CMT test sonucu çıkan hayvanlar ile sağrının hijyen skoru (HS) arasından anlamlı bir ilişki bulunmamıştır (P>0,05). Hijyen skoru (HS) artan hayvanlarda CMT testi sonuçlarında pozitiflik oranı arttığı tespit edilmiştir. Sonuç olarak CMT testi ile subklinik mastitis ile hijyen skoru (HS) arasındaki ilişkinin yetersiz olduğu düşünülmektedir. Subklinik mastitis ile hijyen skoru (HS) arasındaki ilişki için CMT testine ek olarak Somatik Hücre Sayısı (SHS) tespiti ile desteklenirse daha etkili olacaktır.

Anahtar Kelimeler: *CMT testi, Hijyen skoru, Subklinik mastitis, Süt işletmeciliği.*

Introduction

It is known that the most significant economic losses due to milk yield are losses due to mastitis in lactating dairy cows (Abdeen et al., 2021; de Campos et al.,2023; Ijaz et al., 2021). It has been reported that mastitis has many causes (Cobirka et al., 2020). Mastitis varies depending on several factors, including animal breed, age, lactation number, lactation duration, and environmental conditions. For example, Holstein cows are more prone to mastitis than Brown Swiss (Çoban and Tüzemen, 2007). The incidence of mastitis in older animals is higher than in younger animals (Rişvanlı and Kalkan, 2002). Mastitis is divided into two groups: clinical and subclinical (Cobirka et al., 2020; Han et al., 2022). While clinical mastitis is easier to detect by external symptoms, subclinical mastitis continues for a long time without clinical symptoms and goes unnoticed causing yield losses (Ijaz et al., 2021). Clinical mastitis findings are detected by redness in the mammary gland, edema, pain, hardness, and the presence of a vial in milk. Detection methods, including Somatic Cell Count (SCC), measurement of milk electrical conductivity, microbiological examinations, biochemical methods, PCR, and enzyme assays, are used in the diagnosis of subclinical mastitis (Abdeen et al., 2021; Anwar et al., 2022; Cantekin et al., 2015; Özenç, 2019). SCC determination methods are the most commonly used method in practice. Increasing the number of somatic cells (over 400,000 cells/mL) increases the incidence of mastitis in milk. It has been reported that the majority of milk yield loss is due to subclinical mastitis (Çelik and Akçay, 2024; De Graves and Fetrow 1993; Yalçın et al., 2000). Furthermore, SCC is a significant criterion for determining milk quality (Feng et al., 2021; Şafak et al., 2022). CMT is known to be the most common test for identifying SCC in milk and diagnosing subclinical mastitis (dos Santos et al., 2020). Also, they reported that there is a loss of milk yield for each unit increase in the positivity level in CMT (Ayvazoğlu Demir and Eşki, 2019). In addition, the hygiene score of the udder, depending on environmental effects, provides information about mastitis. Unhygienic barn conditions and non-compliance with milking rules increase the incidence of mastitis. The number of somatic cells in milk also increases in animals with increased hygiene scores (Akdag et al., 2017; Çelebi and Akdağ, 2022; Sharif and Muhammad, 2008). It has been reported that the rate of mastitis increases due to high hygiene scores (Mıtev et al., 2013). There are many factors affecting the hygiene score of cows. These include: type of barn, barn conditions, type of bedding, bedding material, grouping, etc. Many factors can be listed. Bedding type and bedding materials are among the important factors. For example, it has been reported that the body parts of cows in bedding types with dry and soft permeable bedding materials are cleaner, while the body parts of cows housed in impermeable, hard, wet bedding are dirtier (Fulwider et al., 2007; Hultgren and Bergsten, 2001; Koçyiğit and Tüzemen, 2015).

Based on this information, we decided to investigate whether there is a relationship between subclinical mastitis using only the CMT test, depending on the hygiene score, in dairy cows in family-type dairy farming.

Material and Methods

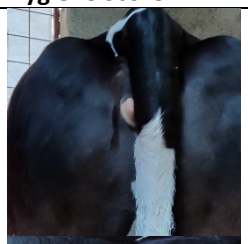
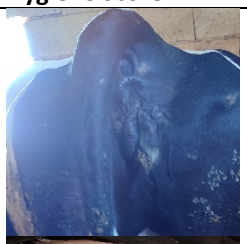
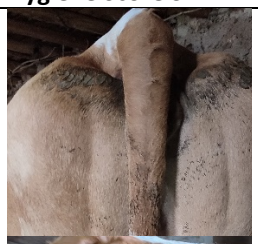
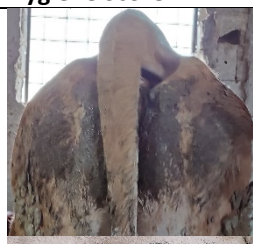
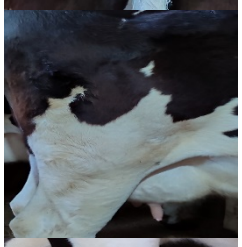
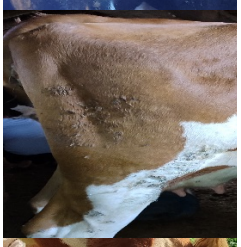
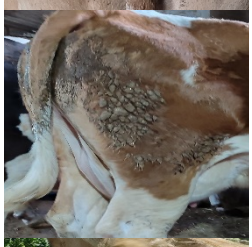
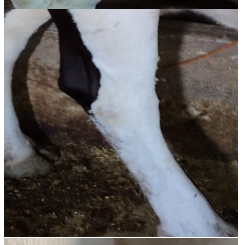
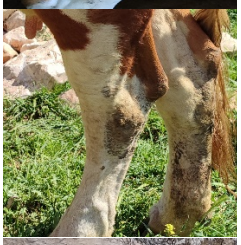
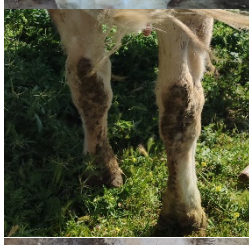
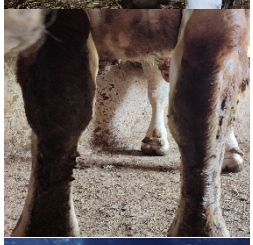
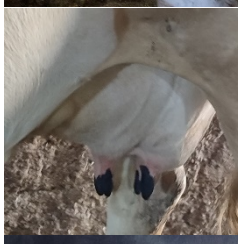
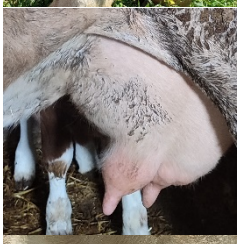
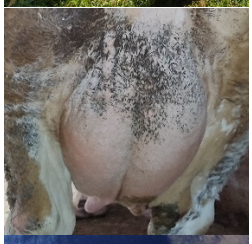
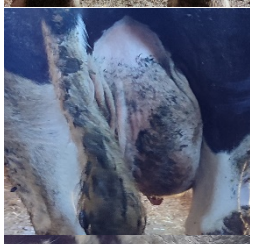
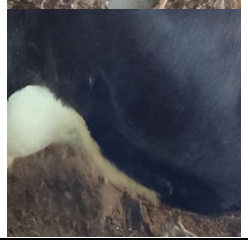
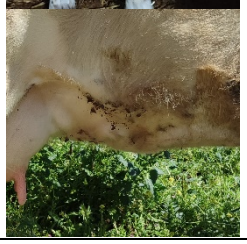
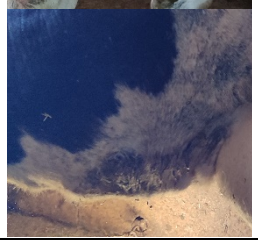
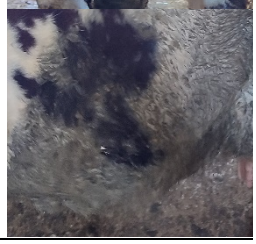
Ethical approval was also obtained from the Harran University Animal Experiments Local Ethics Committee, with decision number 2024/003/01-12 and ethics committee permission dated 16 May 2024, numbered 333691.

This study was conducted on 10 small family dairy farms. The number of animals varied between 2-15 within the borders of Şanlıurfa province. Data records were taken from 80 dairy cows in the study group, including 42 Holstein, 7 Holstein cross, 23 Simmental, 5 Simmental cross, and 3 Brown Swiss cows The ages of the cows ranged from 2 to 9 years. But the majority were between the ages of cows 3 and 5. Lactation periods ranged from 1 month to 12 months. The bedding materials of the barn were recorded according to the type of dairy farm where the animals were kept. Barn types: There were 3 different barn types: Free Stall, Tied Stall, and Semi-open. The bedding materials in the barns were: 1- Concrete bedding, 2- Garden soil, and concrete barn floor and 3- Rubber bedding. There were 3 different bedding bedding. In these family-type dairy farms, there was no application of cleaning the udder before and after milking. All farms were milking twice daily, in the morning (08:00 am) and evening (15:00 pm). After the above information of each animal was taken and recorded in the data notebook, the HS score of each region was given and recorded according to the Hygiene Score in Table 1. Photos were taken of animals on the farm (Fregonesi and Leaver 2001; Schreiner and Ruegg, 2003). The hygiene score is based on the cleanliness of the areas, ranging from the cleanest to the dirtiest. Scores were given as 1 point for the cleanest region and 4 points for the dirtiest region. . TheA sample of milk from each teat was transferred to a California Mastitis Test (Schalm and Noorlander, 1957) container, and CMT (KERBL, Germany) solution was added. The results were then interpreted. The results are scored in 4 different categories: No clotting and color change; negative (-), Mild clotting color change (+), Marked clotting and clumping (++), Immediate and severe clotting, extreme color change (+++).

Statistical Analysis: The hygiene score and Subclinical mastitis data were analyzed using the Kruskal-Wallis test, as they were not normally distributed according to the Shapiro-Wilk test. Additionally, the Pearson Correlation test was applied to show whether there was a relationship between the Hygiene score and Subclinical mastitis data. In addition, group comparisons were analyzed using the Kruskal-Wallis test.

Significance levels were considered $P<0,05$. All data were analyzed. All data were analyzed using the SPSS (version 28.0) statistical program.

Table 1. Hygiene score definition.

	Hygiene Score 1	Hygiene Score 2	Hygiene Score 3	Hygiene Score 4
Rear				
Thigh				
Leg				
Udder				
Belly				

Results

Since the hygiene score differs for each animal's HS region, each region is scored differently (Table 2). CMT results were negative in 50 animals. CMT was positive in a single mammary lobe in 18 animals (+,++,+++). In 10 animals, CMT was positive in two mammary lobes. In one animal, three mammary lobes were positive. Only one animal, all mammary lobes, was positive.

Table 2. Hygiene Score (HS) Points by number of animals

	HS 1	HS 2	HS 3	HS 4
Rear	55	20	4	1
Thigh	25	31	20	4
Leg	13	52	13	2
Udder	57	18	3	2
Belly	51	17	8	4

When the bedding materials in the barn were compared with the hygiene score; It was found that there was a significant relationship between the bedding material of the legs and the belly, respectively [χ^2(2, N=80)=6.60, P=0,037], [χ^2(2, N=80)=7.42, P=0,025]. Apart from these significant relationships, no significant relationship was found between bedding materials and hygiene scores (P>0,05). It was found that there was a significant difference between the hygiene score on the legs and the bedding materials made of concrete garden soil and barn concrete. While the average leg hygiene score of the animals in the barn with concrete bedding material is 2.4, the average leg hygiene score of the animals in the barn with garden soil and barn concrete bedding material is 1.4 (U=87.500; P=0,019). It was found that there was a significant difference between the hygiene score of the belly area and the bedding material rubber bedding and garden soil and barn concrete bedding materials. While the average belly hygiene score of the animals in the barn with rubber bedding is 2.6, the average leg hygiene score of the animals in the barn with garden soil and barn concrete material is 1.5 (U=100.000; P=0.010).

There was no relationship between the hygiene score and the CMT test result in any mammary lobe (P>0,05) (Table 2). It was found that there was a medium positive relationship between the rear and the leg in the hygiene score [*r*(80)=0,487, P<0,01)]. In the hygiene score, it was found that there was a low positive relationship between the rear and the feet (*r*(80)=0.343, P<0,01), udder (*r*(80)=0.269, P<0,05) and belly (*r*(80)=0.328, P<0,01). In the hygiene score, it was found that there was a medium positive relationship between the thigh and the leg (*r*(80)=0.548, P<0,01), udder (*r*(80)=0.505, P<0,01), and belly (*r*(80)=0.553, P<0,01).

It was found that there was a medium positive relationship between the leg and the udder and belly in the hygiene score [*r*(80)=0.411, P<0,01), *r*(80)=0.462, P<0,01)]. It was found that there was a high positive relationship between udder and belly in the hygiene score [*r*(80)=0.665, P<0,01)].

According to the CMT result, it was found that there was a medium positive relationship between the right front udder lobe, the left front udder lobe, and the right rear udder lobe [*r*(80)=0.309, P<0,01), *r*(80)=0.481, P<0,01)]. According to the CMT result, it was found that there was a medium positive relationship between the left front udder lobe and the right rear udder lobe [*r*(80)=0.424, P<0,01)] (Table 3).

No significant relationship existed between the body hygiene score and animals with positive CMT test results in any mammary lobe (P>0,05).

Table 3. Correlation of Hygiene Score and California Mastitis Test.

	HS-R	HS-T	HS-L	HS-U	HS-B	CMT-RFML	CMT- LFML	CMT- RRML	CMT- LRML
HS-R		0,487**	0,343**	0,269*	0,328**	0,056	0,036	0,020	0,032
HS-T			0,548**	0,505**	0,553**	0,102	0,139	0,087	-0,106
HS-L				0,411**	0,462**	0,161	0,074	0,164	-0,119
HS-U					0,665**	0,000	0,076	-0,054	0,036
HS-B						0,140	0,050	0,096	-0,026
CMT-RFML							0,309**	0,481**	0,077
CMT- LFML								0,424**	0,179
CMT- RRML									0,076
CMT- LRML									

HS-R: Hygiene Score Rear, HS-T: Hygiene Score Thigh, HS-L: Hygiene Score Leg, HS U: Hygiene Score Udder, HS-B: Hygiene Score Belly, CMT: California Mastitis Test, RFML: Right Front Mammary Lobe, LAML: Left Front Mammary Lobe, RRML: Right Rear Mammary Lobe, LRML: Left Rear Mammary Lobe ** P<0,01 , * P<0,05.

There is no significant relationship found between animals with positive CMT test results in any mammary lobe in terms of barn type, pad material, breeds, age, lactation periods, lactation duration, and milk yield, respectively (P>0,05).

When the general average hygiene score of each animal was examined according to the CMT result in any udder lobe, it was found that the number of animals with (-) CMT results was higher and the number of animals with low hygiene scores was higher. Animals with CMT results of (+), (++), and (+++) have a high average hygiene score. However, no statistically significant relationship was found between them (Figure 1).

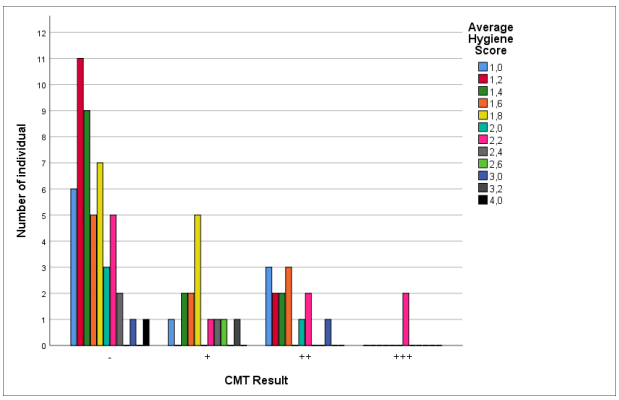


Figure 1. Each animal's average Hygiene Score with relation to the animal's average Hygiene Score in relation to CMT results.

Discussion and Conclusion

According to our results, the hygiene score of each region was different from the others. In addition, hygiene scores in interconnected body parts affect each other positively. In the results reported by Schreiner and Ruegg (2003), it was found that as the udder thigh score, which is considered the hygiene score, increases, the rate of subclinical mastitis increases, and the risk of developing mastitis increases. For example, there is a high-level correlation between the udder area and the abdominal area hygiene score. In addition, a relationship was found between the CMT test and subclinical mastitis between udder lobes in the same direction. We found that the Hygiene score in barns with concrete backing was higher than in barns with other bedding materials. In this study, it showed that the Hygiene scores are also high in concrete-based barns, since the animals are kept tied and always sleep in the same place. It has been reported that the hygiene region is effective with the type of bedding and bedding material in the barn. (Koçyiğit and Tüzemen, 2015). It is supported by our findings. It has been reported that there is a highly significant relationship between udder and foot hygiene in the hygiene score (Schreiner and Ruegg, 2003). This situation is supported by our findings. It is also reported that foot and udder hygiene scores increase in cows that lie in poor barn conditions for longer periods of time (DeVries et al., 2012).

We did not get the results we expected in our findings. We expected the CMT positivity level to be very high in

animals with high hygiene scores. However, since many factors have been shown to be among the causes of subclinical mastitis, making a judgment based only on hygiene will not yield the expected result. It has been reported that SCC (<200,000 µmL1) in milk is low under hygienic milking conditions, and SCC in milk under unhygienic milking conditions increases and increases the incidence of clinical mastitis (Emre et al., 2011). Additionally, are known a high correlation between CMT and SCC. The relationship between subclinical mastitis and the hygiene score with CMT test is thought to be insufficient. In addition to the relationship between the hygiene score and CMT, performing the SCC test will give stronger results. It was understood that the presence of subclinical mastitis by the CMT test was not sufficient to explain the relationship between the hygiene score. We would like to point out that other tests are needed to detect subclinical mastitis.

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Authors Contributions

Motivation / Concept: AU
Design: AU, AO
Control/Supervision: AU, AO
Data Collection and/or Processing: AU
Analysis and / or Interpretation: AO
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Writing the Article: AO
Critical Review: AU

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Ekşi Mayalı Hamur Tozunun Piliç Eti Karkas Özellikleri, Yenilebilir İç Organlar ve Et Kalitesi Renk Özellikleri Üzerine Etkisi

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Özet: Bu çalışma ticari etlik piliç yemlerine ilave edilen ekşi mayalı hamur tozunun piliç eti karkas özellikleri, yenilebilir iç organlar ve et kalitesi renk özellikleri üzerine olan etkisini incelemek amacı ile yapılmıştır. Çalışmada 60 adet RossPM$_3$ genotipi etlik civciv kullanılmış, deneme grubunda yer alan hayvanlar büyütme döneminin son bir haftası hariç deneme süresince %1 oranında ekşi mayalı hamur tozu içeren ticari etlik civciv/piliç yemleri ile, kontrol grubunda yer alan hayvanlar deneme süresince standart etlik civciv/piliç yemleri ile beslenmişlerdir. Her iki grupta yer alan hayvanlar 7 haftalık yaşta standart koşullarda kesilmiş, ön soğutma işleminden sonra bütün karkas ağırlığı ve yenilebilir iç organ ağırlıkları belirlenmiştir. Bütün karkaslar; kemikli göğüs eti, but ve kanat olarak parçalanmış, karkas parçalarının canlı ağırlık ve karkas ağırlığı içindeki payları bulunmuş, göğüs ve but eti renk kalite özellikleri belirlenmiştir. Ekşi mayalı hamur tozu içeren yem ile beslemenin karkas randımanı, karkas parçalarının canlı ağırlık ve karkas ağırlığı içindeki payları üzerine etkisi önemsiz bulunmuş, göğüs etinin derili ya da derisiz olması göğüs eti renk özelliklerinden parlaklık (L*), canlılık/doygunluk (C*) ve ***ΔE*** değerleri üzerine önemli bir etki göstermiştir. Sonuç olarak; ekşi mayalı hamur tozu içeren yem ile besleme piliç eti karkas özellikleri ve et kalitesi renk özellikleri üzerine olumsuz bir etki göstermemiştir. Ekşi mayalı hamur tozunun yeme katılabilecek en uygun karışım oranının belirlenmesi ile piliç etinin besleyici özellikleri üzerine yeni çalışmaların planlanması faydalı olacaktır.

Anahtar Kelimeler: *Ekşi mayalı hamur tozu, Et kalitesi, Karkas, Piliç eti.*

Effects of sourdough powder on carcass characteristics, edible internal organ weights and meat color quality in broiler chickens

Abstract: This study assessed the impact of dietary supplementation of sourdough on carcass, edible internal organs and meat colour characteristics of broiler meat. In total, 60 RossPM$_3$ genotype broiler chickens were used in the study. The chickens in the experimental groups were fed commercial broiler chicken feed containing 1% sourdough powder throughout the trial, except for the last week of the growing period while the chickens in the control group were fed standard broiler feed throughout the trial. All animals in both groups were slaughtered at 7 weeks of age under standard conditions. Whole carcass weight and edible internal organ weights were determined after pre-cooling of the carcasses. The weight of the whole carcass, breast meat, legs and wings were determined. Meat color quality characteristics of breast and leg meat were measured. It was found that the effects of sourdough powder on carcass and carcass yield and edible internal organ weights were found to be not significant. There were significant differences in brightness (L*), chrome (C*) and ***ΔE*** values of breast meat with or without skin between the control and treatment groups. In conclusion; feeding sourdough powder did not have a negative effect on broiler meat carcass characteristics and meat quality color characteristics. It would be beneficial to plan new studies on the nutritional properties of chicken meat by determining the optimum ratio of sourdough that can be added to the ration.

Keywords: *Broiler meat, Carcass, Meat quality, Sourdough powder.*

Giriş

Hayvansal üretimde koruyucu amaçlı antibiyotiklerin yasaklanması ile birlikte bağışıklık sitemini güçlendiren, çevre ve insan sağlığı ile hayvan refahına olumsuz etkileri olmayan, probiyotikler, prebiyotikler, bitki özleri gibi doğal yem katkı maddelerinin hayvan beslemede kullanımı giderek yaygınlaşmaktadır (Rehman ve ark. 2020; Song ve ark. 2022; Taveniello ve ark. 2023). Laktik asit bakterilerini kullanan fermentasyon tekniği hayvan beslemede yaygın bir şekilde kullanılmakta olup (Kljak ve ark. 2023), fermentasyon işlemi ham materyalin yararlanabilirliğini artırarak sindirilebilirliğini geliştirmekte ve hayvanların performansını artırmaktadır. Yüzyıllardır bilinen bir mayalandırma yöntemi olan ekşi hamur; içerisinde laktik asit başta olmak üzere çok sayıda yararlı bakterileri barındırmaktadır (De Vuyst ve ark., 2023). Ekşi hamur; un, su, tuz, maya ve *Saccharomyces cerevisiae* isimli mayayı içeren bir karışımdır. *Saccharamyces cerevisia* sığırlar başta olmak üzere çiftlik hayvanlarında sindirim sistemi faaliyetlerini iyileştirip yemden yararlanmayı geliştirmek için yıllardan bu yana kullanılmaktadır (Bampidis ve ark., 2023; Cai ve ark., 2021; Tun ve ark., 2020). Bilinen yararlarının yanında *Saccharamyces cerevisia* son yıllarda özellikle sığırlarda metan gazı salınımını azaltmak amacı ile çalışılan ürünlerden birisidir (Darabighane ve ark., 2019). Bisküvi hamuru, hamur ekşisi ya da *Saccharamyces cerevisia'nın* bıldırcın ve yumurtacı tavuklarda etkileri üzerine bazı çalışmalar olsa da (Mirakzehi ve ark., 2022; Patil ve ark., 2024; Yousif ve Kloor, 2023) etlik piliç performansı ve piliç eti kalitesi üzerindeki etkileri konusunda yapılmış çalışma sayısı son derece sınırlıdır (Et-Atti ve ark., 2025). Bu çalışma ticari etlik piliç yemlerine ilave edilen ekşi hamur tozunun karkas ve et kalitesi renk özellikleri üzerine etkilerini incelemek amacı ile yapılmıştır.

Materyal ve Metot

Bu çalışma; Bursa Uludağ Üniversitesi Veteriner Fakültesi Hayvan Sağlığı ve Hayvansal Üretim Araştırma Merkezi Araştırma kümesinde gerçekleştirilmiştir. Çalışma için Bursa Uludağ Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan 21.11.2022 tarih ve 2022-16/02 karar no ile araştırma onayı alınmıştır. Ticari koşullarda üretilmiş yemler ile standart besleme programının uygulandığı kontrol grubu ve ticari standart yemlere ekşi mayalı hamur tozunun ilave edildiği, üçer tekrarlı, toplamda 2 ana grup çalışmada yer almıştır. Araştırma grupları pencereli, tünel havalandırma sistemi ve petek-fan serinletme sistemine sahip, doğalgazlı radyanlar ile ısıtılan, otomatik yemleme ve sulama sistemine sahip araştırma kümesinde oluşturulmuştur. Çalışmada; 1x1 m ölçülerinde toplamda 6 deneme bölmesi (tekrarlı gruplar) yer almış, altlık malzemesi olarak 1 m^2 alanda 8 kg., büyük parçacıklı planya talaşı kullanılmıştır. Kontrol ve ekşi mayalı hamur tozu ilave edilen deneme gruplarında 30' ar adet (her tekrarlı grupta 10' ar adet) olmak üzere toplamda 60 adet günlük yaşta erkek RossPM3 genotipi etlik civciv kullanılmıştır.

Deneme gruplarında birim alanda hayvan yoğunluğu ve ışık programı ile diğer çevresel konular "etçi tavukların korunması ile ilgili asgari standartlara ilişkin yönetmeliğe" göre düzenlenmiştir (Resmi Gazete 2018). Gruplarda barındırma yoğunluğu maksimum 33 kg/m^2 canlı ağırlık olarak planlanmıştır. Aydınlatma programı civciv/piliçlerin göz hizasında en az 20 lux yoğunluğunda ışık alacak şekilde uygulanmış, büyütme dönemi başında civciv seviyesinde 33-36 °C sıcaklık sağlanmış, her hafta tedrici olarak sıcaklık 3.0-3.5 °C düşürülerek 4. haftadan itibaren 18-21 °C ´de sabit tutulmuştur. Büyütme döneminde ilk 3 hafta; %40-60, sonrasında %30-40 rutubet sağlanmıştır. Kontrol ve deneme gruplarında büyütme döneminin ilk haftası ve son üç günü 24 saat aydınlatma uygulanmış, diğer günlerde 4 saati kesintisiz olmak üzere 6 saat karanlık:18 saat ışık programı uygulanmıştır. Her iki gruptaki civciv/piliçler deneme süresince ticari bir yem fabrikasından temin edilen etlik civciv/piliç yemleri ile önlerinde sürekli yem olacak şekilde beslenmişlerdir. Deneme başından 10 günlük yaşa kadar etlik piliç başlangıç (%22 protein ve 3000 ME, kcal/kg), 11.-34. günler etlik piliç büyütme (%20 protein ve 3050 ME, kcal/kg), 35.-49. günler arası ise kesim öncesi yem (%18 protein ve 3100 ME, kcal/kg) yem ile besleme yapılmıştır. Kontrol grubundaki hayvanlar deneme süresince standart etlik piliç yemleri ile beslenirken, deneme grubunda yer alan hayvanların yemine, kesim öncesi son hafta hariç, %1 oranında ticari olarak üretilmiş ekşi hamur tozu (toz çavdar ekşisi) katılmıştır. Yemler toz formda hazırlanmıştır. Aşılama ve diğer biyogüvenlik uygulamaları kanatlı işletmeleri için bildirilen standartlara göre yapılmıştır (Tarım ve Orman Bakanlığı; Ticari Etlik ve Yumurtacı Kanatlı İşletmelerinin Biyogüvenlik Talimatı, 2015).

Büyütme dönemi sonunda (49 günlük yaş) gruplarda yer alan hayvanlar bireysel olarak kesim öncesi tartılmış, standart koşullarda kesilmiş (TS 5925, 2014) ve ön soğutma işleminden sonra bireysel tartımlar ile karkas ağırlığı tespit edilmiştir. Bütün karkaslar TSE standartlarına göre (TS 5890, 2014) kemikli göğüs eti, kalçalı butlar ve kanatlar şeklinde parçalanmış, göğüs eti, butlar ve kanatlar bireysel tartılarak ağırlıkları belirlenmiştir. Karkas ağırlığı canlı ağırlığa oranlanarak karkas randımanı bulunmuş, karkas parçalarının canlı ağırlık ve karkas ağırlığı içindeki payları hesaplanmıştır. Göğüs ve but eti örneklerinde renk ölçüm cihazı (PCE-XXM 20, PCE Instruments LTD) ile renk kalite özellikleri belirlenmiştir. Göğüs eti renk özellikleri derili ve derisiz örneklerde, but eti renk özellikleri ise sadece derisiz örneklerde ölçülmüştür. Parlaklık (L*), kırmızı renk koordinatı (a*) ve sarı renk koordinatı (b*) değerleri üç ölçümün ortalamasını dikkate alan Uluslararası Aydınlatma Komisyonu CIELab (Commision Internationele de l'E Clairage) tarafından verilen standartlara göre yapılmıştır (Keskin ve ark. 2017). Buna göre; parlaklık (L*); 0-100; koyu/siyah' tan yaygın beyaza kadar değişen renk tonunu, kırmızı renk koordinatı (a*); -60' a kadar negatif değerler yeşil/mavi, +60'pozitif değerler kırmızının değişik tonlarını, sarı renk koordinatı (b*); -60' a kadar negatif değerler mavinin değişik tonları, +60' pozitif değerler sarının değişik tonlarını göstermektedir (Keskin ve ark. 2017; CIE Lab 1976, King ve ark. 2023, Konica Minolta 2005; Kralik ve ark. 2018).

Elde edilen bu değerler kullanılarak örneklemlerde renk açı değeri (h*, arctan), h◦ =tan-1 (b*/a*).180/π) ve renk doygunluk-canlılık (C*), C*= (a^{*2}+b^{*2})1/2 değerleri ile *ΔE* değerleri (*ΔE* = (L2 + a^2 + b^2)1/2) hesaplanmıştır (Altan ve ark., 2001; Ingram ve ark., 2008).

Karkas randımanı ve karkas parçalarının canlı ağırlık ve karkas ağırlığı içindeki payları ile but eti renk kalite özellikleri bakımından gruplar arası farklılıklar student-t testi ile, göğüs eti renk kalite özellikleri bakımından gruplar arası farklılıklar çok yönlü varyans analizi ile karşılaştırılmıştır (Snedecor ve Cohran, 1989). İstatistiki önem kontrolleri SPSS bilgisayar programında yapılmıştır (IBM Corp, 2021).

Bulgular

Kontrol ve deneme gruplarında karkas ve karkas parçalarının canlı ağırlık içindeki payları Tablo 1' de gösterilmiştir. Karkas randımanı ve karkas parça ağırlıklarının canlı ağırlık içindeki payı bakımından kontrol ve ekşi mayalı hamur tozu içeren deneme grubu arasındaki farklılıklar önemsiz bulunmuştur.

Tablo 1. Karkas randımanı ve karkas parçaları ile yenilebilir iç organların canlı ağırlık içindeki payları (%).

Gruplar	Karkas randımanı	Göğüs	Butlar	Kanatlar	Karaciğer	Kalp	Taşlık
Kontrol	76.57±0.55	32.69±1.98	31.89±1.76	7.70±0.33	2.53±0.18	0.71±0.055	1.21±0.07
Deneme	72.78±0.34	31.12±1.55	30.06±1.24	7.83±0.29	2.50±0.16	0.65±0.043	1.06±0.04
P	*0.467*	*0.478*	*0.332*	*0.774*	*0.862*	*0.407*	*0.125*

Çalışmada yer alan gruplarda karkas parçalarının karkas ağırlığı içindeki payları Tablo 2' de sunulmuştur. Karkas parçalarının bütün karkastaki payı bakımından gruplar arası farklılıklar önemsiz bulunmuştur. Kontrol ve deneme gruplarında kemikli göğüs etinin bütün karkastaki payı sırası ile; %43.41 ve 43.42, butların payı sırası ile %42.52 ve 41.78, kanatların payı sırası ile; %10.30 ve 10.87 hesaplanmıştır.

Tablo 2. Deneme gruplarında karkas parçalarının karkas ağırlığı içindeki yüzde payları.

Gruplar	Göğüs Eti	Butlar	Kanatlar
Kontrol	43.41±2.27	42.52±2.29	10.30±0.51
Deneme	43.42±2.83	41.78±1.99	10.87±0.43
P	*0.994*	*0.656*	*0.207*

Kontrol ve deneme gruplarında elde edilen karkaslarda göğüs ve but eti renk kalite özellikleri Tablo 3 ve Tablo 4' de gösterilmiştir. Ekşi mayalı hamur tozu ile beslemenin göğüs ve but eti renk kalite özellikleri üzerine etkisi önemsiz bulunurken göğüs etinin derili ya da derisiz olması parlaklık, (L*; P<0.001), Chrome (C*, P<0.05) ve ΔE (P<0.001) renk değerlerini önemli düzeyde etkilemiştir. Göğüs eti parlaklık (L*) değeri üzerine besleme x göğüs eti kondisyonu arası interaksiyon etkisi önemli bulunmuştur (P<0.01).

Kontrol ve deneme gruplarında but eti parlaklık (L*) değeri; 58.23 ve 57.72, kırmızı renk koordinatı değerleri; -15.08 ve -15.80, sarı renk koordinatı (b*) -6.00 ve -4.59 bulunmuş, hue açı (h*) ve chrome (C*) değerleri sırası ile 1.33 ve 1.20; 18.25 ve 17.51 hesaplanmıştır.

Tablo 3. Kontrol ve deneme gruplarında derili ve derisiz göğüs eti renk değerleri.

Grup/Özellik	L*	a*	b*	h*	C*	*ΔE*
Besleme						
Kontrol	59.43±1.40	-14.91±4.23	-2.34±0.92	0.686±0.29	20.13±3.42	2099±160
Deneme	62.38±1.38	-16.21±4.22	-3.03±0.91	0.713±0.30	17.88±3.44	2260±159
Göğüs eti						
Derili	66.03±1.40	-19.20±4.20	-1.94±0.96	0.476±0.29	23.95±3.42	2644±158
Derisiz	55.79±1.38	-11.92±4.19	-3.43±0.95	0.922±0.29	14.06±3.45	1714±160
BeslemexGöğüs eti						
KontrolxDerili	61.28±1.96	-14.42±5.61	-2.15±1.36	0.392±0.44	23.61±4.85	2342±224
KontrolxDerisiz	57.59±1.93	-15.39±5.70	-2.53±1.32	0.980±0.42	16.65±4.81	1855±225
DenemexDerili	70.78±1.95	-23.97±5.97	-1.73±1.33	0.561±0.43	24.30±4.88	2945±224
DenemexDerisiz	53.98±1.94	-.8.45±5.80	-4.34±1.34	0.864±0.42	11.47±4.67	1573±222
ANOVA						
Besleme	0.141	0.829	0.606	0.950	0.648	0.481
Göğüs Eti	0.001	0.231	0.270	0.295	0.050	0.001
Besleme x Göğüs Eti	0.002	0.176	0.406	0.737	0.551	0.058

L*: parlaklık, a*: kırmızılık, b*: sarılık, h: açı değeri***ΔE****: renk fark, C*: renk doygunluk düzeyi.

Tablo 4. Deneme ve kontrol gruplarında but eti renk değerleri.

Grup/Özellik	L*	a*	b*	h*	C*	*ΔE*
Kontrol	58.23±0.75	-15.08±2.63	-6.00±0.77	1.33±0.19	18.25±3.42	1899±86
Deneme	57.72±0.76	-15.80±2.62	-4.59±0.75	1.20±0.18	17.51±3.44	1862±85
P	*0.645*	*0.851*	*0.195*	*0.607*	*0.804*	*0.762*

L*: parlaklık, a*: kırmızılık, b*: sarılık, h: açı değeri**ΔE***: renk fark, C*: renk doygunluk düzeyi.

Tartışma ve Sonuç

Piliç eti üretiminde koruyucu amaçlı antibiyotik kullanımının yasaklanmasından sonra (Anonim 2005; FDA, 2015) hayvanların bağışıklık sistemlerini güçlendirmek ve salmonellozis, kolibasillozis, nekrotik enteritis (Diarra ve Maloin, 2015) gibi sindirim sistemi enfeksiyonlarını önlemek amacıyla kullanılan başlıca alternatiflerden birisi *Saccharomyces cerevisiae* isimli mayadır (Soren ve ark., 2024). Bu çalışmada *Saccharomyces cerevisiae* isimli maya hücreleri içeren hamur mayası ile mayalanmış toz çavdar hamur ekşisi içeren rasyonun etlik piliç karkas özellikleri ve bazı et kalitesi özellikleri üzerine etkisi incelenmiştir. Kurutulmuş ekşi mayalı çavdar hamur tozu; doğal çavdar hamurundan üretilmiş bir ürün olup, hamur ürünleri yapımında fırıncı mayasının alternatifidir (Arora ve ark., 2021).

Standart etlik piliç yemleri ile beslenen kontrol grubu ve standart yeme %1 oranında ilave edilen ekşi mayalı çavdar hamur tozu ile beslenen deneme grubunda karkas ve karkas parçalarının canlı ağırlık içindeki payı bakımından gruplar arası farklılıklar önemsiz bulunmuştur. Bu bulgu, maya ve maya ürünlerinin etlik piliç karkas ve karkas parçaları üzerine önemli bir etkisi olmadığını bildiren çalışma bulguları ile benzerdir (Morales-Lopes ve ark., 2009; Yalçın ve ark., 2013). Ekşi mayalı hamur tozu içeren yem ve standart yem ile beslenen gruplarda hesaplanan karkas randımanı ile kemikli göğüs eti ağırlıklarının canlı ağırlık içindeki yüzde oranları Ross 308 genotipi için farklı yemleme programlarında yetiştirilen etlik piliçlerden hesaplanan bulgulardan daha yüksek bulunmuştur (Martinez ve ark., 2021). Karaciğer ve taşlık ağırlıklarının canlı ağırlık içindeki oranları Özbek (2020) tarafından 56 günlük yaşta kesilen hızlı gelişen genotip için bildirilen değerden yüksek iken, kalp ağırlığının payı benzer bulunmuştur.

Saccharomyces cerevisia fırıncı mayası olarak da bilinmekte olup, probiyotik etkiye sahip ve hayvansal üretimde oldukça fazla kullanılan bir maddedir. Bu çalışmada doğal çavdar hamurundan üretilmiş toz çavdar ekşisi kullanılmıştır. Ekşi mayanın glisemik endeksi düşük olup, içerdiği laktik asit, vitamin ve mineraller ile bağışıklık sistemini güçlendirmeye katkı sağlamaktadır. Aynı zamanda ekşi maya probiyotik bir etkiye sahip olup, bıldırcınlarda karaciğer ağırlığını düşürdüğü ve performansı artırdığı bildirilmiştir (Coşkun, 2018).

Kanatlı hayvanlarda et rengini etkileyen başlıca faktörler; genetik yapı, cinsiyet, yaş, besleme, kesim öncesi işlemler ve hayvan kesimi esnasındaki uygulamalardır (Krallik ve ark., 2018). Bu çalışmada; standart etlik piliç yemleri ile standart yeme ilave edilen ekşi hamur tozunun etlik piliç göğüs ve but eti renk kalite özellikleri üzerine etkisi önemsiz bulunmuştur. Göğüs etinin derili ya da derisiz olması L* ve ***ΔE*** değeri gibi bazı renk kalite özelliklerini önemli düzeyde etkilemiştir. Bu çalışmada kontrol ve deneme gruplarından elde edilen göğüs etlerinde ölçülen parlaklık (L*) değeri 56' dan daha büyük olduğundan Lee ve ark. (2022) tarafından yapılan tanımlamaya göre her iki gruptaki etlerin açık/parlak renkli olduğu söylenebilir. Göğüs eti parlaklık değeri aynı zamanda göğüs eti dejenerasyonları (miyopatiler) ve karkas ağırlığından da önemli düzeyde etkilenmektedir (Abdullah ve ark. 2025; Munoz-Lapeira ve ark., 2025). Bütün renk kalite özellikleri ölçüldüğü yere göre de (cranial, caudal, central) farklı olabilir. Grigore ve ark. (2023) broyler yemlerine katılan maya ürünlerinin göğüs eti parlaklık düzeyini önemli düzeyde etkilediğini bildirmişlerdir. ***ΔE*** değeri renkleri birbirinden ayırt etme, Chroma ve hue açı değerleri görsel renk algıları ile yakından ilişkilidir. Hue açı değeri renklerin canlılığını ifade etmekte olup, yüksek olması canlı renkleri, düşük değer renklerin donuk olduğuna işaret etmektedir (Hernandez Salueña ve ark., 2019). Pişmiş ve çiğ piliç etinin rengi, görsel açıdan sağlıklı ve taze piliç etinin en önemli göstergelerinden birisidir ve tüketici tercihini doğrudan etkilemektedir (Font-i-Furnols ve Guerrero, 2014).

Çalışmadan elde edilen bulgular bütünü ile değerlendirildiğinde; çalışmada kullanılan oranda etlik piliç yemlerine katılan ekşi hamur tozunun etlik piliç karkas ve karkas özelikleri ile et kalitesi renk özellikleri üzerine olumsuz bir etkisi olmadığı görülmüştür. Ticari üretim koşullarında, yeme katılacak en uygun ekşi mayalı hamur tozu oranını belirleme amaçlı çalışmalar ile ekşi mayalı hamur tozunun bağışıklık sistemi, hayvan refahı, piliç etinde kalıntı düzeyleri vb. dikkate alan yeni çalışmaların planlanmasının faydalı olacağı düşünülmektedir.

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Histopathological Grading of Idiopathic Gastroenteritis Diseases in Dogs

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Abstract: Idiopathic gastroenteritis is a disease that causes lesions in the stomach and intestines. Twenty dogs of different ages, genders, and breeds showing diarrhea, vomiting, weight loss, and other gastrointestinal findings brought to the Department of Pathology were performed necropsy. For pathological examinations, samples were taken from the cardia, fundus, antrum, and pylorus parts of the stomach and the duodenum, jejunum, ileum, cecum, colon, and mesenteric lymph nodes of the intestines. They were stained with hematoxylin and eosin (H&E). It was diagnosed as lymphocytic-plasmacytic gastritis with eosinophilic enteritis (n: 4), eosinophilic enteritis (n: 4), lymphocytic-plasmacytic gastroenteritis (n: 3), eosinophilic gastroenteritis (n: 4) and lymphocytic-plasmacytic enteritis (n: 5) histopathologically. Thus, it was determined that idiopathic gastroenteritis disease was thought to be common in Turkey and current data on incidence of the disease were provided.

Keywords: *Eosinophilic gastroenteritis, IBD, Lymphocytic-Plasmacytic gastroenteritis, Pathological grading method.*

Köpeklerde İdiyopatik Gastroenterit Hastalıklarının Histopatolojik Derecelendirilmesi

Özet: İdiyopatik gastroenteritis, mide ve bağırsaklarda lezyonlara neden olan bir hastalıktır. Patoloji Anabilim Dalı'na getirilen ishal, kusma, kilo kaybı ve diğer gastrointestinal bulguları gözlenen farklı yaş, cinsiyet ve ırktan yirmi köpeğin nekropsisi yapıldı. Patolojik incelemeler için midenin kardia, fundus, antrum ve pilorus kısımlarından ve bağırsakların duodenum, jejunum, ileum, sekum, kolon ile mezenterik lenf düğümlerinden örnekler alındı. Bunlar hematoksilen ve eozin (H&E) ile boyandı. Histopatolojik inceleme sonucunda eozinofilik enteritis (n: 4), eozinofilik enteritis (n: 4), lenfositik-plazmasitik gastroenteritis (n: 3), eozinofilik gastroenteritis (n: 4) ve lenfositik-plazmasitik enteritis (n: 5) ile lenfositik-plazmasitik gastritis tanısı konuldu. Bu nedenle idiyopatik gastroenteritis hastalığının Türkiye'de yaygın olduğu düşünülmektedir. Ayrıca hastalığın insidansı hakkında güncel veriler sağlanmıştır.

Anahtar Kelimeler: *Eozinofilik gastroenteritis, IBD, Lenfositik-Plazmasitik gastroenteritis, Patolojik dereceleme yöntemi.*

Introduction

Infectious agents (bacterial, viral, fungal, parasitic), toxins, exposure to foreign bodies, genetic predisposition, and food allergens play a role in the etiology of gastroenteritis diseases (Cerquetella et al., 2010), and these factors can be seen in three forms of the disease (peracute, acute, and chronic). However, the etiology of idiopathic inflammatory bowel disease (IBD), which occurs with the progression of the chronic form, is not fully known (Jergens and Simpson, 2012). There are opinions that the main mechanism of etiopathogenesis is that the lymphoid tissues related to the intestines show hypersensitivity to antigens in the gastrointestinal system, causing loss of tolerance (Cerquetella et al., 2010; Hall et al., 2005; Rychlik et al., 2007). In addition, breed predisposition, immune dysregulation and the interaction of environmental factors are also important factors in the etiology of the disease (Niina et al., 2021). The disease can be seen in dogs of all ages and breeds. It is more likely to be seen in Boxers, Border Collies, German Shepherds, Rottweilers (Farray et al., 2020; Kathrani et al., 2011) and in dogs five years of age and younger (Fonseca-Alves et al., 2012). The disease has no gender specificity (Cerquetella et al., 2010).

The lesions of the disease occur in the stomach and intestines. The disease is divided into three types according to the localization of immune system cells in the stomach and intestines: lymphocytic-plasmacytic, eosinophilic and granulomatous (Day et al., 2008, Simpson, 2010). The most common forms in dogs are lymphocytic-plasmacytic enteritis and eosinophilic gastroenteritis. Granulomatous inflammation is less common (Rychlik et al., 2007).

The Canine IBD Activity Index - CIBDAI, a clinical grading method, was developed to objectively correlate laboratory and histological indices of clinical findings in idiopathic gastroenteritis disease. This method is routinely accepted worldwide (Jergens et al., 2003). To better understand and examine IBD disease, a histopathological grading system was developed in the stomach and intestines (Allenspach et al., 2019; McCann et al., 2007). However, histopathological grading studies are rare (Farray et al., 2020). In this system, cell infiltration, eosinophil presence, fibrosis, atrophy of intestinal villi, expansion of crypts, and increase in goblet cells were evaluated (Fonseca-Alves et al., 2012; Lyles et al., 2009; McCann et al., 2007; Rychlik et al., 2007).

For the treatment to be applied to animals with IBD to have positive results, it is essential to grade the severity of the disease pathologically. The aim of the study was to examine the breed, gender, and age distribution in dogs with suspected IBD or showing gastrointestinal findings and to classify the inflammation in the stomach and intestines by grading.

Materials and Methods

In this study, 20 dogs of different ages, genders, and breeds showing diarrhea, vomiting, weight loss, and other gastrointestinal findings brought to the Department of Pathology were performed necropsy. This study was approved by the Ankara University Animal Experiments Local Ethics Committee, Ankara, Türkiye (Decision No: 2023-11-100).

Macroscopic examinations:

For pathological examinations, samples were taken from the cardia, fundus, antrum, and pylorus parts of the stomach and the duodenum, jejunum, ileum, cecum, colon, and mesenteric lymph nodes of the intestines. The mucosal surfaces of the stomach and intestines were examined macroscopically for parasites, hemorrhages, etc. Tissue samples were fixed in 10% buffered formalin.

Histopathological examinations:

After the routine tissue process, the cells were embedded in paraffin wax and sectioned at 4 µ. They were stained with hematoxylin and eosin (H&E). The results were evaluated under a light microscope (Leica DM 4000M) and photographed (Leica DFC-280).

Results

Breed, gender and age distributions of dogs:

The breed distribution of dogs (n:20) was as follows; mixed breed (n:5), German shepherd dog (n:3), Terrier (n:2), Rottweiler (n:2), Labrador (n:2), Pomeranian (n:1), Springer spaniel (n:1), Catalan shepherd dog (n:1), English cocker (n:1), Anatolian shepherd dog (n:1) and Pug (n:1). Age distribution was as follows; under 12 months (n:7), between 1-10 years (n:8) and over 10 years (n:5). The genders were determined as male (n:10) and female (n:10).

Macroscopic findings:

The lumens of the dogs' stomachs and intestines were opened entirely, and the gastrointestinal system was examined in detail. Foreign bodies (n:2), volvulus (n:1), and hemoabdomen (n:5) were observed in the stomach. Hemorrhages (n:3) were detected in the stomach and intestines, especially in the jejunum. Yellowish-greenish mucoid content was detected in the lumens of the intestines (n:15). However, there were no prominent ulcer areas in the GI tract. Parasites were not observed in any case.

Microscopic findings:

All stomach and intestinal sections were examined as modified according to the parameters in the histopathological scoring system of Allenspach et al. (2019) and McCann et al. (2007) (Table 1).

Accordingly, in the stomach; lymphocytes and plasma cells, eosinophils, neutrophils in lamina propria, fibrosis and intraepithelial lymphocytes; in small intestines; crypt dilatation, lymphocytes and plasma cells, eosinophils, neutrophils in lamina propria, in large intestines (colon); crypt dilatation, fibrosis, goblet cell count, lymphocyte, plasma cell, eosinophil and macrophage in lamina propria were examined (Fig. 1a-h) and converted into numerical data per x400 field (Table 2). Each parameter was graded as 0 (Normal), 1 (Mild), 2 (Moderate) and 3 (Marked) and they were diagnosed.

Table 1. Modified scoring system for defining gastrointestinal inflammation.

Location	Histopathological Parameters	Grade			
		0	1	2	3
		(Normal)	(Mild)	(Moderate)	(Marked)
Stomach (Fundus)	Fibrosis (number of fibrocytes separating glands)	≤2	3–5	6–10	≥11
	Intraepithelial lymphocytes (lymphocytes per stretch of 50 epithelial cells)	≤2	3–10	11–20	≥21
	Lamina propria lymphocytes and plasma cells (cells per 400× field)	≤20	21–50	51-100	≥101
	Lamina propria eosinophils (cells per 400× field)	≤2	3–20	21–50	≥51
	Lamina propria neutrophils (cells per 400× field)	0	≤20	21–50	≥51
Stomach (Antrum)	Fibrosis (number of fibrocytes separating gastric pits or mucous glands)	≤10	11–15	16–20	≥21
	Intraepithelial lymphocytes (lymphocytes per stretch of 50 epithelial cells)	≤2	3–5	4–10	≥11
	Lamina propria eosinophils (cells per 400× field)	≤2	3–10	11–50	≥51
Duodenum and ileum	Crypt dilation (% of crypts that were dilated, distorted, or containing eosinophilic material/degenerate neutrophils)	≤2	3–10	11–25	≥26
	Lamina propria lymphocytes and plasma cells (% area of one 400× villous field or cells between crypts)	≤25, ≤2	26–50, 3–5	51–75, 6–10	≥76, ≥11
	Lamina propria eosinophils (cells per 400× field)	≤3	4–10	11–20	≥21
	Lamina propria neutrophils (cells per 400× field)	0	≤10	11–30	≥31
Colon	Crypt dilation and distension (% of crypts per section)	0	≤25	26–50	≥51
	Fibrosis (number of fibrocytes separating crypts)	≤2	3–5	6–10	≥11
	Goblet cell numbers (% reduction from normal)	0	≤25	26–50	≥51
	Lamina propria lymphocytes and plasma cells (cells between crypts)	≤5	6–10	11–20	≥21
	Lamina propria eosinophils (cells per 400× field)	≤2	3–10	11–20	≥21
	Lamina propria macrophages (cells per 400× field)	≤2	3–20	21–50	≥51

The cardia and fundus and the antrum and pylorus sections of the stomach were evaluated together because they gave the same results. Grading could not be done because the stomach (n: 1) was autolytic (Case 5). Therefore, histopathological grading was done on 19 cases.

The intestines, duodenum, jejunum, ileum, and colon were evaluated separately and concluded. The jejunum and ileum generally showed similar results. Since one of the samples in the ileum (Case 10) and colon (Case 17) was autolytic, the results were assessed in 19 cases.

No parasites, hemorrhages, inclusion bodies, bacterial clusters, or dense neutrophil leukocyte infiltrations were found in the stomach or intestinal sections.

After all these microscopic scoring and examinations, inflammation in the intestines was observed in all dogs. It was diagnosed as lymphocytic-plasmacytic gastritis with eosinophilic enteritis (n: 4), eosinophilic enteritis (n: 4), lymphocytic-plasmacytic gastroenteritis (n: 3), eosinophilic gastroenteritis (n: 4) and lymphocytic-plasmacytic enteritis (n: 5) histopathologically.

Discussion and Conclusion

IBD, or idiopathic gastroenteritis, is a disease that causes lesions in the stomach and intestines. Due to its etiological uncertainty, it is an area to be investigated, especially in animals. Different methods must be used to define the disease because clinical findings and diagnostic tests are not specific and because of the possible side effects of the drugs used in treatment (Dye et al., 2013).

The distribution of dogs in the study by gender (n: 10 each) and age (5 years and under (n: 11)) supports previous studies (Cerquetella et al., 2010). In addition, the disease was more common in Rottweilers, German shepherds, and mixed breeds in this study and detected similar results to previous studies (Kathrani et al., 2011; Minnat et al., 2017).

Macroscopic findings in IBD are mostly detected in the proximal part of the stomach and intestines (German et al., 1999). Mild redness, erosion and ulcer areas, occasional folds, and diphtheroid formations are seen in the mucosa (Rychlik et al., 2007). Additionally, mild thickening in the duodenum and areas of serosal hyperemia in the ileum have been observed (McTavish, 2002). Microscopic findings show that all layers of the small intestine are thicker than usual, there are mononuclear cell infiltrations, connective tissue hyperplasia, fibrosis, atrophy of intestinal villi and expansion of crypts, disruption of the integrity of the gastric and intestinal epithelium, leukocytosis and epithelial cell necrosis in the mucosa in general (Fonseca-Alves et al., 2012; McCann et al., 2007; Rychlik et al., 2007). An increase in eosinophils, lymphocytes and plasma cells was observed as a differential diagnosis (Lyles et al., 2009). The results obtained were mainly consistent with the macroscopic and microscopic findings.

In a study of Kanat and Ortatatlı (2022) in examination of histopatlogical in intestines, the cases in which epithelial degeneration, desquamation, hyperaemia, oedema, neutrophil granulocyte and macrophage infiltrations in propria are preponderant, were described as acute; mononuclear cell infiltrations and fibrosis/atrophy formed cases as chronic; and the cases formed with only dense

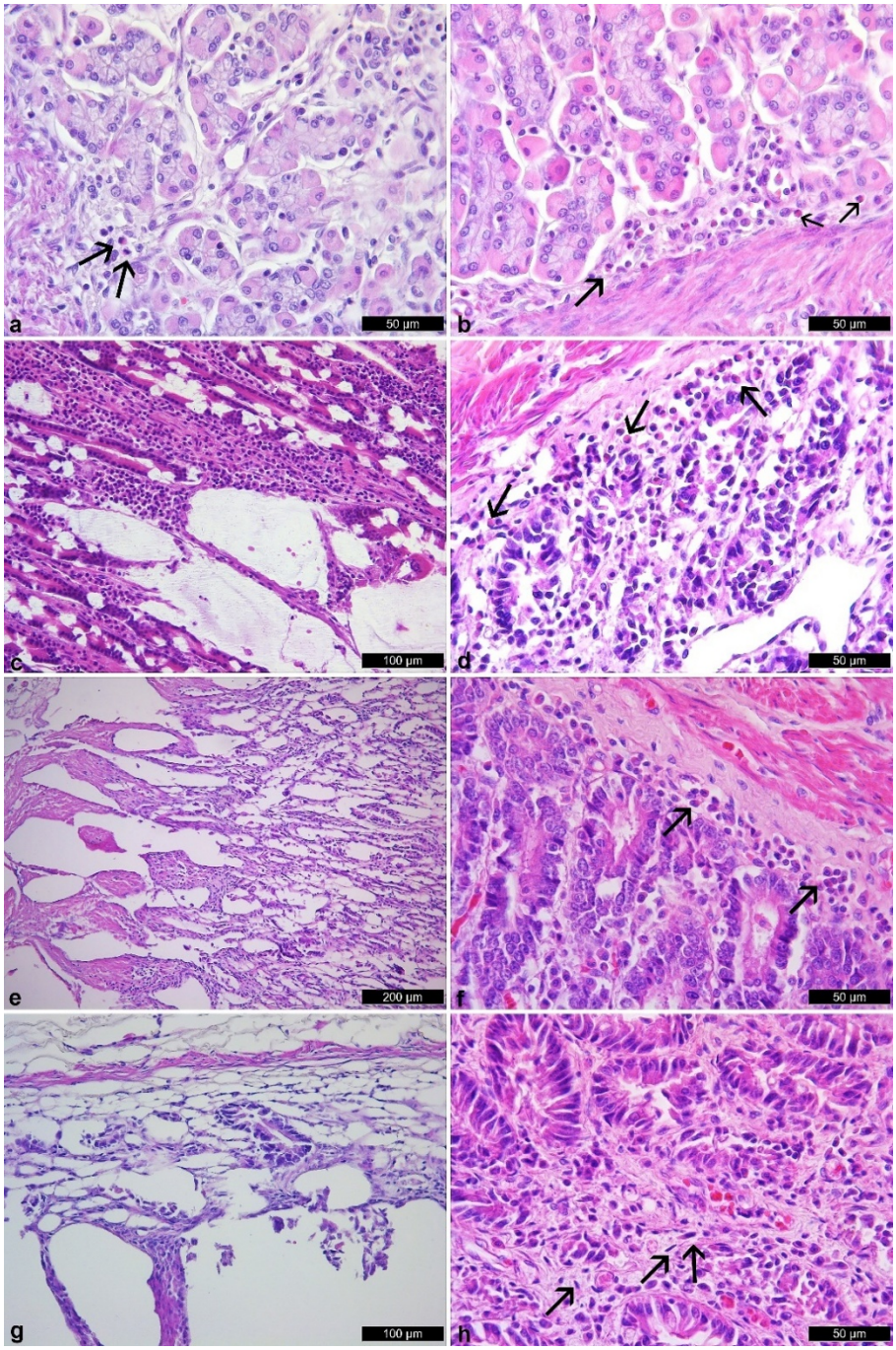

Figure 1a: Few eosinophils (arrows) in the lamina propria of the stomach (grade 1), b: Many eosinophils (arrows) in the lamina propria of the stomach (grade 3), c: Crypt dilatation in the duodenum (grade 2), d: Eosinophils (arrows) in the lamina propria of the duodenum (grade 2), e: Crypt dilatation in the jejunum (grade 3), f: Eosinophils (arrows) in the lamina propria of the jejunum (grade 2), g: Crypt dilatation in the colon (grade 3), h: Connective tissue cells (arrows) that cause fibrosis in the colon (grade 2), HE.

lymphocyte and plasma cell accumulations in mucosa were defined as lymphocytic and plasmacytic enteritis. However, since this study aimed to examine idiopathic gastroenteritis diseases in dogs histopathologically, the stomach sections were reviewed along with the intestines. Here, some parameters in the histopathological scoring system of Allenspach et al. (2019) and McCann et al. (2007) were used. Accordingly, in the stomach; lymphocytes and plasma cells, eosinophils, neutrophils in lamina propria, fibrosis and intraepithelial lymphocytes; in small intestines; crypt dilation, lymphocytes and plasma cells, eosinophils, neutrophils in lamina propria, in large intestines (colon); crypt dilatation, fibrosis, goblet cell count, lymphocyte, plasma cell, eosinophil and macrophage in lamina propria were examined and they were diagnosed only as eosinophilic or lymphocytic-plasmocytic gastro and/or enteritis, (Table 2). Thus, the results and diagnoses were compatible with the previously applied scoring system.

Table 2. Histological grading and diagnosis in all cases histological grading and diagnosis in all cases.

Histopathologic Parameter		**Case No**																			
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Stomach (Cardia + Fundus)	Fibrosis	0	0	0	0	*	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Intraepithelial lymphocytes	2	0	0	0	*	1	1	0	0	0	0	3	1	0	0	0	2	0	0	0
	Lymphocytes and plasma cells in LP	3	0	1	1	*	1	1	0	0	1	0	3	1	1	2	0	2	0	0	0
	Eosinophils in LP	1	0	0	0	*	0	2	0	0	0	0	1	1	0	1	0	0	0	1	1
	Neutrophils in LP	1	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stomach (Pylorus)	Fibrosis	0	0	0	0	*	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0
	Intraepithelial lymphocytes	0	0	0	0	*	1	1	0	0	1	0	2	2	1	0	0	1	0	0	0
	Eosinophils in LP	1	0	1	0	*	0	1	0	0	0	0	3	1	0	0	0	0	0	0	0
Duodenum	Crypt dilation	0	0	1	0	0	1	1	2	0	0	1	0	2	1	1	0	2	3	1	1
	Lymphocytes and plasma cells in LP	3	1	2	1	1	3	3	3	1	2	3	3	3	3	2	3	3	1	2	3
	Eosinophils in LP	3	0	1	0	0	2	2	1	0	0	0	3	3	2	2	3	3	0	3	1
	Neutrophils in LP	0	0	1	0	1	1	0	1	0	0	0	1	0	0	1	1	1	1	0	0
Ileum	Crypt dilation	0	0	0	0	0	1	2	1	0	*	1	0	2	1	1	2	2	3	1	0
	Lymphocytes and plasma cells in LP	3	2	2	1	1	3	3	3	1	*	3	3	2	2	1	3	1	1	2	3
	Eosinophils in LP	3	1	0	0	0	2	3	2	0	*	2	2	3	2	3	1	0	0	3	0
	Neutrophils in LP	1	1	1	0	1	1	0	1	0	*	0	0	0	0	1	0	0	1	0	0
Colon	Crypt dilation	1	1	1	1	1	1	2	1	0	2	2	1	3	1	1	1	*	3	1	1
	Fibrosis	0	0	0	0	0	1	2	0	0	1	0	3	0	2	2	0	*	1	1	0
	Goblet cell numbers	3	3	1	2	2	3	2	1	2	0	2	3	3	1	2	2	*	0	1	1
	Lymphocytes and plasma cells in LP	3	2	2	1	1	2	3	1	1	1	2	2	1	2	3	2	*	1	1	2
	Eosinophils in LP	0	1	1	0	0	3	3	0	0	0	1	1	1	2	2	1	*	0	0	0
	Macrophages in LP	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	*	0	0	0
Histopathological Diagnosis		**EGE**	**EE**	**LPGE**	**LPE**	**LPE**	**LPG+EE**	**EGE**	**EE**	**LPE**	**LPGE**	**EE**	**EGE**	**EGE**	**LPG+EE**	**LPG+EE**	**EE**	**LPGE**	**LPE**	**LPG+EE**	**LPE**

LP:Lamina propria L: Lymphocyte count, P: Plasma cell count, *: Autolytic, EGE: Eosinophilic Gastroenteritis, EE: Eosinophilic Enteritis, LPGE: Lymphocytic-Plasmocytic Gastroenteritis, LPE: Lymphocytic-Plasmocytic Enteritis, LPG+EE: Lymphocytic-Plasmocytic Gastritis and Eosinophilic Enteritis

Lymphocytic-plasmacytic gastroenteritis, the most common type of IBD in dogs, consists of numerous lymphocytes, plasma cells, and other inflammatory cells in the lamina propria and submucosa of the stomach and intestines (Bhavani et al., 2023; Lee et al., 2021; Rousseau, 2005). Eosinophilic gastroenteritis is characterized by an increase in eosinophils in the lamina propria and submucosa and is the second most common type of IBD (Fonseca-Alves et al., 2012; Sattasathuchana and Steiner, 2014). There is no specific diagnostic method for eosinophilic gastroenteritis. Since eosinophils in dogs are increased in parasitic diseases (*Physaloptera* spp., *Ollulanustricuspis* spp., *Gnathostoma* spp. and *Spirocerca* spp. for eosinophilic gastritis; *Ancylostoma caninum* for eosinophilic enteritis), these diseases need to be eliminated, and the disease is diagnosed according to clinical findings (Neiger, 2008; Simpson, 2010). In addition to parasitic diseases, mast cell tumors and lymphomas also cause the release of cytokines that secrete eosinophil polymorphonuclear leukocyte chemotaxis factors. As a result of this stimulation, paraneoplastic eosinophilia and eosinophilic infiltrations occur in the gastrointestinal system (Marchetti et al., 2005; Ozaki et al., 2006; Tomiyasu et al., 2010). In a study in Turkey, the oral mucosa, esophagus, stomach, and intestines of dogs were examined, and the results related to microbiological, parasitological, parvoviral enteritis, and distemper diseases were investigated (Kanat and Ortatatlı, 2011; Kanat and Ortatatlı, 2022). All these diseases were excluded in this study, and idiopathic diagnoses were examined as a preliminary diagnosis. Twenty dogs with no suspected viral, bacterial or parasitic diseases and showing idiopathic IBD or gastrointestinal findings were used in this study. Considering both anamnesis/clinical and macroscopic findings and histopathological results of dogs, viral, bacterial or parasitic diseases were easily excluded. No parasites, hemorrhages, inclusion bodies, bacterial clusters or dense neutrophil leukocyte infiltrations were found in the stomach and intestinal examinations. In all these dogs, gastritis, enteritis or gastroenteritis was diagnosed histopathologically. Most of the materials diagnosed with enteritis were eosinophilic form (n: 12) and 4 of them were mixed (eosinophilic gastroenteritis). Of the lymphocytic-plasmacytic gastroenteritis, 5 were only in the intestines (enteritis), and 3 were in the mixed form (lymphocytic-plasmacytic gastroenteritis), and a total of 8 lymphocytic-plasmacytic enteritis were detected. In short, although it is mentioned in the literature that lymphocytic-plasmacytic enteritis is more common (Rychlik et al., 2007), in this study, it was determined that eosinophilic gastro/enteritis was more frequently defined in routine necropsy materials.

In this study, breed, gender, and age distribution were examined, and histopathologically graded and diagnosed. Thus, idiopathic gastroenteritis disease was thought to be common in Turkey and current data on incidence were provided. The limitation of this study is the small number of samples. However, further studies are thought to provide more information and data on this subject.

For a positive treatment outcome, pathological grading of the severity of the disease is very significant. Idiopathic gastroenteritis disease is still a subject clear to research in the field of clinical pathology in terms of histopathological grading and appropriate pharmacological drug treatment for the severity of the disease obtained, and it has become a subject that requires pathology and pharmacology branches to focus more on this subject and work towards preventing the disease.

Conflict of Interest

The authors declare no conflicts of interest with respect to the publication of this manuscript.

Ethical Approval

This study was approved by the Ankara University Animal Experiments Local Ethics Committee, Ankara, Türkiye (2023-11-100).

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Authors Contribution

Motivation / Concept: AST
Design: CÇ
Control/Supervision: AST
Data Collection and/or Processing: CÇ, AST
Analysis and / or Interpretation: CÇ, AST
Literature Review: CÇ
Writing the Article: CÇ
Critical Review: AST

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Hormonal and metabolic effects on antral follicle count in dairy cows

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Abstract: This study aimed to investigate the relationship between antral follicle count (AFC) and hormonal profiles as well as metabolic status in dairy cows. A total of 32 Holstein cows aged between 2 and 4 years and in their 60th day of lactation were included. Based on ultrasonographic evaluations, the cows were divided into two groups according to their AFC: low AFC (<24) and high AFC (≥24). Following ovulation synchronization, follicular development was monitored on the day of ovulation (Day 0) and subsequently on Days 3, 6, and 9. Blood samples collected on these days were analyzed for serum anti-Müllerian hormone (AMH), estradiol (E2), progesterone (P4), beta-hydroxybutyrate (BHBA), and cholesterol (CHOL) levels. The results showed no significant differences in AMH levels between the low and high AFC groups during the study period ($P > 0.05$). However, E2 levels on Day 9 were significantly higher in the low AFC group compared to the high AFC group ($P = 0.004$). Additionally, P4 levels were significantly higher in the low AFC group on Days 0, 6, and 9 ($P < 0.05$). In contrast, BHBA levels were significantly elevated in the high AFC group across all time points ($P < 0.05$). Cholesterol levels were consistently higher in the low AFC group throughout the study ($P < 0.05$). In conclusion, the findings suggest that AFC has a significant effect on hormonal profiles and energy metabolism in dairy cows. Particularly, the elevated steroid hormone levels observed in the low AFC group may reflect a compensatory mechanism to sustain reproductive function in conditions of limited follicular reserve.

Keywords: *Anti-Müllerian hormone, Antral follicle count, Dairy cows, Energy metabolism, Hormone profile, Metabolic parameters.*

Sütçü İneklerde Antral Folikül Sayısının Hormonal ve Metabolik Etkileri

Özet: Bu çalışma, süt ineklerinde antral folikül sayısı (AFC) ile hormonal profiller ve metabolik durum arasındaki ilişkiyi araştırmayı amaçlamıştır. Çalışmaya, 2 ile 4 yaşları arasında ve laktasyonun 60. gününde bulunan toplam 32 Holstein ineği dahil edilmiştir. Ultrasonografik değerlendirmelere göre inekler, AFC değerlerine göre düşük AFC (<24) ve yüksek AFC (≥24) olmak üzere iki gruba ayrılmıştır. Ovulasyon senkronizasyonunun ardından foliküler gelişim, ovulasyon günü (Gün 0) ve ardından 3., 6. ve 9. günlerde takip edilmiştir. Bu günlerde alınan kan örneklerinde serum anti-Müllerian hormonu (AMH), östradiol (E2), progesteron (P4), beta-hidroksibutirat (BHBA) ve kolesterol (CHOL) seviyeleri analiz edilmiştir. Sonuçlar, çalışma süresi boyunca düşük ve yüksek AFC grupları arasında AMH seviyelerinde anlamlı bir fark bulunmadığını göstermiştir ($P > 0.05$). Ancak, 9. günde E2 seviyeleri düşük AFC grubunda, yüksek AFC grubuna kıyasla anlamlı derecede daha yüksek saptanmıştır ($P = 0.004$). Buna ek olarak, 0., 6. ve 9. günlerde düşük AFC grubunda P4 seviyeleri anlamlı derecede daha yüksek bulunmuştur ($P < 0.05$). Buna karşılık, tüm zaman noktalarında yüksek AFC grubunda BHBA seviyeleri istatistiksel olarak anlamlı düzeyde daha yüksek tespit edilmiştir ($P < 0.05$). Kolesterol seviyeleri ise çalışma boyunca düşük AFC grubunda tutarlı bir şekilde daha yüksek bulunmuştur ($P < 0.05$). Sonuç olarak, elde edilen bulgular AFC'nin süt ineklerinde hormonal profiller ve enerji metabolizması üzerinde önemli bir etkisi olduğunu göstermektedir. Özellikle düşük AFC grubunda gözlemlenen artmış steroid hormon seviyeleri, sınırlı foliküler rezerv koşullarında üreme fonksiyonunun sürdürülebilmesi için bir telafi mekanizmasını yansıtıyor olabilir.

Anahtar Kelimeler: *Anti-Müllerian hormon, Antral folikül sayısı, Enerji metabolizması, Hormon profili, Metabolik parametreler, Süt inekleri.*

Introduction

Reproductive performance in dairy cows is a critical factor for enhancing economic efficiency in livestock production (Tohumcu and Tohumcu, 2024). It directly influences herd renewal rates and milk yield, while also playing a decisive role in reducing production costs and ensuring the sustainability of farm profitability. In this context, antral follicle count (AFC) has emerged as a significant biomarker for evaluating reproductive potential. AFC, which represents the number of follicles visible on ultrasonography, is widely utilized in fertility assessments due to its ability to reflect ovarian reserve (Guanga et al., 2022; Ireland et al., 2008; Moon et al., 2024).

The high repeatability of AFC and its substantial inter-individual variability enhance its value as a predictive tool for reproductive performance (Gobikrushanth et al., 2017; Koyama et al., 2018). Antral follicles form a pool of follicles capable of responding to gonadotropins and achieving ovulation. Consequently, AFC not only serves as an indicator of an individual's reproductive potential but also reflects metabolic status, with implications for both biological outcomes and economic efficiency (Morotti et al., 2022).

Hormones, which regulate various biological processes, are chemical compounds produced and secreted by multiple structures, including the ovaries. The developing follicles and other ovarian structures play a significant role in hormone production and secretion (Clark et al., 2022). Anti-Müllerian hormone (AMH), a glycoprotein secreted by granulosa cells, is a key indicator of follicular development (Juengel et al., 2021). Although the relationship between AMH levels and AFC has been explored in several studies (Guanga et al., 2022; Ireland et al., 2008; Moon et al., 2024), there remains limited information on the dynamic nature of this relationship and its variations across different stages of the estrous cycle. Steroid hormones, such as estradiol (E2) and progesterone (P4), are central to the regulation of the reproductive cycle (Bosolasco et al., 2021). The synthesis and metabolism of these hormones are closely tied to cholesterol metabolism (Patel et al., 2019). In addition, beta-hydroxybutyrate (BHBA) levels are considered an important marker of negative energy balance and metabolic stress (Ducháček et al., 2023).

This study was conducted under the hypothesis that AFC significantly affects hormone levels and metabolic status in dairy cows. The aim was to elucidate the relationships between AFC and key hormonal (AMH, E2, and P4) and metabolic parameters (BHBA and cholesterol), thereby contributing to a better understanding of the interplay between reproductive and metabolic processes in dairy cows.

Materials and Methods

Animals and Experimental Design: This study was conducted on 32 lactating Holstein cows housed at the Food and Livestock Application and Research Center. The cows were selected based on the following criteria: being 2 to 4 years old, at the 60th day of lactation, having a body condition score (BCS) between 2.75 and 3.50, having no history of reproductive or metabolic disorders, and displaying regular estrous cycles in the last two cycles. Following ultrasonographic evaluations, the cows were divided into two groups according to their antral follicle counts: the low AFC group (n=16, AFC<24) and the high AFC group (n=16, AFC≥24) (Sakaguchi et al., 2018). The study was approved by the Atatürk University Local Ethics Committee of Animal Experiments (Decision Number: 2021/91). All cows were housed under identical conditions and were fed a total mixed ration (TMR) formulated to meet NRC (2001) requirements. Throughout the study, ad libitum access to clean water was provided. Routine veterinary care was meticulously maintained, and all cows were deemed healthy based on pre-experiment clinical examinations. The cows were milked twice daily according to a standard milking protocol, and reproductive health records were systematically maintained.

Synchronization Protocol and Follicular Wave Monitoring: A standard 7-day ovulation synchronization (Ov-Synch) protocol, in combination with P4, was applied to all cows. The protocol included an initial GnRH injection on day -10, followed by PGF2α administration on day -3, and a second GnRH injection on day -1. During the Ov-Synch procedure, an intravaginal progesterone-releasing device (PRID® Delta, Ceva Sante Animale, Libourne, France) was inserted under aseptic conditions, and 10 mcg of buserelin acetate (Receptal®, MSD, Unterschleissheim, Germany) was administered intramuscularly. Seven days later, the PRID device was removed, and an intramuscular injection of 0.075 mg cloprostenol sodium (Estropur®, Bioveta, Ivanovice na Hane, Czech Republic) was given (Figure 1). Forty-eight hours after PRID removal, an additional 10 mcg of buserelin was administered intramuscularly (Hölper et al., 2023). Ovulation was monitored twice daily (at 09:00 and 21:00) using transrectal ultrasonography with a 7.5 MHz linear probe (Z60®, Mindray, Jiangsu, China). Cows in which ovulation was confirmed were included in the study, with the day of ovulation designated as day 0 (d 0). To monitor follicular development, further ultrasonographic examinations were performed on days 3, 6, and 9. during each examination, antral follicles with a diameter of ≥3 mm were identified, counted, and recorded.

Blood Sampling and Hormonal Analysis: Blood samples were collected from the coccygeal vein on the days specified in Figure 1. Samples were then centrifuged at 1200 x g at 4 °C for 10 minutes, and the sera were stored at -80 °C for subsequent analysis of anti-Müllerian hormone (AMH) (#EA0241BO, BT LAB), estrogen (E2) (#EA0093BO, BT LAB), progesterone (P4) (#EA0008BO, BT LAB), beta-hydroxybutyrate (BHBA) (#E0267BO, BT LAB), and cholesterol (CHOL) (#E2030BO, BT LAB). All serum measurements were performed using bovine ELISA kits according to the manufacturer's protocols (Bioassay Technology Laboratory).

Statistical analysis: Data normality was assessed with the Shapiro-Wilk test. A two-way repeated measures ANOVA

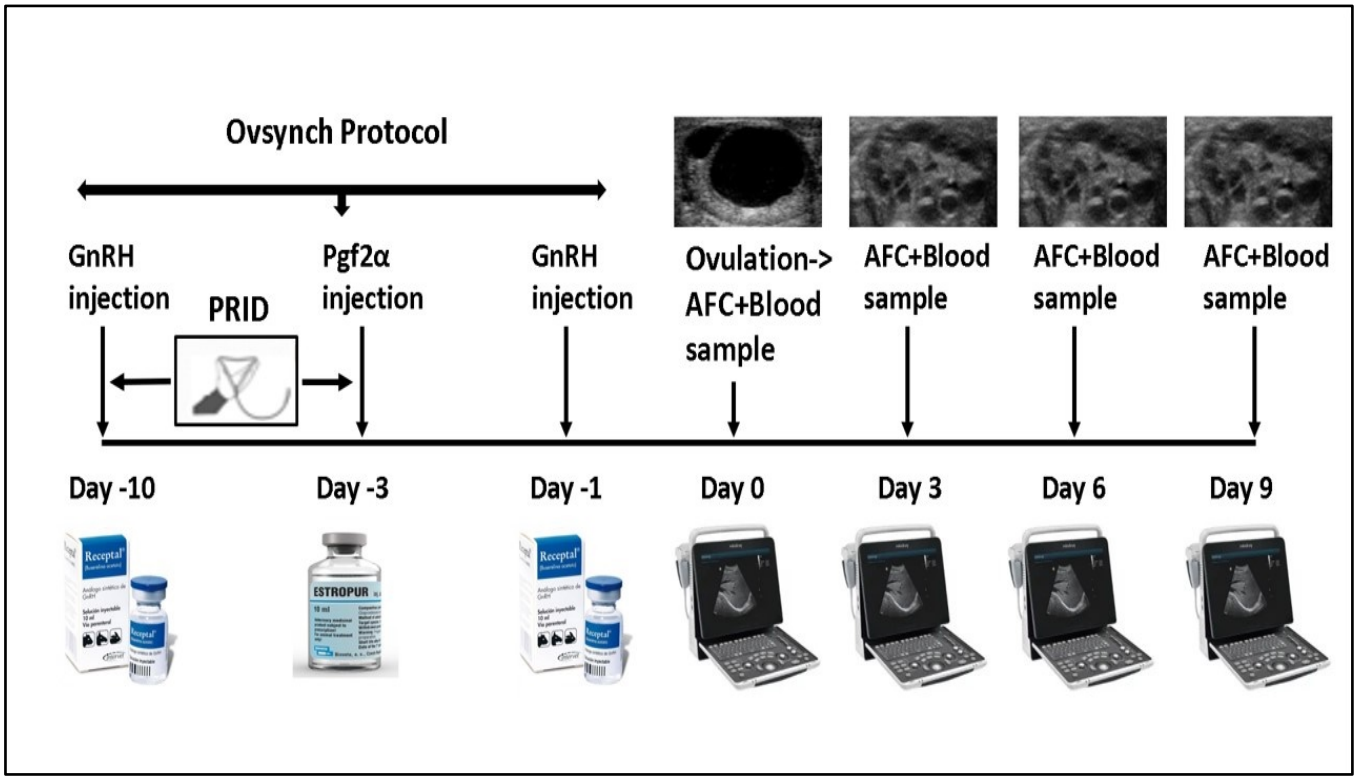

Figure 1. Synchronization Protocols, Blood Sampling, and Monitoring of Follicular Waves.

was employed to evaluate the effects of AFC group (low vs. high) and time (days 0, 3, 6, and 9) on hormone and metabolic parameters. Post-hoc comparisons were performed using Bonferroni correction. Pearson correlation analysis was used to examine the relationship between AFC and hormonal/metabolic profiles. All statistical analyses were performed using Medcalc version 20.2 (Medcalc Software Ltd., Ostend, Belgium) and SPSS version 25.0 (IBM Company, SPSS, IL, USA). A significance threshold of $P < 0.05$ was applied to all statistical tests (Table 1).

Table 1. Plasma anti-Müllerian hormone (AMH), estradiol (E2), progesterone (P4), beta-hydroxybutyrate (BHBA) and cholesterol (CHOL) measurement in low antral follicle count (AFC) and high AFC.

Hormones	Groups	Time-points				
		T_0	T_3	T_6	T_9	$P^{\dagger}$
AMH (ng/L)	Low AFC	51 ± 22.8	42.5 ± 16.1	50 ± 20.4	46 ± 19.9	**0.245**
	High AFC	45.1 ± 10.7	50.4 ± 16	52.2 ± 23.8	55 ± 23.1	**0.277**
P^*		**0.356**	**0.177**	**0.728**	**0.236**	
E2 (ng/L)	Low AFC	146 ± 58.2	130 ± 57	118 ± 48.1	126 ± 21.1^a	**0.290**
	High AFC	113 ± 54.6	100 ± 36.4	99 ± 35.8	95.2 ± 34^b	**0.230**
P^*		**0.103**	**0.085**	**0.219**	**0.004**	
P4 (ng/mL)	Low AFC	15.7 ± 6.32^a	13.8 ± 6	12.1 ± 4.40^a	12.87 ± 3^a	**0.115**
	High AFC	9.80 ± 3.72^b	12 ± 5.82	9.27 ± 3.47^b	9.37 ± 4.12^b	**0.063**
P^*		**0.003**	**0.404**	**0.05**	**0.010**	
BHBA (nmol/mL)	Low AFC	186 ± 84^a	169 ± 66^a	190 ± 102^a	183 ± 67^a	**0.438**
	High AFC	268 ± 121^b	252 ± 85^b	259 ± 88^b	262 ± 90^b	**0.871**
P^*		**0.033**	**0.004**	**0.047**	**0.009**	
Chol (mg/dl)	Low AFC	156 ± 90^a	173 ± 100^a	155 ± 78.2^a	146 ± 68.2^a	**0.572**
	High AFC	89.5 ± 19.9^b	87.9 ± 22.9^b	85.8 ± 34.8^b	82.2 ± 24.4^b	**0.560**
P^*		**0.007**	**0.002**	**0.003**	**0.001**	

Data were expressed as mean ± standard deviation. Different letters indicated significant difference between groups at the same time ($p < 0.05$). P^* values showed significant difference between groups at the same time while $P^{\dagger}$ values demonstrated significant difference between different time-point in same groups.

Results

Hormonal and Metabolic Parameters in Low versus High AFC Groups

AMH Profile

No significant differences were observed in AMH concentrations between low and high AFC groups throughout the study period (P > 0.05). In the low AFC group, mean (± SD) AMH concentrations at T0, T3, T6, and T9 were 51 ± 22.8, 42.5 ± 16.1, 50 ± 20.4, and 46 ± 19.9 ng/L, respectively. Corresponding values in the high AFC group were 45.1 ± 10.7, 50.4 ± 16.0, 52.2 ± 23.8, and 55 ± 23.1 ng/L. Temporal changes in AMH concentrations were not significant within either group (P = 0.245 for low AFC; P = 0.277 for high AFC) (Table 1).

Steroid Hormone Profiles

Estradiol

A significant difference in E2 concentrations between groups was observed only at T9, with the low AFC group showing higher concentrations compared to the high AFC group (126 ± 21.1 vs 95.2 ± 34.0 ng/L, respectively; P = 0.004). No significant differences were detected at other time points (P > 0.05). Temporal changes in E2 concentrations were not significant within either group (P = 0.290 for low AFC; P = 0.230 for high AFC) (Table 1).

Progesterone

The low AFC group exhibited significantly higher P4 concentrations compared to the high AFC group at T0 (15.7 ± 6.32 vs 9.80 ± 3.72 ng/mL; P = 0.003), T6 (12.1 ± 4.40 vs 9.27 ± 3.47 ng/mL; P = 0.050), and T9 (12.87 ± 3.00 vs 9.37 ± 4.12 ng/mL; P = 0.010). No significant difference was observed at T3 (P = 0.404). Temporal changes in P4 concentrations were not significant within either group (P = 0.115 for low AFC; P = 0.063 for high AFC) (Table 1).

Metabolic Parameters

Beta-hydroxybutyrate

BHBA concentrations were consistently higher in the high AFC group compared to the low AFC group across all time points: T0 (268 ± 121 vs 186 ± 84 nmol/mL; P = 0.033), T3 (252 ± 85 vs 169 ± 66 nmol/mL; P = 0.004), T6 (259 ± 88 vs 190 ± 102 nmol/mL; P = 0.047), and T9 (262 ± 90 vs 183 ± 67 nmol/mL; P = 0.009). No significant temporal changes were observed within either group (P = 0.438 for low AFC; P = 0.871 for high AFC) (Table 1).

Cholesterol

The low AFC group maintained significantly higher cholesterol concentrations compared to the high AFC group throughout the study period: T0 (156 ± 90 vs 89.5 ± 19.9 mg/dL; P = 0.007), T3 (173 ± 100 vs 87.9 ± 22.9 mg/dL; P = 0.002), T6 (155 ± 78.2 vs 85.8 ± 34.8 mg/dL; P = 0.003), and T9 (146 ± 68.2 vs 82.2 ± 24.4 mg/dL; P = 0.001). Temporal changes in cholesterol concentrations were not significant within either group (P = 0.572 for low AFC; P = 0.560 for high AFC) (Table 1).

Discussion and Conclusion

The lack of a significant difference in AMH concentrations between AFC groups was an unexpected finding, as AMH is widely regarded as a reliable marker of follicular reserve (Schwarzmann et al., 2023). This outcome contrasts with previous studies that have reported positive correlations between AFC and AMH levels (Baldrighi et al., 2014; Guanga et al., 2022; Ireland et al., 2008; Rico et al., 2009). However, our results align with research suggesting that changes in follicular development or hormone concentrations may be influenced by metabolic status (İleritürk & Kaynar, 2023; Rosa et al., 2021; Song et al., 2021).

Unlike findings from other studies (Modina et al., 2014; Sakaguchi et al., 2019), elevated E2 concentrations were observed in the low AFC group on day 9 (T9). Bonato et al. (2022) reported larger dominant follicle diameters in cows with low AFC, which could explain the increased E2 production (De los Reyes et al., 2006). The elevated E2 levels observed on T9 may therefore indicate distinct follicular dynamics in these cows. Similarly, the persistently high P4 levels observed in the low AFC group could be associated with differences in CL size (Bonato et al., 2022). While our findings partially parallel studies by Jimenez-Krassel et al. (2015) and Modina et al. (2014), which documented varying steroidogenic capacities among animals with differing AFC, they also diverge from studies linking low AFC to reduced P4 levels (Mossa et al., 2012; Mossa & Ireland, 2019; Sakaguchi et al., 2018). The elevated steroid hormone concentrations in cows with low AFC may represent a compensatory mechanism, reflecting increased steroidogenic activity per follicle to sustain reproductive function despite a reduced follicle count (Bonato et al., 2022; Mossa et al., 2012). Additionally, when coupled with more favorable metabolic profiles (lower BHBA levels), the higher P4 concentrations in low AFC cows suggest improved luteal function and potentially enhanced fertility, even with a diminished follicular reserve.

In dairy cows, circulating BHBA is a well-established biomarker of negative energy balance (NEB), which is known to impair follicular development (Gong et al., 2022; Missio et al., 2022). BHBA levels are generally considered normal when below 1 mmol/L (1000 nmol/mL) (Fiore et al., 2020). In vitro studies have demonstrated that direct injection of BHBA into follicles reduces follicle diameter and decreases E2 and P4 production by granulosa cells (Missio et al., 2022). Moreover, BHBA negatively impacts follicular development by reducing IGF-1 levels (Matoba et al., 2012). In our study, BHBA levels were within the normal range across all cows. However, the persistently higher BHBA levels in the high AFC group likely reflect a more pronounced energy deficit compared to the low AFC group. This observation could be attributed to the increased glucose utilization required for follicular development, as a higher number of antral follicles imposes a greater metabolic energy demand (Gamarra et al., 2015). Furthermore, the elevated cholesterol concentrations in the low AFC group, along with higher E2 and P4 levels compared to the high AFC group, are consistent with the positive association between cholesterol and steroid hormone synthesis. Previous studies have reported that embryos from high AFC cows contain less cholesterol than those from low AFC cows (Rosa et al., 2021). These findings suggest that the increased cholesterol levels observed in low

AFC cows may reflect differences in precursor availability for steroidogenesis or alterations in hepatic lipid metabolism (Anderson et al., 2015; Mathey et al., 2017).

In conclusion, AFC appears to have a significant impact on hormonal and metabolic processes in dairy cows. Notably, the high cholesterol and low BHBA levels observed in the low AFC group suggest that this group may possess a more favorable metabolic profile and improved luteal function. On the other hand, the increased follicular development in the high AFC group seems to elevate metabolic energy demands, potentially leading to higher BHBA levels. These findings highlight the potential of AFC as a critical biomarker for both reproductive performance and energy metabolism.

Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

Ethical Approval

This study was approved by the Ataturk University Animal Experiments Local Ethics Committee (20.04.2021, 2021/91 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

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Data Collection and / or Processing: VT, DTO, AYÇ, ŞA
Analysis and / or Interpretation: VT, MC, DTO
Literature Review: VT, DTO, AYÇ, ŞA
Writing the Article: VT, MC
Critical Review: MC

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Comparative anatomy of the pelvic cavity of rat strains in a translational aspect

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Abstract: The anatomy of the pelvic cavity has significant importance in daily clinical applications and surgical interventions such as determining dystocia, surgery of rectal cancers and mesorectal excision, treatment of twisted pouch syndrome and percutaneous sacroiliac screw fixation, and pelvic floor disorders. This study aims to determine the diameters and area calculations of the pelvic cavity in mostly preferred rat strains (Wistar Albino, Brown Norway, Sprague Dawley, and Lewis) and investigate the suitability of rats in translational studies in which anatomical conditions are not a cause of dystocia. In this study, pelvis bones were used. Each group consisted of six rats. They were examined morphologically and morphometrically. According to the Kruskal-Wallis analysis to determine whether there is any difference between strains, significant differences were observed between the strains for the length of the symphysis, oblique diameter, true conjugate, anatomical conjugate, and diagonal conjugate parameters ($p<0.05$). In conclusion, anatomically, Lewis is the most suitable laboratory rat strain that does not predispose to labor dystocia, followed by the Wistar Albino strain. These two strains may be a choice for studies on physiological dystocia. On the other hand, Sprague Dawley is less suitable for experimental studies involving the pelvic inlet, particularly those related to labor dystocia caused by anatomical factors.

Keywords: *Diameter, Dystocia, Pelvic inlet, Rat, Translational anatomy.*

Ratlarda pelvik boşluğun karşılaştırmalı anatomisi

Özet: Pelvik boşluğun anatomisi, klinik uygulamalar ve rektal kanserlerin cerrahisi, mezorektal eksizyon, torsiyonlu kese sendromu tedavisi, perkütan sakroiliak vida tespiti ve pelvik taban bozuklukları gibi cerrahi müdahalelerde önemli bir yere sahiptir. Bu çalışma, en çok tercih edilen sıçan türlerinde (Wistar Albino, Brown Norway, Sprague Dawley ve Lewis) pelvik boşluğunun çaplarını ve alan hesaplamalarını belirlemeyi ve anatomik koşulların güç doğuma neden olmadığı translasyonel çalışmalarda sıçanların uygunluğunu araştırmayı amaçlamaktadır. Çalışmada pelvis kemikleri kullanılmıştır. Her grup altı sıçandan oluşmuştur. Kemikler morfolojik ve morfometrik olarak incelenmiştir. Türler arasında fark olup olmadığını belirlemek için yapılan Kruskal-Wallis analizi sonuçlarına göre LS, DO, CV, CA ve CD parametrelerinde türler arasında anlamlı farklar gözlenmiştir ($p<0.05$). Sonuç olarak, anatomik açıdan Lewis, güç doğuma eğilim göstermeyen en uygun laboratuvar sıçan türü olup, bunu Wistar Albino türü izlemektedir. Bu iki tür, fizyolojik güç doğum ile ilgili çalışmalarda tercih edilebilir. Öte yandan, Sprague Dawley, özellikle anatomik faktörlere bağlı güç doğum ile ilgili pelvik giriş çalışmalarında daha az uygun bir türdür.

Anahtar Kelimeler: *Çap, güç doğum, pelvik boşluk, sıçan, translasyonel anatomi.*

Introduction

The pelvic cavity in rats is structured cranio-ventrally by the hip bone, dorsally by the sacrum, and the caudal vertebrae. The hip bone consists of the ilium in the cranial region, the pubis in the cranio-ventral region, and the ischium in the caudo-ventral region. The ilium represents the most cranial component of the os coxa. Its cranial end expands laterally into the ventral iliac spine and joins the sacroiliac joint. The two pubic bones are joined at the midline through the symphysis pelvina. On the ventral edge of the pubis, just cranial to the pubic symphysis and near the anterior margin of the obturator foramen, lies a distinct iliopectineal eminence, which serves as the attachment site for the pectineus muscle. The dorsal margin of the ischium is elevated, forming the ischiatic spine. The obturator foramen, a large opening, separates the ischium from the pubis. The triangular-shaped sacrum forms the first part of the pelvic roof and consists of four sacral vertebrae. The ventral surface of the sacrum is flat, in contrast to humans. The first few caudal vertebrae form the second part of the pelvic roof (Chiasson, 1994).

Anatomy of the pelvic cavity has significant importance in daily clinical applications and surgical interventions such as determining dystocia (Narumoto et al., 2015), surgery of rectal cancers and mesorectal excision (Baltus et al., 2025; Bolshinsky et al., 2024; Faisal Bin Abdur Raheem et al., 2024; Hong et al., 2020), treatment of twisted pouch syndrome (Holubar, 2024) and percutaneous sacroiliac screw fixation (Link et al., 2024) and pelvic floor disorders (Handa et al., 2003; Maccioni, 2012). Mainly, dystocia refers to difficult or obstructed labor, which is a significant cause of maternal and fetal morbidity and mortality globally. The dimensions of the pelvic cavity are a determinant of the occurrence of vaginal labor in humans. One of them is the narrowest diameter of the pelvis, the true conjugate, which is the shortest distance from the sacral promontory to the pubic symphysis (Oğuz and Desticioğlu, 2021). Early identification and management are crucial to minimize adverse outcomes. Various experimental studies have been carried out for decades for this purpose. This study aims to determine the diameters and area calculations of the pelvic cavity in mostly preferred rat strains (Wistar Albino, Brown Norway, Sprague Dawley, and Lewis) and investigate the suitability of rats in translational studies in which anatomical conditions are not a cause of dystocia.

Materials and Methods

In this study, pelvis bones belonging to four different male rat strains (Wistar Albino, Brown Norway, Sprague Dawley, and Lewis) aged 12 months old and weighing between 850 and 900 grams were used. The bones included in the study were obtained from rat cadavers used in various projects conducted at the Dokuz Eylül University Faculty of Medicine Multidisciplinary Experimental Animal Laboratory. It was taken into ensuring that the skeletal integrity of these cadavers remained intact. Therefore, each group consisted of six rats. Twenty-four pelvis were examined morphologically and morphometrically. Dokuz Eylül University Local Ethics Committee for Animal Experiments granted the ethics committee approval (22/2022) for the study. The skeletons were macerated by boiling for 30 minutes. After the maceration process, the soft tissues on the skeletons were carefully cleaned. Then, the bones were soaked in 3% hydrogen peroxide for 5 minutes and dried at room temperature (Üstündağ et al., 2024a).

Osteometric Measurements: Morphometric measurements of the pelvis of each animal were taken in millimeters using a calibrated electronic digital caliper with a sensitivity of 0.01 mm, an accuracy of ±0.01 mm (<100 mm), and a repeatability of 0.01 mm. Among the morphometric measurements, the length of the pelvic symphysis (LS), oblique diameter (DO), true conjugate (CV), anatomical conjugate (CA), diagonal conjugate (CD), vertical diameter (DV), dorsal transversal diameter (DTD), medial transversal diameter (DTM), ventral transversal diameter (DTV), anterior diameter (AD), medial diameter (MD), external bi-ischial length (EBB), posterior diameter (PD), pelvic girdle area (PGA) and pelvic inlet area (IPA) was measured. Diameters are shown in Figure 1. Moreover, the formulas based on Silva et al. (2019) study calculated pelvic girdle and pelvic inlet areas. The formulas are given below:

$$PGA = \frac{DTM}{2} x \frac{CA}{2} x\, \pi$$

$$IPA = \left(\frac{(DTD + DTM)}{2} x \frac{CA}{2}\right) + \left(\frac{(DTM + DTV)}{2} x \frac{CA}{2}\right)$$

Statistical analysis: All linear morphometric data were subjected to a homogeneity test of variances. Data were presented as the mean ± standard deviation, and the $p<0.05$ value was considered significant. Length of the pelvic symphysis, all the diameters, external bi-ischial breadth, pelvic girdle area, and pelvic inlet area were analyzed by the Kruskal-Wallis test following the Mann-Whitney U test to define the diversity between the groups (Üstündağ et al., 2024b). In addition, Pearson correlation analysis was applied to pelvic areas to identify the correlation between pelvic areas and the length of the pelvic symphysis, all the diameters, and external bi-ischial breadth (Silva et al., 2019).

Results

According to the Kruskal-Wallis analysis to determine whether there is any difference between strains, significant differences were observed between the strains for LS, DO, CV, CA, and CD parameters ($p<0.05$). The differences between the strains are shown in Table 1.

The Pearson correlation test aimed at assessing the relationship between PGA and IPA with the examined parameters yielded the following findings. In the Wistar Albino strain, a positive correlation was noted between PGA and DTD ($p<0.05$), while no correlation was detected

Table 1. Results of Kruskal-Wallis test.

Parameters	Wistar Mean±SD	Brown Norway Mean±SD	Sprague Dawley Mean±SD	Lewis Mean±SD	p
LS	6,04 ± 0,53^{ab}	5,94 ± 0,34^{ab}	5,4 ± 0,63^{a}	6,8 ± 0,58^{b}	0,027*
DO	29,62 ± 1,11^{ab}	28,5 ± 0,62^{b}	29,48 ± 0,68^{ab}	30,7 ± 099^{a}	0,027*
CV	38,6 ± 1,78^{abc}	37,84 ± 1,49^{ab}	36,48 ± 1,71^{ab}	40,4 ± 1,05^{c}	0,018*
CA	35,3 ± 2,02^{abc}	35,14 ± 0,89^{ab}	33,9 ± 1,18^{ab}	36,94 ± 0,75^{c}	0.032*
CD	42,2 ± 1,62^{abc}	41,74 ± 0,97^{ab}	39,86 ± 2,15^{ab}	43,56 ± 0,51^{c}	0.026*
DV	10,06 ± 1,42	10,22 ± 0,9	9,18 ± 1,23	11,1 ± 1,75	NS
DTD	14,56 ± 1,12	14,0 ± 0,95	15,28 ± 0,5	15,3 ± 1,04	NS
DTM	14,2 ± 0,68	13,8 ± 0,7	13,96 ± 1,24	14,04 ± 1,09	NS
DTV	11,18 ± 0,76	9,74 ± 0,74	10,84 ± 0,58	11,7 ± 2,69	NS
AD	15,2 ± 0,46	14,96 ± 0,71	15,34 ± 1,76	14,1 ± 2,24	NS
MD	16,44 ± 0,77	18,22 ± 1,2	16,56 ± 1,95	17,44 ± 1,31	NS
PD	20,1 ± 2,7	19,3 ± 1,85	18,7 ± 2,3	22,06 ± 3,62	NS
EBB	24,02 ± 2,17	23,76 ± 1,34	22,4 ± 2,04	25,4 ± 2,56	NS
PGA	393,30 ± 26,75	380,9 ± 26,45	370,72 ± 24,32	406,65 ± 24,02	NS
IPA	1655,69 ± 165,58	1428,91 ± 167,68	1530,06 ± 150,03	1786,66 ± 353,81	NS

***:** Significant at p<0.05 level, **NS:** Non-Significant, **LS:** the length of the pelvic symphysis, **DO:** oblique diameter, **CV:** true conjugate, **CA:** anatomical conjugate, **CD:** diagonal conjugate, **DV:** vertical diameter, **DTD:** dorsal transversal diameter, **DTM:** medial transversal diameter, **DTV:** ventral transversal diameter, **AD:** anterior diameter, **MD:** medial diameter, **PD:** posterior diameter, **EBB:** external bi-ischial length, **PGA:** pelvic girdle area and **IPA:** pelvic inlet area

Table 2. Results of pearson correlation analysis of pelvic areas.

		LS	DO	CV	CA	CD	DV	DTD	DTM	DTV	AD	MD	PD	EBB
Wistar Albino	**PGA**	0,326	0,540	0,839	0,700	0,671	-0,831	0,896*	0,548	0,256	0,659	0,274	0,529	0,214
	IPA	0,552	0,723	0,707	0,409	0,459	-0,538	0,674	0,704	0,720	0,486	-0,237	0,128	-0,263
Brown Norway	**PGA**	-0,105	0,576	0,781	0,808	0,687	-0,750	0,519	0,952*	0,609	0,875	0,570	-0,097	0,771
	IPA	-0,003	0,696	0,716	0,819	0,605	-0,509	0,658	0,799	0,904*	0,824	0,528	-0,377	0,590
Sprague Dawley	**PGA**	-0,536	0,128	-0,216	-0,653	-0,269	-0,792	0,657	0,964**	0,777	0,676	-0,900	0,124	0,470
	IPA	-0,426	0,266	-0,142	-0,723	-0,168	-0,837	0,725	0,959*	0,927*	0,470	-0,235	0,360	-0,950
Lewis	**PGA**	0,383	-0,587	-0,682	-0,855	-0,798	0,488	0,225	0,990**	0,400	0,158	-0,342	-0,750	-0,797
	IPA	0,892*	-0,287	0,397	0,189	0,109	0,857	-0,363	0,172	0,967**	-0,886*	0,635	0,126	-0,410

Correlation is significant at the 0.05 level (2-tailed), p< 0.05, * Correlation is significant at the 0.01 level (2-tailed), p< 0.01, **PGA:** Pelvic girdle area and **IPA:** Pelvic inlet area

Table 3. Results of Pearson correlation analysis of pelvic diameters.

WistarAlbino	**LS**	**DO**	**CV**	**CA**	**CD**	**DV**	**DTD**	**DTM**	**DTV**	**AD**	**MD**	**PD**	**EBB**
CD	0.640	0.308	.949*	0.844	1								
DTD	-0.117	0.390	0.539	0.541	0.353	-.932*	1						
AD	-0.241	0.700	0.428	0.683	0.249	-.917*	0.774	0.087	-0.014	1			
EBB	-0.739	-0.341	-0.055	0.278	0.003	-0.602	0.533	-0.069	-0.788	0.431	0.851	.903*	1
BrownNorway	**LS**	**DO**	**CV**	**CA**	**CD**	**DV**	**DTD**	**DTM**	**DTV**	**AD**	**MD**	**PD**	**EBB**
CA	0.323	0.616	.936*	1									
CD	0.306	0.702	0.777	.904*	1								
DTM	.903*	0.479	0.580	0.588	0.471	0.098	0.444	1					
AD	0.844	0.366	0.509	0.490	0.257	-0.060	0.555	.948*	0.609	1			
MD	-0.033	-0.070	.912*	0.730	0.470	-0.212	-0.230	0.397	0.396	0.419	1		
EBB	0.074	0.726	-0.158	0.168	0.209	-0.756	0.710	-0.146	0.580	-0.112	-0.377	-.966**	1
Sprague Dawley	**LS**	**DO**	**CV**	**CA**	**CD**	**DV**	**DTD**	**DTM**	**DTV**	**AD**	**MD**	**PD**	**EBB**
DV	0.390	-0.572	0.624	.954*	0.525	1							
DTD	0.000	0.822	-0.652	-.988**	-0.265	-.916*	1						
DTM	-0.431	0.372	-0.398	-0.831	-0.332	-.918*	0.830	1					
PD	-0.144	-0.763	0.815	0.635	0.511	0.496	-0.577	-0.132	-0.120	0.644	.947*	1	
EBB	-0.253	-0.877	0.718	0.720	0.345	0.559	-0.682	-0.220	-0.299	0.661	.981**	.968**	1
Lewis	**LS**	**DO**	**CV**	**CA**	**CD**	**DV**	**DTD**	**DTM**	**DTV**	**AD**	**MD**	**PD**	**EBB**
CA	-0.011	0.639	.927*	1									
DV	.950*	-0.691	-0.014	-0.179	-0.099	1							
DTM	0.298	-0.617	-0.771	-.919*	-0.837	0.421	0.249	1					
AD	-0.758	0.154	-0.688	-0.542	-0.440	-0.713	0.583	0.266	-.971**	1			
MD	0.312	0.487	.886*	0.770	0.523	0.162	-0.257	-0.468	0.744	-0.728	1		
PD	-0.017	0.822	0.778	.927*	.901*	-0.270	0.109	-0.816	0.314	-0.383	0.743	1	
EBB	-0.281	.890*	0.809	.912*	0.764	-0.484	0.032	-0.852	0.155	-0.232	0.721	.947*	1

*Correlation is significant at the 0.05 level (2-tailed), p< 0.05, ** Correlation is significant at the 0.01 level (2-tailed), p< 0.01, **CA:** Anatomical conjugate, **CD:** Diagonal conjugate, **DV:** Vertical diameter, **DTD:** Dorsal transversal diameter, **DTM:** Medial transversal diameter, **AD:** Anterior diameter, **MD:** Medial diameter, **PD:** Posterior diameter, **EBB:** External bi-ischial length

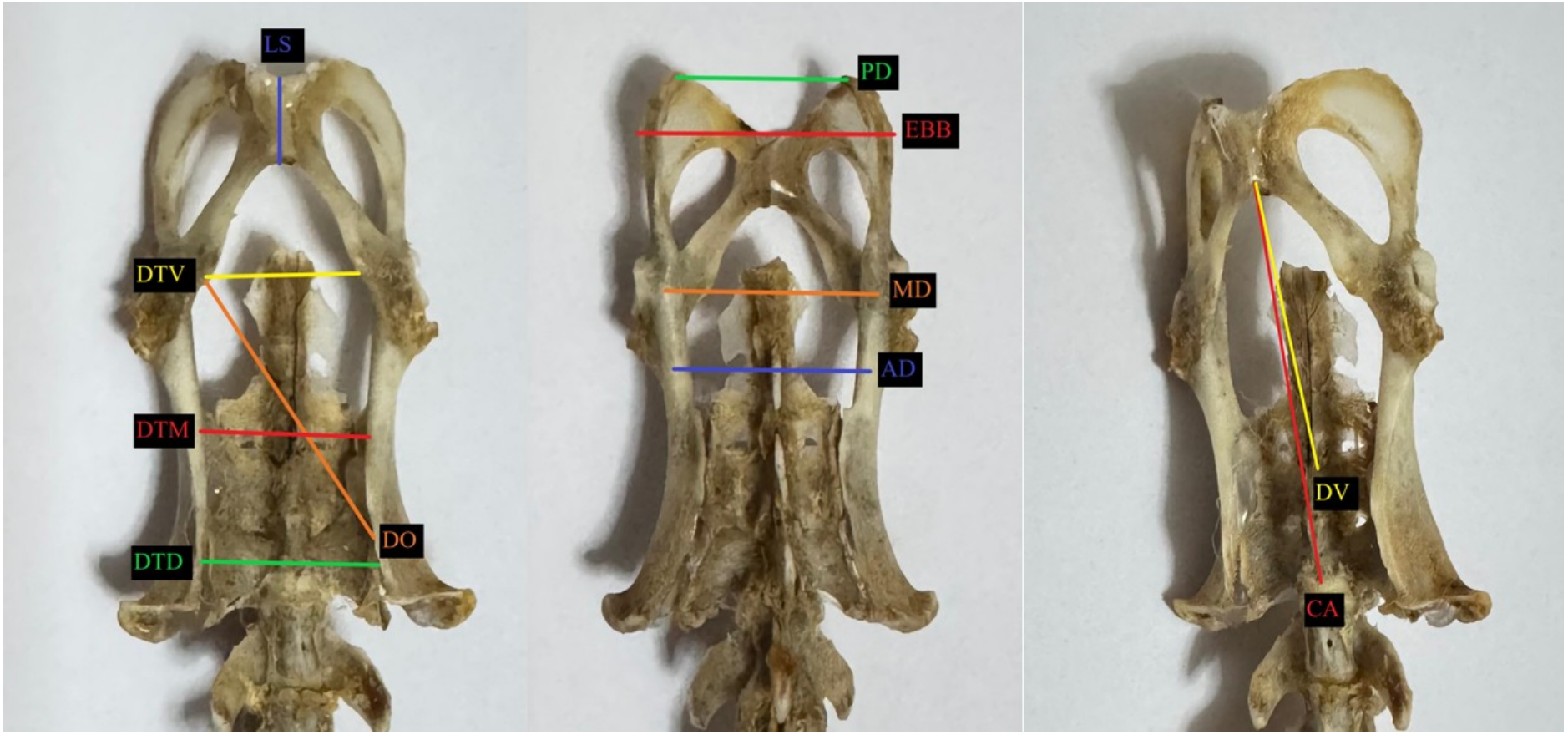

Figure 1. Measured diameters of the pelvis.

LS: The length of the pelvic symphysis, **CA:** Anatomical conjugate, **DV:** Vertical diameter, **DO:** Oblique diameter, **DTD:** Dorsal transversal diameter, **DTM:** Medial transversal diameter, **DTV:** Ventral transversal diameter, **AD:** Anterior diameter, **MD:** Medial diameter, **PD:** Posterior diameter, **EBB:** External bi-ischial length

between IPA and any parameter. In the Brown Norway strain, positive correlations were found between PGA and DTM ($p<0.05$) and between IPA and DTV ($p<0.05$). For the Sprague Dawley strain, a significant positive correlation emerged between PGA and DTM ($p<0.01$) as well as between IPA and both DTM and DTV ($p<0.05$). In the Lewis strain, positive correlations were identified between PGA and DTM ($p<0.01$), along with IPA and DTV ($p<0.01$), LS, and AD ($p<0.05$). The correlations are summarised in Table 2.

The results of the Pearson correlation test conducted to evaluate the relationships among diameters revealed several significant findings. In the Wistar strain, a positive correlation was observed between CD and CV and between EEB and PD. Conversely, a negative correlation was identified between DV and DTD, as well as between DV and AD ($p < 0.05$). Positive correlations were noted for the Brown Norway strain between LS and DTM, CV and CA, and MD with both CD and AD ($p < 0.05$). In contrast, a negative correlation was found between PD and EBB ($p < 0.01$). In the Sprague Dawley strain, positive correlations were observed between CA and DV, and MD and PD ($p < 0.05$), along with stronger correlations between EBB and both MD and PD ($p < 0.01$). However, negative correlations were identified between DV and both DTD and DTM, as well as between CA and DTD ($p < 0.01$).In the Lewis strain, positive correlations were established between LS and DV, CV with both CA and MD, DO with EBB, CA with DTM, PD with EBB, and CD with PD, and another correlation between PD and EBB ($p < 0.05$). A negative correlation was found between DTV and AD ($p < 0.01$). The correlations are summarised in Table 3.

Discussion and Conclusion

Rats are not thought to be anatomically predisposed to labor dystocia. There are many anatomical reasons supporting this idea. One reason for this is the shape of the cranial aperture of the pelvis. According to the morphological findings of this study, the cranial aperture of the pelvis resembles the anthropoid pelvis in humans, which serves the most suitable labor (Handa et al., 2003; Salk et al., 2016). Additionally, taking into consideration the diameters originating from the promontory, when compared to humans, the long and inclined diameters in rats, as well as their flat pelvic floor, do not contribute to obstructed labor. Furthermore, unlike humans, the flat sacrum extends to the middle of the dorsal part of the pelvis, and the caudal vertebrae's upward orientation during birth is a facilitating factor for delivery. As known in humans, the dorsal part of the pelvis consists of a ventrally concave sacrum and coccyx (Gruss and Schmitt, 2015).

However, it should be noted that the present study exclusively analysed male rats, as the specimens were obtained from previously conducted research. While this limitation may initially suggest a lack of comparative evaluation, it is essential to acknowledge the well-documented presence of sexual dimorphism. In general, male rats tend to be larger than females. Previous studies have demonstrated that male Sprague Dawley rats exhibit greater body size and wider pelvic structure than their female counterparts (Berdnikovs et al., 2007; Routzong et al., 2024). Consequently, it can be inferred that female rats likely possess a narrower pelvic cavity.

The dimensions of the pelvic cavity are a determinant of the occurrence of vaginal labor in humans. One of them is the narrowest diameter of the pelvis, the true conjugate, which is the shortest distance from the sacral promontory to the pubic symphysis (Oğuz and Desticioğlu, 2021). According to the results of our study, Lewis has the most extended dimensions and a larger pelvic inlet area; in contrast, Sprague Dawley has the shortest dimensions and narrower pelvic inlet area compared to other strains.

In Tresch et al. (2024) study, CA is between 8.5 cm and 10.5 cm and is considered subnormal in humans. As the distance decreases, the pelvic inlet narrows, and the horizontal angle required for vaginal delivery becomes more vertical, which is undesirable. DTM should be greater than 12 cm, which refers to a gynaecoid pelvis, the most suitable shape for cephalopelvic proportion (Pavličev et al., 2020). According to our results, in rats, CA's natural incline serves standard vaginal delivery. Also, the anthropoid-shaped cranial aperture does not cause any obstruction due to the rats' narrow, elongated cranium structure due to their suitable cephalopelvic proportion.

Considering the study by Handa et al. (2003), a wide transverse diameter combined with a short anatomical conjugate of the pelvic inlet increased susceptibility to pelvic floor disorders. Based on this information, our study observed that in rats, the transverse diameters were narrow and similar across strains, while the longest anatomical conjugate was identified in the Lewis strain. The shortest diameter, however, was observed in the Sprague Dawley strain. Therefore, it can be suggested that the Lewis strain, followed by the Wistar Albino strain, is the most suitable for studies on pelvic floor disorders. Conversely, the Sprague Dawley strain should not be preferred.

Rectal cancer is one of the most commonly observed pathologies in the pelvic region, and its primary treatment is total mesorectal excision. In this process, understanding pelvic dimensions significantly impacts the surgical procedure's success rate, as the pelvis's bony structure is a critical factor that directly limits surgical access to the rectum. Pelvic types described as narrow and deep negatively affect surgical interventions (Baltus et al., 2025; Bolshinsky et al., 2024; Faisal Bin Abdur Raheem et al., 2024; Hong et al., 2020). Based on our findings, it is anticipated that rats, the most commonly used in laboratory studies, could be preferred for developing new surgical approaches for rectal cancer treatment. Also, the posterior pelvic region is crucial in addressing twisted pouch syndrome, a condition observed in humans (Holubar, 2024). Recent studies indicate that this syndrome has not yet been experimentally investigated. However, it has been noted that rats are suitable subjects for developing new surgical techniques similar to those employed in rectal cancer treatments.

Another field of experimental studies is lumbopelvic pain, a cause of mechanical dystocia in vaginal birth. Because it mainly affects the muscles of the pelvic floor and causes pain in the vaginal opening, thereby presenting a mechanical

obstacle during labor (Brown and Johnston, 2013; Dufour et al., 2018). Therefore, we believe using rats in experimental lumbopelvic pain is appropriate regardless of the strain. Because the dimensions of the MD, PD and EBB are pretty similar in each strain, and it is the area on which the vagina is located. Gruss and Schmitt's study (2015) also indicates that pelvic dimensions influence thermoregulation and play a significant role in regulating heat loss from the body surface. Our results suggest that rats could be preferred in studies investigating the effects of pelvic width and depth on the surface area-to-mass ratio and heat loss.

In conclusion, anatomically, Lewis is the most suitable laboratory rat strain that does not predispose to labor dystocia, followed by the Wistar Albino strain. These two strains may be a choice for studies on physiological dystocia and pelvic inlet disorders. On the other hand, Sprague Dawley is less suitable for experimental studies involving the pelvic inlet, particularly those related to labor dystocia caused by anatomical factors. The limitation of this study is that it was conducted exclusively on male subjects. It should be emphasized that the study could be replicated in female subjects to provide a more comprehensive analysis. However, for further studies, evaluation of the pelvic diameters and inlet area by micro-CT imaging is strongly recommended.

Conflict of Interest

The authors stated that they did not have any real, potential or perceived conflict of interest.

Ethical Approval

This study was approved by the Dokuz University Animal Experiments Local Ethics Committee (08.07.2022, 2022/22 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Bibliometric Analysis of Postgraduate Theses on Foot Diseases in Cattle in Turkey

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Abstract: The aim of this study is to evaluate postgraduate theses on foot diseases observed in the cattle population in Türkiye within a holistic framework using the bibliographic analysis method, and to contribute to the academic studies that will follow.

As the material and method used in the research, a search was made by typing the keyword “foot diseases” in the box where search term was made in the postgraduate theses section of the official website of the National Thesis Center of the Higher Education Institution.

As a result of the search, it was determined that 16 of the n=25 theses, which were conducted between 1990 and 2024, were related to foot diseases in cattle, theses were related to foot diseases in sheep and goats, and one thesis, which was conducted in 1989, was related to medicine. In this research, the full text of 15 (93.75%) of the theses on the subject was reached, while one (6.25%) could not be reached. Twelve (75%) of the theses were found to be master's theses, and 4 (25%) were found to be doctoral theses.

It was thought that very few of the postgraduate theses on the investigation of foot diseases in cattle were published as articles, and the studies conducted remained mainly at the master's level and did not contribute much to the doctoral overlap, and the impact of the literature as a publication should be increased.

Keywords: *Bibliographic analysis, Bovine foot diseases, Higher education institution, Postgraduate thesis.*

Türkiye’de Sığırlarda Görülen Ayak Hastalıklarının Araştırılmasına Yönelik Yapılmış Lisansüstü Tezlerin Bibliyometrik Analizi

Özet: Yapılan çalışmanın amacı; Ülkemizdeki sığır popülasyonunda gözlemlenen ayak hastalıklarına yönelik yapılmış lisansüstü tezleri bibliyografik analiz yöntemiyle bütüncül çerçevede değerlendirip sonrasında yapılacak akademik çalışmalara katkı sunmasını sağlamaktır.

Metod: Araştırmada kullanılan gereç ve yöntem olarak; Yüksek Öğretim Kurumu Ulusal Tez Merkezi’nin resmi internet sitesinde lisanüstü tezler bölümündeki, tarama teriminin yapıldığı kutucuğa “ayak hastalıkları” anahtar kelimesi ‘yazılarak arama yapıldı.

Bulgular: Yapılan tarama sonucu ulaşılan n=25 tezin; 1990 ile 2024 yılları arasında yapılan 16’sının sığırlarda görülen ayak hastalıkları ile ilgili olduğu, 8 tezin koyun ve keçilerde görülen ayak hastalıklarıyla alakalı olduğu, 1989 yılında yapılmış bir tezin ise tıpla alakalı olduğu saptanmıştır. Yapılan bu araştırmada konuyla ilgili tezlerin 15’nin (%93,75) tam metinine ulaşılmış, birine ise (%6,25) ulaşılamamıştır. Taramada saptanan tezlerin 12’si (%75) Yüksek lisans, 4’ü ise (%25) doktora tezi olarak bulunmuştur. İncelenen lisansüstü tezler araştırma metodolojisi açıdan değerlendirildiğinde 9’u (%56,25) insidans, 6’sı (%37,5) prevalans, 1 tanesi (%6,25) ise karşılaştırma şeklinde değerlendirmiştir.

Sonuç: Sığırlarda görülen ayak hastalıklarının araştırılmasına yönelik yapılmış lisansüstü tezlerin çok az bir kısmının makale olarak yayınlanıp, yapılan çalışmaların daha çok yüksek lisans düzeyinde kalarak doktora çakışmalarına çok fazla katkı sağlamadığı, literatüre yayın olarak etkisinin arttırılması gerektiği düşünülmüştür.

Anahtar Kelimeler: *Sığır Ayak hastalıkları, Bibliyografik Analiz, Yüksek Öğretim Kurumu, Lisansüstü Tez.*

Introduction

Foot diseases in cattle are one of the most critical health problems in the world, manifesting themselves with the emergence of lameness in animals and significantly affecting their quality of life. Factors such as decreased milk production and reproductive performance, medical costs, culling, mortality, and the risk of developing other diseases due to these diseases, especially in dairy cattle, are considered factors that put industrial productivity at risk economically. In addition, in meat production, besides the decrease in live weight gain in animals, economic losses are also considered as reasons that lead to the reduction in market value and product quality and undesirable situations such as high mortality and early slaughter (Dionizio et al., 2022; Whay and Shearer, 2017).

Bibliometric analysis has gained tremendous popularity in research in recent years. This popularity has led to the advancement, availability, and accessibility of bibliometric software such as Gephi, Leximancer, VOSviewer, and scientific databases such as Scopus Web of Science, and the spread of bibliometric methodology across disciplines from information science to business research (Donthu et al., 2021a). Scholars use bibliometric analysis to uncover emerging trends and research components in article and journal performance and to explore the intellectual structure of a particular field in the existing literature. The data that take center stage in the bibliometric analysis are usually extensive and objective. Still, their interpretation is often based on subjective assessments generated through objective and informed techniques and procedures. Bibliometric analysis is used to make sense of large volumes of unstructured data and to reveal and map its nuances. Therefore, bibliometric studies enable scholars to get a one-stop overview, derive new ideas for research, and position their intended contributions to the field (Donthu et al., 2021b).

Analyzes applied to the studies obtained from literature searches through web-based search engines will also provide added value to current approaches regarding new research and the development of cooperation between authors (Donthu et al., 2021c). The presence of thesis and articles prepared on bovine foot diseases in cattle breeding in Türkiye reveals the importance of this phenomenon in terms of both animal welfare and economic aspects. In this research, theses on bovine foot diseases were prepared from the theses on bovine foot diseases registered in the National Thesis Center on the official website of the Council of Higher Education.

The aim of this research is to make a bibliometric analysis of the theses on the subject in Türkiye and to provide different perspectives for new studies to be conducted on this subject.

Materials and Methods

The study material consisted of 16 postgraduate theses in veterinary surgery in Türkiye between 1990 and 2024, which were designed to investigate foot diseases in cattle.

Since no experimental or non-experimental intervention was used in the study, and similar published studies were used as references; no ethics committee report was needed in terms of systematic methodology.

When the research is evaluated methodologically, it aims to investigate the subject using the bibliometric analysis technique, which has started to be used in the field of veterinary surgery in Türkiye in recent years. For this purpose, on 06.01.2025, on the official website of the National Thesis Center of the Higher Education Institution on the official website of the Higher Education Institution on 06.01.2025, searches were made by typing the relevant words such as “bovine foot disease, bovine foot diseases, foot disease” in the box where the search terms were written in the postgraduate theses section, but a sufficient number of theses could not be reached. Afterward, n=25 postgraduate theses were reached by typing the keywords “foot diseases.” It was determined that 16 were related to foot diseases seen in cattle between 1990 and 2024. These theses were evaluated separately in terms of year, content, institution, thesis level, scientific method, sample size, and content.

Statistically, the data obtained from the theses scanned in this study were transferred to Microsoft Excel form, and visuals were created. The percentage and frequency values of the requested analyses to be converted into statistical data were performed using SPSS for Windows 21.0 (IBM, Inc., Chicago, IL, USA).

Results

After the research, the data related to the variables obtained from n=16 theses were uploaded to the SPSS statistical program, and the averages related to the analyses were prepared in tables and figures and presented for evaluation.

Within the scope of the study, the statistical expression of the features related to the “n” number of variables in the theses made for the examination of foot diseases detected in cattle in Türkiye is shown in Table 2. It was thought that it would be appropriate to present the expressions related to these variables under a single title instead of in separate tables.

The data presented for the variables in the table above shows that the first thesis on the subject was done in 1990, and the last thesis was done in 2024, but 37.5% of these theses were presented in 2009, 2020, and 2022. It was determined that 75% of the theses were at the master's thesis level, 62.5% of the academic titles of the thesis advisors were professors, and 37.5% were conducted in the Eastern Anatolia Region in terms of the location of Türkiye where the research was conducted. It was also determined that 31.3% of the theses were studied within the Faculty of Veterinary Medicine of Fırat University as the university where the thesis was prepared academically. After the examinations, it was determined that 37.5% of the theses presented were converted into articles and published in

Table 1. General distribution of the variables in the number "n" in the theses analyzed in the study

NO	THE NATIONAL THESIS CENTER THESIS NO	YEAR	TYPE OF THESIS	SUPERVISOR TITLE	REGION OF EXECUTION	UNIVERSITY OF EXECUTION	THE YEAR THESIS WAS CONVERTED INTO AN ARTICLE	NAME OF THE JOURNAL IN WHICH THE ARTICLE WAS PUBLISHED	NUMBER OF CITATIONS RECEIVED BY THE ARTICLE	THE NUMBER OF ANIMALS EXAMINED	NUMBER OF FOOT DISEASES DETECTED	NUMBER OF DEFORMED NAILS DETECTED	NUMBER OF FEMALE ANIMALS DIAGNOSED WITH DISEASE	NUMBER OF MALE ANIMALS WITH DISEASE DETECTED	NUMBER OF DISEASES DETECTED THE FRONT FOOT	NUMBER OF DISEASES DETECTED IN THE HIND FOOT	NUMBER OF DISEASES BY SEASON				NUMBER OF DISEASES BY BREED			NUMBER OF AGE-RELATED DISEASES				
																	SPRING	SUMMER	AUTUMN	WINTER	CULTURE BREED	LOCAL BREED	HYBRID BREED	ONE YEAR OLD	TWO YEARS OLD	THREE YEARS OLD	FOUR YEARS OLD	FİVE YEARS AND OLDER
1	905126	2024	MD	Prof. Dr.	MR	Bingöl	-	-	-	2592	596	-	-	-	-	-	113	47	161	275	-	-	-	-	-	-	-	-
2	744387	2022	MD	Assist. Prof	CA	Kırıkkale	-	-	-	3047	100	57	-	-	38	62	-	-	-	-	84	16	-	-	-	-	-	-
3	724925	2022	MD	Prof. Dr.	EAR	Fırat	-	-	-	4000	383	145	292	73	-	-	-	-	-	-	324	3	38	-	-	-	-	-
4	676863	2020	MD	Prof. Dr.	EAR	Yüzüncü Yıl	-	-	-	1560	215	110	146	69	-	-	-	-	-	-	180	13	22	-	-	-	-	-
5	616214	2020	MD	Prof. Dr.	EAR	Kafkas	-	-	-	1303	45	51	-	-	10	49	-	-	-	-	32	3	-	5	13	13	9	5
6	536105	2019	MD	Prof. Dr.	SAR	Fırat	-	-	-	6000	332	432	465	97	104	459	124	64	77	297	528	3	31	-	-	-	-	-
7	428897	2016	MD	Prof. Dr.	GAB	Fırat	2016	Fırat Univ. Health Sci. Vet. Journal	18	1912	213	235	209	4	31	182	52	26	37	98	209	3	1	5	47	54	42	65
8	448902	2015	MD	Prof. Dr.	GAB	Harran	-	-	-	570	43	14	-	-	16	27	-	-	-	-	28	2	13	-	-	-	-	-
9	532178	2009	MD	Assoc. Prof.	MR	Yüzüncü Yıl	-	-	-	1344	81	-	-	-	-	-	-	-	-	-	26	26	29	-	-	-	-	-
10	532177	2009	MD	Prof. Dr.	AR	Yüzüncü Yıl	-	-	-	1090	71	-	-	-	-	-	-	-	-	-	35	20	16	-	-	-	-	-
11	193492	2007	MD	Assist. Prof	AR	Kocatepe	2009	Kocatepe Veterinary Journal	23	1800	117	175	-	-	25	170	-	-	-	-	150	20	25	-	-	-	-	-
12	157558	2004	PhD	Assoc. Prof.	SAR	Fırat	2004	Firat Üniv. DAUM Journal	17	1638	232	277	184	48	39	189	130	34	53	104	126	55	51	11	14	21	43	123
13	108176	2001	MD	Assoc. Prof.	SAR	Yüzüncü Yıl	2001	Yüzüncü Yıl Un. Health Sci Inst J	21	1800	321	1450	-	-	-	-	85		236		267	54		-	-	-	-	-
14	99113	2000	PhD	Assoc. Prof.	SAR	Fırat	2002	Fırat Univ. Health Sci. Vet. Journal	25	1688	153	239	144	65	32	207	109	26	47	83	123	53	33	13	23	32	29	106
15	40426	1995	PhD	Prof. Dr.	MarR	Uludağ	-	-	-	1798	262	-	-	-	19	250	-	-	-	-	-	-	-	-	-	-	-	-
16	18102	1990	PhD	Prof. Dr.	MarR	İstanbul	-	-	-	2000	69	97	44	25	10	59	22	6	22	19	65	-	4	6	19	6	7	32

*CHE: Conuncıll of Higher Education *MD: Master's Degree, PhD: Doctorate ***MR: Mediterranean Region, EAR: Eastern Anatolia Region, CA: Central Anatolia Region, SAR: Southeastern Anatolia Region, AR: Aegean Region, MarR: Marmara Regi

Table 2. Statistical analysis of some variables obtained from the post-research thesis

Features	n	%
Thesis year		
1990	1	6,3
1995	1	6,3
2000	1	6,3
2001	1	6,3
2004	1	6,3
2007	1	6,3
2009	2	12,5
2015	1	6,3
2016	1	6,3
2019	1	6,3
2020	2	12,5
2022	2	12,5
2024	1	6,3
Thesis type		
MD	12	75
PhD	4	25
Consultant title		
Assistant. Prof. Dr.	2	12,5
Assoc. Prof. Dr.	4	25
Prof. Dr.	10	62,5
The region where the thesis is conducted		
Mediterranean Region	2	12,5
Marmara Region	2	12,5
Aegean Region	2	12,5
Eastern Anatolia Region	6	37,5
Southeastern Anatolia Region	3	18,8
Central Anatolia Region	1	6,3
University where the thesis was done		
Fırat	5	31,3
Bingöl	1	6,3
Harran	1	6,3
Uludağ	1	6,3
İstanbul	1	6,3
Kırıkkale	1	6,3
Kafkas	1	6,3
Afyon Kocatepe	1	6,3
Van Yüzüncü Yıl	4	25
From thesis to article		
Yes	6	37,5
No	10	62,5
The journal in which the article was published		
National Journal	6	100
Number of article citations (min-max): 17-25	X±SS= 20.80±3,34	

journals. It was seen that all of the articles of these completed theses were published in national journals but not in international journals such as SCI and SCI-Expand. The average number of citations (min:17-max:25) of these articles published in national journals was 20.80±3.34.

The findings regarding the evaluation of the theses obtained after the research in terms of foot diseases are presented in Table 3. Accordingly, it was determined that the average number of animals examined was 2133,87±1303,83, the average number of foot diseases was 202,06±151,28, and the average number of deformed nails was 273,50±388,44. In addition, the average number of female animals was 212±134,41, and the average number of male animals was 54,42±31,44. In addition, the average number of foot diseases in the front feet was 36.54±29.32, and the average number of foot diseases in the hind feet was 176.27±127.93. When the distribution of the cases according to the seasons was analyzed, it was determined that foot

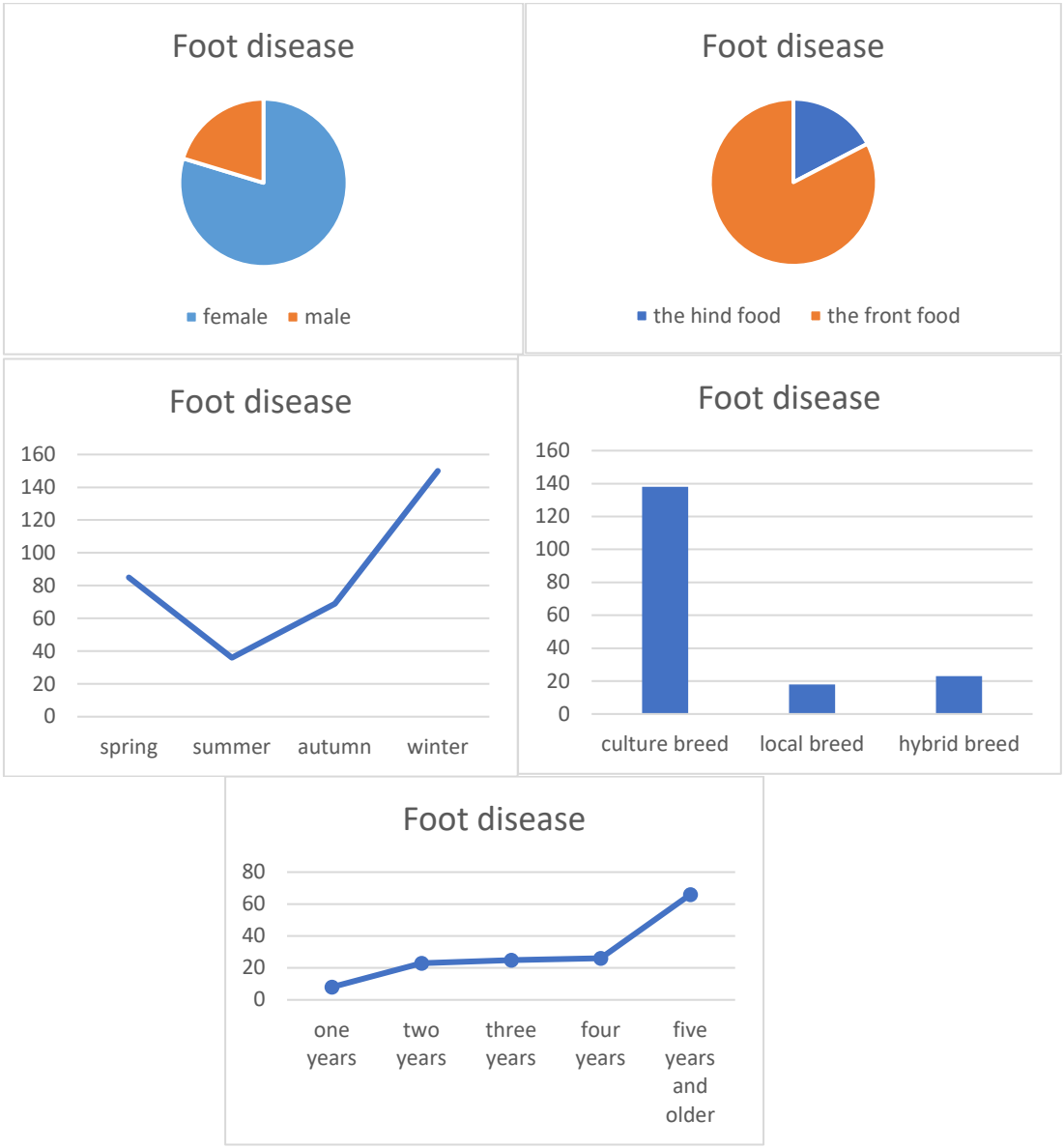

Figure 1. Graphical distribution according to variables causing foot diseases

diseases were most common in winter (149,50±97,25) and least common in summer (35,50±17,15). When the animals in the studies were evaluated in terms of breed characteristics, it was found that the animals with the disease were mostly seen in cultivated cattle (137,71±142,27) and the least in local cattle (18,07±17,91). In the theses examined, it was determined that the most common foot disease in cattle was observed in animals aged five years and over (66.20±49.31) and the least in animals aged one year (8±3.74) (Table 3) (Figure 1).

Discussion

Bibliometric analysis presents the intellectual structure and emerging trends in a topic or research area by summarizing large amounts of data, using qualitative and quantitative techniques, making it possible to apply them to the data. Although this type of analysis efficiently summarizes and synthesizes data from the literature, it has some limitations. First, given that it considers articles published in the languages in which the analysis is

conducted, this research will consider articles with abstracts or keywords. The other limitation is that the data obtained from the databases is not produced exclusively for this type of analysis and contains errors that may affect the analysis results, such as duplicate data and incorrect entries. Therefore, researchers should consider standardizing and structuring the data to reduce such errors. Another limitation is that qualitative data are highly subjective, and researchers should carefully support the results with appropriate analysis (Donthu et al., 2021c). Despite this analysis's limitations, detailed knowledge and rigorous data standardization make it possible to efficiently interpret large amounts of data, which can be very useful in research. Foot diseases in cattle are of great importance in the sectoral sense, especially in terms of cattle breeding, as they cause losses in terms of both animal welfare and economic value. In the search made on the official website of YÖK National

Thesis Center, it was determined that 16 studies were conducted at the postgraduate thesis level between 1990 and 2024. When the number of thesis on the subject is generally examined, there is a linearly increasing trend.

When the results of 53 studies on foot diseases from 6 continents focusing mainly on Europe and North America over about 30 years were examined, it was found that the prevalence of those who had problems due to foot disease

Table 3. Evaluation of thesis in terms of foot diseases.

Variables	Minimum	Maximum	X±SS
Number of animals examined	570	6000	2133,87±1303,83
Foot disease	43	596	202,06±151,28
Deformed nail	14	1450	273,50±388,44
Female animal	44	465	212±134,41
Male animal	4	97	54,42±31,44
The front foot	10	104	36,54±29,32
The hind foot	27	459	176,27±127,93
Spring	22	130	85,25±41,12
Summer	6	64	35,50±17,15
Autumn	22	161	68,75±46,07
Winter	19	297	149,50±97,25
Culture breed	18	528	137,71±142,27
Local breed	2	55	18,07±17,91
Hybrid breed	1	51	23,41±14,24
One year old	5	13	8±3,74
Two years old	13	47	23,20±13,89
Three years old	6	54	25,20±18,78
Four years old	7	43	26±17,34
Five years and older	5	123	66,20±49,31

in the study group, including 414950 cows from 3945 herds varied between 5.1% and 45%. The average prevalence was 22.8% (Thomsen et al., 2023). Silva et al. (2020) reported that digital dermatitis was the most studied worldwide when they analyzed the studies on bovine foot diseases and hoof deformities published in the Scopus database over approximately 50 years. The statistical study showed that studies on bovine foot and hoof diseases among disease groups have gradually increased. Since the 1980s, they have been studied much more than infectious diseases (Silva et al., 2020).

The fact that studies on foot diseases are published in reputable journals with high impact factors, such as Preventive Veterinary Medicine, Animals, and Veterinary Journal, can be considered an indication of the global interest in the studies carried out in the field. This international interest has also encouraged researchers to conduct new studies to discover treatment and prevention methods in this field (Fürmann et al., 2024; Koflerü et al., 2024; Magrin et al., 2020).

Although the published research articles emphasized the importance of factors such as age, seasonal effect, and gender, which were included as variables in the thesis and play a role in the etiology of foot diseases, it was observed that some theses did not examine these factors much.

The academic studies examined in the literature reviews conducted to emphasize the importance of the variables in the theses in terms of foot diseases revealed that the diseases are more common, especially in winter and autumn months (Garvey, 2022; Jury et al., 2021; Magrin et al., 2020). Our study determined that the effect of seasons on diseases was most common in winter and least common in summer.

Academic studies emphasize that cattle are more frequently exposed to foot diseases due to the decrease in the structural features of the hooves of older animals due to age (Browne et al., 2022; Fürmann et al., 2024; Sadiq et al., 2021). The study observed that older animals aged 5 years and older were more frequently affected by foot diseases than young animals.

The articles emphasized that foot diseases were more common in female animals than in male animals (Erickson et al., 2024; Griffiths et al., 2018; Mahd-Gharehbagh et al., 2020). In the theses examined in this study, foot diseases were more common in female animals than male cattle.

Studies have stated that foot diseases in animals are localized, especially in the hind feet (Beaver et al., 2021; Rukol et al., 2021). The study determined that foot diseases in cattle are generally more common in the hind feet than in the front feet.

Academic studies have reported that foot diseases detected in cattle are much more common in animals belonging to high-yielding breeds (Islam et al., 2020; Roziyev et al., 2022; Zavadilová et al., 2021). The theses found that

the disease cases were mostly seen in cultivated animals and the least in local animals.

Conclusion

This study will be a reference source for researchers interested in foot diseases in cattle. However, it should not be ignored that this research has some limitations, such as that it was conducted from a single database and only Turkish theses were included in the data set. In addition, as a result of the examination of the theses reached as a result of the research, the relevant ones were used, while the irrelevant ones were excluded from the study. The data obtained from the study will be a template for determining future trends on the subject. Especially if these research institutions develop appropriate policies on the study of the subject and support it with resources, it will draw more attention to the subject and help to increase interest. This will provide added value in the fight against foot diseases in cattle for both animal welfare and economic losses.

Conflict of Interest

None.

Ethical Permission

Since experimental animals were not used in this study, HADYEK was not required.

Similarity Rate

We declare that the similarity rate of the article is 13% (Ithenticate) as stated in the report uploaded to the system.

Author Contributions

Idea/Concept/Design/Supervision/Consultancy/Data Collection and/or Processing/Analysis and/or Interpretation/Source Scan/Writing of the Article/Critical Review/: Öİ

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Freezing of Honamli Goat Buck Sperm with Commercial and Laboratory Extenders and Evaluation of In Vitro Spermatological Parameters

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Abstract: This study aimed to evaluate the effectiveness of commercial versus laboratory-made extenders for cryopreserving Honamli buck semen. Semen was collected from three 2-3-year-old bucks and pooled. The pooled semen was divided into two portions: one with seminal plasma removed and diluted with Tris egg yolk-based extender (TEYBE+), and the other with seminal plasma retained, extended with commercial Bioxcell (B), Tris egg yolk-based extender (TEYBE-), and Tris lecithin-based extender (TLEBE). The extended semen was stabilized at +4 °C for 2 hours, cryopreserved by exposure to liquid nitrogen steam at -120 °C for 12 minutes, and frozen. After thawing, motility, viability, plasma membrane and acrosome integrity (PMAI), high mitochondrial membrane potential (HMMP), and mitochondrial reactive oxygen species (MROS) levels were evaluated.
Group B exhibited the lowest post-thaw motility, viability, PMAI, and HMMP values ($p < 0.001$). The highest viability rate was recorded in the TEYBE+ group ($p < 0.05$). TEYBE- showed superior motility, PMAI, HMMP, and viability compared to B, whereas TLEBE did not outperform TEYBE+. The lowest MROS levels were observed in the TEYBE+ group ($p < 0.05$, compared to B). In conclusion, the findings indicated that the separation of the seminal plasma used in the freezing of buck semen had a beneficial impact on the freezing process. Conversely, the B extender, which is commercially employed for freezing bull semen, was ineffective in cryopreservation of the buck semen when compared to extenders containing lecithin and egg yolk.
Keywords: *Bioxcell, Cryopreservation, Flow cytometry, Honamli.*

Honamlı Teke Spermasının Ticari ve Laboratuvarda Hazırlanan Sulandırıcılar ile Dondurulması ve İn Vitro Spermatalojik Parametrelerinin Değerlendirilmesi

Özet: Bu çalışmanın amacı Honamlı Teke spermasının kriyoprezervasyonunda ticari ve laboratuvar sulandırıcılarını etkinliğini değerlendirmektir. Çalışmada 2-3 yaşlarında üç Honamlı tekesinden spermalar toplandı ve birleştirildi. Birleştirilen sperma iki eşit parçaya bölündü; Bir kısım seminal plazma uzaklaştırıldı ve tris yumurta sarısı bazlı sulandırıcı (TYSBS+) ile sulandırıldı; diğer kısım seminal plazması uzaklaştırılmadı ve Bioxcell (B), tris yumurta sarısı bazlı sulandırıcı (TYSBS-) ve tris lesitin bazlı sulandırıcı (TLBS) ticari sulandırıcı ile sulandırıldı. Sulandırılan sperma örnekleri +4 derecede 2 saat boyunca ekilibrasyona ve sıvı nitrojen buharında (-120°C'de 12 dakika) kriyoprezervasyona tabi tutularak donduruldu. Çözdürme işleminden sonra spermaların motilite, canlılık, plazma membranı ve akrozom bütünlüğü (PMAI), yüksek mitokondriyal membran potansiyeli (HMMP) ve mitokondriyal reaktif oksijen türleri (MROS) seviyeleri değerlendirildi.
B grubu, çözdürme sonrası en düşük motilite, canlılık, PMAI ve HMMP değerlerini göstermiştir ($p < 0.001$). En yüksek canlılık oranı TYSBS+ grubunda kaydedilmiştir ($p<0.05$). TYSBS-, B'ye kıyasla daha yüksek motilite, PMAI, HMMP ve canlılık gösterirken, TLBS, TYSBS+'den daha iyi performans sergileyememiştir. En düşük MROS seviyeleri TYSBS+'de gözlenmiştir ($p < 0,05$, B ile karşılaştırıldığında). Sonuç olarak, teke spermasının dondurulması sürecinde seminal plazmanın uzaklaştırılmasının dondurma başarısı üzerinde olumlu bir etkisi olduğu belirlenmiştir. Buna karşılık, boğa spermasının dondurulmasında yaygın olarak kullanılan ticari B sulandırıcısının, teke spermasının kriyoprezervasyonu açısından, yumurta sarısı ve lesitin içeren sulandırıcılara kıyasla etkisiz olduğu tespit edilmiştir.
Anahtar Kelimeler: *Bioxcell, Kriyoprezervasyon, Akış sitometrisi, Honamlı.*

Introduction

The cryopreservation of buck and ram semen is a crucial component of genetic resource management. The dilution process in buck and ram semen significantly influences the efficiency of artificial insemination (AI) and the overall reproductive efficiency. Moreover, the choice of semen diluent is a key factor impacting fertility rates, yet the development of optimal protocols continues to pose a considerable challenge (Ferreira et al., 2014).

Semen dilution is critical for preserving sperm viability and enhancing fertility outcomes. Research has highlighted the significant impact of various diluents and dilution protocols on sperm quality and reproductive success. Notably, studies have demonstrated that yolk-citrate diluents achieve higher pregnancy rates compared to fresh semen, underscoring the importance of optimizing sperm concentration and diluent composition (Ferreira et al., 2014; Madrigali et al., 2021; Mohamed and Moustafa, 2017).

In bucks, the combination of egg yolk and seminal plasma may negatively affect sperm viability, unlike what has been observed in other species (Gangwar et al., 2016). A protein identified in seminal plasma (SBUIII, the glycoprotein BUSgp60) and an enzyme released by the bulbourethral glands (phospholipase-A, also referred to as the egg yolk coagulating enzyme) have been implicated in spermatozoa toxicity in semen diluents derived from milk and egg yolk (Ferreira et al., 2014; Purdy, 2006). When using semen diluents containing egg yolk or milk for goat semen storage, seminal fluid should be removed by centrifugation to minimize the detrimental effects of bulbourethral gland secretions (Purdy, 2006).

These findings underscore the importance of selecting appropriate diluents to enhance sperm survival and fertilization potential during artificial insemination, and the choice of diluent is critical for maintaining sperm quality during the freezing and thawing processes. To maximize survival rates and fertility, protocols for cryopreservation of goat semen must consider species-specific factors, including diluent composition, pH, osmolality, cryoprotectants, and freeze-thaw techniques (Gangwar et al., 2016).

Egg yolk is frequently incorporated into extenders for preserving ram semen through cryopreservation (Alçay et al., 2015; Anand et al., 2014; de Paz et al., 2010; Gholami et al., 2012; Salamon and Maxwell, 2000; Watson, 2000). Although egg yolk is commonly employed, its application is not without challenges, including the risk of pathogen transmission and variability between batches. Additionally, concerns about biosecurity and its impact on sperm analysis have prompted the pursuit of alternative solutions (Aires et al., 2003; Layek et al., 2016).

Soybean lecithin has emerged as an alternative to animal-origin extender as a plant-origin extender. Soybean lecithin has been recognized as an effective substitute for egg yolk in semen extenders used for the cryopreservation of ram and bull sperm. Researchs have demonstrated that extenders formulated with soybean lecithin can achieve equal or better outcomes than those containing egg yolk, particularly regarding post-thaw sperm quality parameters including motility, viability, and fertility (Forouzanfar et al., 2010; Khatun et al., 2021; Masoudi et al., 2016). Additionally, some studies indicates that the effectiveness of Bioxcell (B) in preserving bull spermatozoa is influenced by its composition, particularly the presence of soy lecithin (Akhter et al., 2010; Kaka et al., 2017). Fernández-Novo et al. (2021) demonstrated that B outperformed other extenders in maintaining the viability of spermatozoa at lower temperatures, specifically 5 °C, which is critical for preserving sperm quality during preservation.

This study aimed to assess the effectiveness of B, a commercial semen extender for bulls, in comparison to Tris-lecithin and Tris-egg yolk-based (with and without centrifugation of seminal plasma) extenders in Honamli buck semen.

Material and Method

Animals and Experimental Design

This research was conducted on three Honamli bucks aged 24–36 months. All bucks were kept under identical care and feeding conditions. Semen samples were obtained biweekly throughout the mating season in Burdur, Turkey, using an electroejaculator. The experiment was repeated five times. The study protocol was approved by Local Ethics Committee Animal Experiments of the Burdur Mehmet Akif Ersoy University (approval number 07.12.2020/201-13.11.2024-1388).

Semen Collection and Processing

Macroscopic and microscopic examinations were performed on the semen collected from each buck. Only fresh semen with normozoospermic conditions (concentration ≥ 2.0x 10^9/ml, mass activity ≥ +++3, volume ≥ 1.0 ml and motility ≥ 70% was used. The pooled semen was split into two parts. One portion was left uncentrifuged for seminal plasma separation and was extended with a Bioxcell (2A23440, BioShop, Canada) extender, along with an egg yolk- and soybean lecithin-based (Sigma, P5638) solution. The second semen portion underwent centrifugation to separate the seminal plasma and was then extended with an egg yolk-based solution. The same Tris buffer [299 mM Trizma (Sigma, T1503), 90 mM citric acid (Sigma, C0759), 20 mM glucose (Sigma, G7528) and distiled water] was used in extenders containing egg yolk and lecithin. The final group configurations are outlined in Table 1.

Following dilution, samples were loaded into 0.25 ml straws to achieve a concentration of around 200×10^6 spermatozoa/ml and then equilibrated at +4 °C for two hours. The straws were then placed in liquid nitrogen vapor (approximately 10 cm above the liquid nitrogen, ~ -120 °C) for 12 minutes, followed by storage in a nitrogen tank at -196 °C. The thawing process for spermatological evaluation involved placing semen straws from each experimental group in a 37 °C water bath for 30 seconds, at least two months post-freezing.

Table 1. Compositions of the groups.

Groups	Compositions
Bioxcell (B)	Bioxcell + 6% Glycerol (GLY) (Non-centrifuged semen)
TLEBE-	Tris-based extender + 1% Lecithin (LC) + 6% GLY (Non-centrifuged semen)
TEYBE-	Tris-based extender + 20% eggyolk (EY) + 6% GLY (Non-centrifuged semen)
TEYBE+	Tris-based extender + 20% eggyolk (EY) + 6% GLY (Centrifuged semen)

Table 2. Post-thaw spermatological parameters of Honamli buck semen frozen with different semen diluents.

GROUP	MOTILITY(%)	PMAI(%)	HMMP(%)	MROS(%)	VIABILITY (%)
BIOXCELL	12.7±1.2^{c}	10.4±0.2^{b}	11.6±0.6^{b}	83.8±6.5^{a}	26.1±2.5^{c}
TLEBE-	58.5±1.9^{ab}	29.8±2.6^{a}	38.8±1.9^{a}	73.8±1.7^{ab}	61.2±1.9^{b}
TEYBE-	53.9±2.3^{b}	33.1±1.9^{a}	33.4±5.5^{a}	68.1±5.8^{ab}	55.9±1.8^{b}
TEYBE+	60.9±0.6^{a}	32.1±1.6^{a}	39.7±2.5^{a}	63.1±4.6^{b}	68.3±0.7^{a}
p	**	*	*	*	*

P<0.05 *, p<0.001 **,
$^{a.b.c.}$ The statistical significance is shown in the same column.
TLEBE-: Non-centrifuged Tris 1% Lecithin 6% Glycerol
TEYBE-: Non-centrifuged Tris 20% Egg yolk 6% Glycerol
TEYBE+: Centrifuged Tris 20% Egg yolk 6% Glycerol
PMAI: Plasma Membrane Integrity
HMMP: High mitochondrial membrane potential
MROS: Mitochondrial reactive oxygen species

Evaluation of In Vitro Spermatological Parameters

Motility

Semen motility (%) was assessed with a heated stage set to 37 °C and viewed at 400x magnification in the phase-contrast microscope (Inanc et al., 2023).

Evaluation of Flow Cytometric Analysis

Flow cytometric evaluation was conducted using a (Beckman Coulter) CytoFLEX flow cytometer, which had three channels with emission filters set at 610 ± 20 585 ± 42, 525 ± 40 nm, 488 nm blue laser. Around 10,000 events were analyzed per sample. Debris was eliminated by using the forward scattering area (FSC-A). All analyses were evaluated using software of CytExpert 2.3.

Preparation solutions were prepared using 0.153 mM JC-1 (Invitrogen, T3198), FITC-PNA 100 µg/ml (Sigma, L7381), Sybr-14 (1:10), propidium iodide (PI) (2.99 mM) (Invitrogen, L7011) and 5 µM MitoSOX (Invitrogen, M36008) dissolved in DMSO. The solution was then aliquoted into 50 µl portions and stored at -20 °C until needed. The plasma membrane, acrosome ingetrity (PMAI) in spermatazoa was assessed using a FITC-PNA/PI double staining protocol. Semen samples were extended with phosphate-buffered saline (PBS; 492 µl) to achieve a final concentration of 5×10^6 spermatozoa/ml. To this mixture, 5 µl FITC, 3 µl PI were added, bringing the final volume to 500 µl. After a 15-minute incubation in a 37 °C water bath in a dark environment. The FITC(-)/PI(-) population was identified as PMAI (Inanc et al., 2023).

Spermatozoa exhibiting high mitchondrial membrane potential (HMMP) were evaluated using the JC-1 protocol. Diluted sperm in PBS (495 µl) were to a final concentration of 5×10^6 spermatozoa/ml, followed by the addition of 5 µl JC-1 staining solution, bringing the total volume to 500 µl. The mixture was incubated for 15 minutes in a 37°C water bath in a dark environment before being analyzed by flow cytometry. HMMP levels were assessed (Inanc et al., 2023).

Spermatozoa viability was assessed using the Sybr-14 and PI double staining protocol. The sperm samples were extended in PBS (492 µl) to 5×10^6 spermatozoa/ml. Then, 5 µl Sybr-14 ,3 µl of PI were added to the semen mixture, was incubated for 15 minutes in a 37°C water bath in a dark environment before being analyzed by flow cytometry to evaluate sperm viability. Viability was determined as SYBR(-)/PI(+) population (Inanc et al., 2023).

Mitochondrial reactive oxygen species (MROS) in spermatozoa were assessed using the MitoSOX/PI double staining method. MitoSOX (5 µL) and PI (3 µL) were mixed with 492 µL PBS, and 10 µL of semen was added to the solution, bringing the sperm concentration to 5×10^6 spermatazoa/ml. The mixture was incubated at 15 minutes in a 37 °C water bath in a dark environment. The MITOSOX+/PI- population (%) was considered as mitochondrial ROS (Dönmez and İnanç, 2024).

Statistical Analysis

The Shapiro–Wilk test was performed to assess normality, and Levene's test was used to evaluate variances homogeneity. ANOVA identified group differences, followed by Duncan's test for post-hoc evaluation. The statistical analyses were evaluated 5% significance level, with a p-value of <0.05 indicating significance. The results are presented as means (X) ± standard deviations (SD), while non-parametric data are shown as means (X) ± standard error of the mean (SEM).

Results

The post-thaw spermatological parameters of Honamli bucks are shown in Table 2 based on the study's findings. The

findings revealed that the B extender group had the lowest motility, PMAI, HMMP, and viability rates after thawing, and these differences were found to be statistically significant ($p<0.001$). The TEYBE+ group exhibited the highest post-thaw viability rate in this study ($p<0.05$). Furthermore, the TEYBE- group demonstrated superior motility, PMAI, HMMP, and viability rates post-thaw when compared to the B group, although no advantage was observed relative to the TLEBE- and TEYBE+ groups. A comparison of MROS levels showed that the TEYBE+ group had significantly lower values than the B group ($p<0.05$).

Discussion and Conclusion

This study aimed to evaluate the efficacy of B, a commercial semen extender for bulls, in maintaining the quality of Honamli buck semen. Its performance was compared to Tris-lecithin and Tris-egg yolk-based diluents.

B extender is originally produced for bull semen, but the suitability of the diluent for buck semen has been questioned. Studies suggest that B extenders can notably enhance the motility and viability of sperm from bucks after freezing and thawing. In this study, the B group showed the lowest post-thaw sperm motility (12.7%) ($p<0.01$). Additionally, the B group exhibited the lowest PMAI value (10.4 ± 0.2%), the lowest HMMP value (11.6±0.6%) and the lowest viability (26.1 ± 2.5%) ($p<0.05$). Nethenzheni et al. (2021) reported post-thaw motility values for B-treated sperm as 68.2±13.5% for seminal plasma centrifuged samples and 85.0±3.4% for non-centrifuged samples. Moreover, a notable reduction in the percentage of viable and normal spermatozoa was found in the centrifuged B group (5.2 ± 4.9%) compared to the non-centrifuged B group (45.7 ± 21%). It was reported that the HMMP value for the non-centrifuged B group was 49.8±20.1%. Sarıözkan et al. (2010) reported that both centrifuged and non-centrifuged B groups gave better total motility than Tris groups. In the study by Emamverdi et al. (2015) the B group demonstrated a lower level of total motility in comparison to the tris-lecithin-based diluent group. However, no significant difference was found in contrast to the tris-egg yolk-based diluent group. This work also reported total motility as 47.6% and viability as 32.08% for the B group. Daşkın et al. (2011) reported the motility of 14 ± 2.2% and the plasma membrane integrity of 14 ± 3.4 for 200 × 10^6 spermatozoa per ml ratio dilution, the outcomes of this study support the findings observed in the present research. The reduced performance of B with Honamli buck spermatozoa may be attributed to their high content of polyunsaturated phospholipids, which increases susceptibility to oxidative stress. As a result, the glutathione levels in the B group were inadequate to shield the sperm from oxidative stress during freezing and thawing. The discrepancy in outcomes can be attributed to various factors, including the method of semen collection, season, and breed considerations, thereby generating additional inquiries concerning the suitability of B for ovine semen. Nonetheless, the considerable discrepancy between the studies is a matter of considerable interest.

The motility rates for the TEYBE- and TLEBE- groups were recorded as 53.9±2.3% and 58.5±1.9%, respectively in the present study. While the difference in motility between these groups was not statistically significant, the TLEBE- group showed a slight increase in motility. This evaluation is consistent with previous research indicating that the removal of seminal plasma from buck semen enhances motility in frozen-thawed sperm samples (Ferreira et al., 2014; Inanc et al., 2023; Sen and Tekin, 2015). The high content of unsaturated fats in buck spermatozoa membranes, combined with adverse interactions between sperm extenders and seminal plasma components, continues to hinder the success of cryopreservation. To minimize sperm toxicity caused by enzymes, such as egg yolk-coagulating enzymes, and specific proteins, it is recommended to remove seminal plasma via centrifugation when using extenders containing egg yolk or milk (Çevik and Tuncer, 2005; Inanc et al., 2023; Üstüner et al., 2020).

In Norduz goats, centrifugation of seminal plasma has been found to reduce sperm motility while increasing the percentage of abnormal spermatozoa (Sen and Tekin, 2015). Additionally, the characteristics of freshly collected semen, especially sperm motility and morphological integrity, are closely linked to post-thaw viability (Dorado et al., 2009). For instance, the addition of preservatives such as trehalose has been shown to enhance acrosome integrity and overall viability in cryopreserved ram semen (Zhao et al., 2020).The results from this study were comparable to the post-thaw values of Saanen breed buck semen frozen with skim milk containing 5% and 7% glycerol, as reported by Kulaksiz et al. (2013). Concurrently, the results showed thatthe post-thaw motility values of semen frozen by Büyükleblebici et al. (2014) using 6% glycerol in Ankara bucks were comparable to those observed in the present study. The present study revealed that the plasma membrane integrity values obtained from the TLEBE-, TEYBE-, and TEYBE+ groups (29.80%, 33.10%, and 32.10%, respectively) were significantly higher than those observed in group B (10.40%) ($P < 0.05$). The limited use of the B commercial extender in freezing studies restricts the evaluation of the results. However, similar results were achieved with the data obtained from lecithin and Tris egg yolk extender groups (Büyükleblebici et al., 2014; Emamverdi et al., 2015; Sharma and Sood, 2019; Üstüner et al., 2020), which indicated its similar effect on the preservation the plasma membrane structure. When the data on the viability rates of the study were evaluated, it was observed that the lowest value was obtained in group B (26.10%), while the TEYBE+ group (68.30%) was statistically higher than the TEYBE- (55.90%) and TLEBE- (61.20%) groups ($p<0.05$). The viability rates obtained by Sun et al. (2020) were determined to be 37.57% and 43.13% in the lecithin-containing groups and were found to be lower than those in present study. Although the values of our TEYBE- and control groups were similar, they were lower than those of the TEYBE+ group. The highest level of MROS was found in B (83.8±6.5%) ($p<0.05$). In addition, it was determined that the lowest MROS ratio was obtained in the TEYBE+ group, and the removal of seminal plasma

components from the environment increased the cryoprotective activity of the egg yolk.

Consequently, it was determined that the removal of seminal plasma in the extenders used for freezing buck semen had a beneficial impact on the freezing process, while the B extender was less effective in preserving buck semen compared to those containing lecithin and egg yolk. In light of the findings of this study and the discrepancies observed among the studies, it is not recommended to utilize B for freezing goat semen. Nevertheless, new studies are required to compare the effectiveness of commercial diluent in freezing buck semen.

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Conflict of Interest

The authors stated that they did not experience any real, potential or perceived conflict of interest.

Ethical approval

For this study, permission was obtained from Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee with the number 07.12.2020/201-13.11.2024-1388. In addition, the authors declared that the Research and Publication Ethics were complied with.

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Author Contributions

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Data Collection and/or Processing: RÖ, ŞG
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Literature Review: FM, MH
Manuscript Writing: FM, MH
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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Effects of Nano Zinc Oxide-Enriched Diets on Bone Morphometry and Biomechanical Strength in Japanese Quails

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Abstract: Zinc is an essential trace element critical for numerous biological functions, including bone development, enzyme activity, and immune response. This study investigated the effects of zinc oxide (ZnO) and nano zinc oxide (NZn) supplementation on bone morphometry and biomechanical properties in Japanese quails (*Coturnix coturnix japonica*). A total of 118 one-day-old quails were divided into three groups: control (C), zinc oxide (Zn), and nano zinc oxide (NZn), with each group further subdivided into replicates. Diets were formulated to provide 75 mg/kg zinc, with additional zinc sources added to achieve the desired levels. Morphometric and biomechanical analyses were conducted using a 3-point bending test to evaluate tibiotarsus bone strength and structural properties. Results indicated that the NC group exhibited a significantly higher external mediolateral diameter (ExtMLD) compared to the Zn and C groups ($P = 0.003$), suggesting enhanced periosteal bone growth with NZn supplementation. However, no significant differences were observed in internal diameters or biomechanical parameters such as breaking force, moment of inertia, strength, stiffness, and elastic modulus among the groups ($P>0.05$). Sex-based comparisons revealed that female quails in the Zn and C groups had significantly higher breaking force and moment of inertia compared to males ($P < 0.05$). Still, no such differences were observed in the NZn group. These findings suggest that while NZn may positively influence specific morphometric parameters, its impact on biomechanical strength remains limited. The study highlights the need for further research to elucidate the mechanisms underlying zinc's effects on bone development.

Keywords: *Biomechanic, Bone morphometry, Japanese quails, Zinc.*

Nano Çinko Oksit ile Zenginleştirilmiş Diyetlerin Japon Bıldırcınlarında Kemik Morfometrisi ve Biyomekanik Dayanıklılık Üzerindeki Etkileri

Özet: Çinko, kemik gelişimi, enzim aktivitesi ve bağışıklık yanıtı da dahil olmak üzere birçok biyolojik fonksiyon için kritik olan temel bir eser elementtir. Bu çalışma, çinko oksit (ZnO) ve nano çinko oksit (NZn) takviyesinin Japon bıldırcınlarında (Coturnix coturnix japonica) tibiotarsus kemiğinin morfometrisi ve biyomekanik özellikleri üzerindeki etkilerini araştırmıştır. Toplam 118 adet bir günlük bıldırcın, kontrol (C), çinko oksit (Zn) ve nano çinko oksit (NZn) olmak üzere üç gruba ayrılmış ve her grup kendi içinde alt gruplara bölünmüştür. Diyetler, 75 mg/kg çinko sağlayacak şekilde formüle edilmiş ve belirlenen düzeylere ulaşmak için ek çinko kaynakları kullanılmıştır. Morfometrik ve biyomekanik analizler, kemik dayanıklılığı ve yapısal özellikleri değerlendirmek amacıyla üç nokta eğme testi kullanılarak gerçekleştirilmiştir. Sonuçlar, NZn grubunun dış mediolateral çapının (ExtMLD) Zn ve C gruplarına kıyasla anlamlı derecede daha yüksek olduğunu ($P = 0,003$) göstermiştir, bu da NZn takviyesinin periosteal kemik büyümesini artırabileceğini düşündürmektedir. Ancak, gruplar arasında iç çaplar veya kırılma kuvveti, atalet momenti, mukavemet, rijitlik ve elastik modül gibi biyomekanik parametrelerde anlamlı bir farklılık gözlenmemiştir ($P > 0,05$). Cinsiyete bağlı karşılaştırmalarda, Zn ve C gruplarındaki dişi bıldırcınların kırılma kuvveti ve atalet momenti değerlerinin erkeklere kıyasla anlamlı derecede daha yüksek olduğu tespit edilmiştir ($P < 0,05$), ancak NZn grubunda benzer bir fark bulunmamıştır. Bu bulgular, NZn'nun belirli morfometrik parametreler üzerinde olumlu etkiler gösterebileceğini ancak biyomekanik dayanıklılık üzerindeki etkisinin sınırlı olduğunu ortaya koymaktadır. Çalışma, çinkonun kemik gelişimi üzerindeki etkilerini açıklığa kavuşturmak amacıyla daha ileri araştırmalara ihtiyaç olduğunu vurgulamaktadır.

Anahtar Kelimeler: *Biyomekanik, çinko, Japon bıldırcını, kemik morfometrisi.*

Introduction

Zinc is a crucial trace element involved in over 2,000 transcription factors, essential for maintaining structural and functional integrity. It plays vital roles in bone development, enzyme activity, hormonal regulation, reproduction, growth, immune response, and more (Abbasi et al., 2017; McDowell, 2003). Zinc deficiency can adversely affect health and productivity in livestock (Suttle, 2010).

Nanotechnology involves manipulating matter at the nanoscale (1-100 nm), leading to innovative physical, chemical, and biological properties (Feng et al., 2009; Patil et al., 2012). Nano minerals, produced through physical, chemical, or biological methods, are widely used in agriculture, livestock, and food systems. They offer advantages such as enhanced absorption, reduced excretion, and multifunctional effects like growth promotion, immunomodulation, and antibacterial activity, even at lower doses than conventional minerals (Swain et al., 2015). Studies have demonstrated the efficacy of nano zinc, nano selenium, and nano chromium in animal nutrition (Swain et al., 2015).

Bones contain a significant amount of zinc, essential for normal bone development. Increased dietary zinc levels have been reported to improve bone strength (Bahtiyarca et al., 2007). While some studies have demonstrated that organic zinc minerals positively influence nutrition and growth, others have found no such effect (Ammerman et al., 1995; Cao et al., 2000). The mineral requirements of animals and their bioavailability depend on various factors, including species, breed, age, sex, growth rate, production type, and level, the chemical form and solubility of the mineral, diet composition (nutrient balance, presence of anti-nutritional factors), interactions between minerals, ambient temperature, and the criteria used to assess needs (e.g., maximum growth or bone mineralization) (Yazgan, 1990).

The Japanese quail (Coturnix japonica) has been widely used as an experimental model in various disciplines within the life sciences for decades (Padgett et al., 1959; Minvielle, 2004). As an animal avian model, the Japanese quail is frequently used in studies on the toxicology of chemical compounds and the effects of environmental endocrine disruptors or to study physiological processes in birds (Donaldson et al., 2015; El-Kholy et al., 2019; Tomaszewska et al., 2018,). Quails are also used as an animal model to study bone formation and development in both pre-hatch and post-hatch studies (Hiyama et al., 2019; Kawai et al., 2018; Miller and Bowman, 1981; Ohashi and Kusuhara, 1991; Pourlis et al., 1998; Simmons and Pankovich, 1963; ˇSkrob´anek et al., 2005; Zibr´ın et al., 2003).

A comprehensive assessment of bone tissue's mechanical properties requires analysis of geometric parameters and implementation of standardized mechanical testing protocols. The biomechanical parameters obtained from these tests, including force-displacement relationship, stiffness, ultimate strength, and elastic modulus, are crucial for characterizing the structural and material properties of bone tissue.

The slope of the elastic region of the force-displacement curve represents the extrinsic. Stiffness or rigidity of the structure. The elastic modulus is a measure of the intrinsic stiffness of the material. The maximum stress the bone can sustain is referred to as the ultimate strength; these strength values are independent of the size and shape of the bone. However, the force required to break the bone differs from intrinsic strength because this breaking load or fracture force varies with bone size. It is crucial to keep this distinction in mind because intrinsic strength and breaking load can exhibit different trends in drug studies, especially if the drug affects the size of the bone (Turner and Burr, 1993).

Limited information is available regarding the effects of zinc sources on bone structure and development in quails (Kolaş et al., 2013).

This study aimed to compare the effects of zinc oxide and nano zinc oxide supplementation in different diets on bone strength.

Material and Methods

This study was carried out with the permission of Aydın Adnan Menderes University, Animal Experiments Local Ethics Committee, number 64583101/2023/46.

Animal Material

This study utilized a total of 118 one-day-old Japanese quails (Coturnix coturnix japonica). The quails were allocated into three groups with similar body weights: a control group, a zinc oxide group, and a nano zinc oxide group (Table 1). Experimental diets were formulated in accordance with the standards of the National Research Council (NRC). To achieve a zinc level of 75 mg/kg in the diets, an additional 60 mg/kg of zinc oxide or nano zinc oxide containing 72% elemental zinc was incorporated into the basal diet, accounting for the zinc present in the feed ingredients.

Table 1. Body and carcass weight values according to gender and groups.

	C		Zn		NZn	
	Female (n=22)	**Male (n=18)**	**Female (n=22)**	**Male (n=17)**	**Female (n=27)**	**Male (n=12)**
Body weight (gr)	239.91±17.63	198.56±19.44	232.50±26.60	192.82±17.02	233.70±28.28	196.17±27.04
Carcass weight (gr)	153.01±12.96	140.42±14.58	150.02±13.23	135.10±12.30	152.61±18.66	136.69±18.60

Nano zinc oxide, with a particle size of <50 nm, was procured from US Research Nanomaterials, Inc. (Houston, Texas). The study was conducted at the Poultry Research Unit of the Faculty of Veterinary Medicine, Aydın Adnan Menderes University.

Three-point bending test

Tibiotarsus bones were thawed at room temperature prior to mechanical testing. The midpoint of each bone was determined using a digital calliper and designated as the load point. The mediolateral and craniocaudal outer diameters of the bone were measured at the identified load point. The Zwick/Roell Z0.5 mechanical testing device, located at the TARBIYOMER facility of Adnan Menderes University, was utilized for mechanical tests (Figure 1).

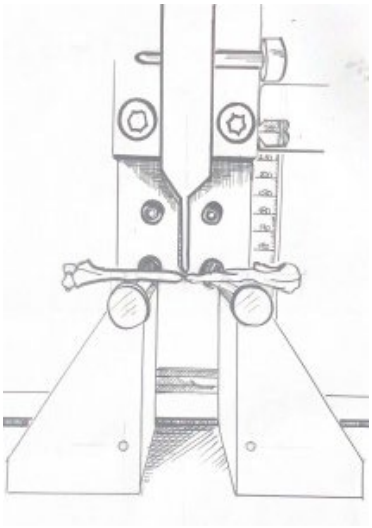

Figure 1. The Three-point bending mechanical testing device.

The distance between the support points for the three-point bending test was determined based on the length and diameter values of the bones examined. During the test, a 2 N preload was applied, followed by a load at a 1 mm/min rate until the bones fractured. A force (N)-deformation (mm) graph was generated for each bone. After testing, mediolateral and craniocaudal inner diameters were measured on the fractured bones. The cross-sectional moment of inertia was calculated using the endosteal and periosteal diameters.

Stiffness was determined from the linear regression of the force-displacement graph using the testXpert software (Zwick/Roell, Ulm, Germany). Ultimate strength and elastic modulus were calculated using stiffness, the moment of inertia, bone diameter, and the distance between the support points, based on the formulas outlined in the references (An and Draughn, 2020; ASABE Standards, 2007; Sharir et al., 2008; Turner and Burr, 1993).

Statistical Analysis

Statistical analyses were performed using the SPSS statistical package program (version 22.0, SPSS Inc., Chicago, II, US) and R Studio software (version 4.4.2, Inc, Boston, MA, USA). The normal distribution of the data was checked using the Shapiro-Wilk test. Paired t-tests were used for gender comparisons of dependent variables when the data were parametric, while the Wilcoxon test was applied for non-parametric data. The homogeneity of variances was checked using the Levene test. For comparisons between groups, one-way analysis of variance (ANOVA) was utilized for parametric data, and the Kruskal-Walli's test was employed for non-parametric data. The homogeneity of variances was checked with the Welch test. If variances were homogeneous post-hoc Bonferroni test results were used. If variances were not homogeneous post-hoc Tamhane's T2 test results were used. The data are presented as mean value ± Standard deviation (MV±SD) and 95% confidence intervals in the tables. Statistical significance (P) was accepted as $P<0.05$.

Table 2. Abbreviations of measurements.

Abbreviation	Unit	Description
$Ext_{ML}D$	mm	Medio-lateral external diameter
$EXT_{CrCd}D$	mm	Crani-caudal external diameter
$Int_{ML}D$	mm	Medio-lateral internal diameter
$Int_{CrCd}D$	mm	Crani-caudal internal diameter
CI_{ML}	%	$[(Ext_{ML}D- Int_{ML}D)/ Ext_{ML}D] *100$
CI_{CrCd}	%	$[(Ext_{CrCd}D- Int_{CrCd}D)/ Ext_{CrCd}D] *100$
L	mm	Bone length
F	N	Bending force
I	mm^4	Moment of inertia
σ	MPa	Ultimate Strength
S	N/mm	Stiffness
E	MPa	Elastic Modulus

Results

Morphometric Measurements

The analysis of morphometric parameters revealed significant differences in specific measurements among the groups (Table 3). The external mediolateral diameter ($Ext_{ML}D$) was significantly greater in the nano zinc oxide (NZn) group (2.89 ± 0.03 mm) compared to the control (C) group (2.78 ± 0.02 mm) and the zinc oxide (Zn) group (2.77 ± 0.03 mm) (P= 0.003). However, no significant differences were observed in the internal mediolateral diameter ($Int_{ML}D$) or the external and internal craniocaudal diameters ($Ext_{CrCd}D$ and $Int_{CrCd}D$) among the groups ($P > 0.05$).

In gender comparison between groups, statistically significant differences were detected in the Cgroup for both outer and inner diameter values in the medio-lateral (ML) direction (P=0.040, P=0.006, respectively). However, no significant differences were observed in the Zn and NZn groups.

In the cranio-caudal (CrCd) direction, statistical analysis revealed significant differences in the outer diameter measurements of the Zn group (P=0.009). Significant differences were identified in both C (P=0.001) and NZn (P=0.007) groups regarding inner diameter values.

Analysis of cortical index values demonstrated a statistically significant difference (P=0.015) exclusively in the C group in the ML direction, while significant gender differences were observed in all groups in the CrCd direction (P=0.000, P=0.002, P=0.017, respectively) (Table 4).

The biomechanical analysis highlighted some group-related trends, although many differences were not statistically significant (Table 5). The breaking force (F) was

Table 3. Morphometric values between groups.

	C (n:40)	Zn (n:39)	NZn (n:39)	P
$Ext_{ML}D$ (mm)*	2.78±0.02[b]	2.77±0.03[b]	2.89±0.03[a]	
	(2.73-2.83)	(2.71-2.83)	(2.84-2.95)	**0.003**
$Int_{ML}D$ (mm)*	1.83±0.03	1.85±0.03	1.86±0.03	
	(1.77-1.88)	(1.80-1.91)	(1.80-1.93)	0.665
$Ext_{CrCd}D$ (mm)*	2.73±0.02	2.72±0.03	2.80±0.03	
	(2.68-2.77)	(2.66-2.78)	(2.732.86)	0.141
$Int_{CrCd}D$ (mm)*	1.85±0.03	1.83±0.03	1.87±0.03	
	(1.79-1.90)	(1.78-1.89)	(1.81-1.93)	0.642
L (mm)*	51.99±0.25	52.14±0.19	52.29±0.29	
	(51.48-52.51)	(51.76-52.52)	(51.70-52.88)	0.701
CI_{ML}*	34.09±1.01	32.82±1.12	35.47±1.17	
	(32.05-36.15)	(30.56-35.08)	(33.11-37.84)	0.240
CI_{CrCd}*	32.03±1.17	32.36±1.03	32.32±1.21	
	(29.66-34.40)	(30.28-34.44)	(30.32-35.20)	0.900

[a,b] :Means within a row that do not share a common superscript differ significantly (P < 0.05). *Abbreviations in the table are as explained in Table 2.

Table 4. Morphometric values in the female and male.

Parametre	Gender	C (n:40)	Zn (n:39)	NZn (n:39)
$Ext_{ML}D$ (mm)*	**Female**	2.77±0.03	2.80±0.04	2.88±0.03
		(2.71-2.84)	(2.71-2.89)	(2.82-2.95)
	Male	2.78±0.03	2.74±0.04	2.92±0.06
		(2.72-2.85)	(2.66-2.82)	(2.80-3.04)
	P	**0.040**	0.318	0.531
$Int_{ML}D$ (mm)*	**Female**	1.76±0.03	1.82±0.04	1.85±0.04
		(1.69-1.83)	(1.75-1.90)	(1.77-1.94)
	Male	1.91±0.04	1.90±0.04	1.88±0.05
		(1.83-1.98)	(1.82-1.99)	(1.78-1.98)
	P	**0.006**	0.170	0.685
$EXT_{CrCd}D$ (mm)*	**Female**	2.75±0.03	2.79±0.04	2.79±0.04
		(2.69-2.81)	(2.71-2.87)	(2.71-2.87)
	Male	2.70±0.03	2.63±0.04	2.80±0.07
		(2.62-2.78)	(2.55-2.72)	(2.65-2.96)
	P	0.268	**0.009**	0.862
$Int_{CrCd}D$ (mm)*	**Female**	1.76±0.03	1.81±0.04	1.82±0.03
		(1.69-1.84)	(1.73-1.88)	(1.75-1.89)
	Male	1.95±0.03	1.87±0.04	1.99±0.05
		(1.88-2.01)	(1.78-1.96)	(1.89-2.10)
	P	**0.001**	0.239	**0.007**
L (mm)*	**Female**	52.39±0.33	52.38±0.23	52.69±1.92
		(51.69-53.08)	(51.90-52.85)	(52.03-53.34)
	Male	51.52±0.37	51.84±0.31	51.41±0.55
		(50.75-2.29)	(51.19-52.50)	(50.19-52.63)
	P	0.088	0.164	**0.040**
CI_{ML} (%)*	**Female**	36.28±6.53	34.62±7.03	35.58±7.56
		(20.33-50.52)	(18.60-45.95)	(23.24-50.87)
	Male	31.43±5.30	30.49±6.35	35.24±6.95
		(21.15-39.63)	(20.00-42.55)	(20.71-45.43)
	P	**0.015**	0.066	0.895
CI_{CrCd} (%)*	**Female**	35.66±7.08	35.06±5.95	34.65±6.43
		(20.88-47.02)	(24.15-47.23)	(22.14-46.18)
	Male	27.58±5.09	28.86±5.32	28.52±8.33
		(18.18-35.46)	(22.22-43.46)	(10.50-42.15)
	P	**0.000**	**0.002**	**0.017**

*Abbreviations in the table are as explained in Table 2.

Table 5. Biomechanic values between groups.

	C (n:40)	Zn (n:39)	NZn (n:39)	P
F (N)*	49.87±1.68 (46.48-53.26)	48.00±1.53 (44.91-51.10)	50.30±1.78 (46.69-53.91)	0.587
I (mm⁴)*	2.20±0.09 (2.03-2.38)	2.21±0.11 (1.99-2.44)	2.54±0.13 (2.27-2.82)	0.055
S (N/mm)*	73.93±2.24 (69.40±78.47)	73.35±2.12 (69.05-77.65)	78.63±2.49 (73.59-83.66)	0.209
σ (MPa)*	158.94±5.14 (148.55-169.33)	153.25±4.44 (144.27-162.23)	144.78±4.64 (135.40-154.17)	0.110
E (MPa)*	5769.74±167.71 (5429.526107.96)	5799±.93±191.02 (5413.22-6186.64)	5463.64±200.02 (5058.73-5868.56)	0.376

*Abbreviations in the table are as explained in Table 2.

marginally higher in the C and NZn groups than the Zn group; however, these differences were not statistically significant ($p = 0.587$). The moment of inertia (I) tended to be higher in the NZn group (2.54 ± 0.13 mm⁴) compared to the Zn (2.21 ± 0.11 mm⁴) and Zn groups (2.20 ± 0.09 mm⁴), approaching significance ($p = 0.055$). The ultimate strength was highest in the C group (158.94 ± 5.14 N) compared to the NZn (144.78 ± 4.64 N) and Zn (153.25 ± 4.44 N) groups, though these differences were not statistically significant ($p = 0.110$). Similarly, stiffness and elastic modulus values did not show significant differences between the groups ($p = 0.209$ and $p = 0.376$, respectively).

Sex-based differences were most evident in the C and Zn groups (Table 6). Female quails in the Zn group exhibited significantly higher breaking force ($p = 0.001$), moment of inertia ($p = 0.003$) and stiffness ($p = 0.002$) compared to males. A similar trend was observed in the C group, where females displayed higher breaking force ($p = 0.001$), moment of inertia ($p = 0.038$) and stiffness ($p = 0.020$) than males. In contrast, in the NZn group, sex-related differences were less pronounced, and no significant variations were detected in most parameters ($p > 0.05$).

Figure 2 presents correlation matrices constructed using R statistical software, depicting the relationships between variables for females (A) and males (B). The correlation coefficients, ranging from -1 to +1, are visualized through circles where the magnitude and direction of correlations are indicated by the size and color intensity of the circles. Analysis revealed predominantly weak to moderate correlations among variables in both groups.

Discussion

Quails are not only raised for egg or meat production but are also used as experimental animals and valuable birds for research purposes. The use of Japanese quails in biomedical research is increasingly widespread (Minvielle, 2004) and is widely used in biological and genetic studies. Bones contain significant amounts of zinc necessary for normal bone development. Increased dietary zinc levels have been reported to improve bone strength (Bahtiyarca et al., 2007). Mechanical tests and bone geometry are used to reveal the external and internal strength of bone tissue. External properties include strength, deformation values and geometric properties, while elastic modulus and strength values are used to evaluate internal properties. In particular, elastic modulus and strength data (internal properties) are evaluated when drug applications affecting bone tissue are applied (Turner and Burr, 1993). Since dietary zinc supplementation affects bone strength (Bahtiyarca et al., 2007), it is necessary to compare the internal properties of bone tissue to investigate the effect of nanozinc in the study.

In bone biomechanics, structural properties of bones contribute significantly to bone strength. Changes in cortical bone, such as density and porosity, directly affect the mechanical assessment of bone (Iolascon et al., 2013; Kralich and Zemel, 2020). Parameters such as F (fracture force) and I (moment of inertia) are directly related to cortical thickness. The geometrical properties of bones, especially the area moment of inertia, determine their behavior under mechanical loading. Cortical components play an important role in bone strength, and the moment of inertia values characterize the resistance of bones to deformation (Muszyński et al., 2018). In our study, cortical index assessments revealed that higher cortical thickness observed in female individuals leads to higher values of moment of inertia (I) and fracture force (F). The stiffness (S) parameter reflects the total deformation characteristic of the bone under load. Due to the positive correlation between the cortical index and the F and S parameters, S values were found to be significantly higher in female individuals. Moment of inertia (I) characterizes the resistance of the bone to deformation under mechanical loading. The high cortical thickness observed in female individuals under the applied load in the craniocaudal (CrCd) direction also causes an increase in the resistance values in this direction. The microstructural properties of the bone tissue affect the elastic modulus and strength parameters. The thickness and structure of the cortical component significantly affect these parameters (Muszyński et al., 2018). In addition, the increase in resistance (I) leads to a decrease in the elasticity property per unit area (mm²) of the bone. In our study, bone geometry and mechanical properties showed significant differences between female and male individuals. Cortical thickness and

Table 6. Biomechanic values in the female and male.

	F (N)*		P	I (mm⁴)*		P	S (N/mm)*		P	σ (MPa)*		P	E (MPa)*		P
	Female	Male		Female	Male		Female	Male		Female	Male		Female	Male	
C (n:40)	54.53±2.27	44.17±1.75		2.36±0.12	2.00±0.12		78.95±3.31	67.80±2.24		163.64±7.56	153.19±6.68		5728.49±245.61	5817.93±228.15	
	(49.80-59.25)	(40.48-47.86)	**0.001**	(2.12-2.60)	(1.76-2.25)	**0.038**	(72.06-85.84)	(63.06-72.53)	**0.020**	(147.91-179.36)	(139.09-167.29)	0.318	(5217.73-6239.26)	(5336.58-6299.28)	0.545
Zn (n:39)	52.35±1.77	42.37±1.98		2.50±0.16	1.85±0.10		78.80±2.64	66.30±2.68		153.11±6.70	153.44±5.57		5537.62±260.98	6139.39±265.25	
	(48.68-56.03)	(38.17-46.56)	**0.001**	(2.16-2.83)	(1.63-2.07)	**0.003**	(73.30-84.30)	(60.61-71.99)	**0.002**	(139.18-167.03)	(141.63-165.25)	0.971	(4994.87-6080.36)	(5577.08-6701.71)	0.120
Nzn (n:39)	52.46±2.08	45.46±3.12		2.56±0.14	2.51±0.31		81.84±3.06	71.39±3.60		147.48±4.90	138.72±10.43		5513.20±172.75	5352.15±537.43	
	(48.19-56.72)	(38.59-52.32)	0.069	(2.27-2.85)	(1.83-3.19)	0.875	(75.56-88.13)	(63.47-79.31)	0.051	(137.41-157.56)	(115.75-161.68)	0.390	(5157.10-5868.29)	(4169.27-6535.02)	0.715

*Abbreviations in the table are as explained in Table 2.

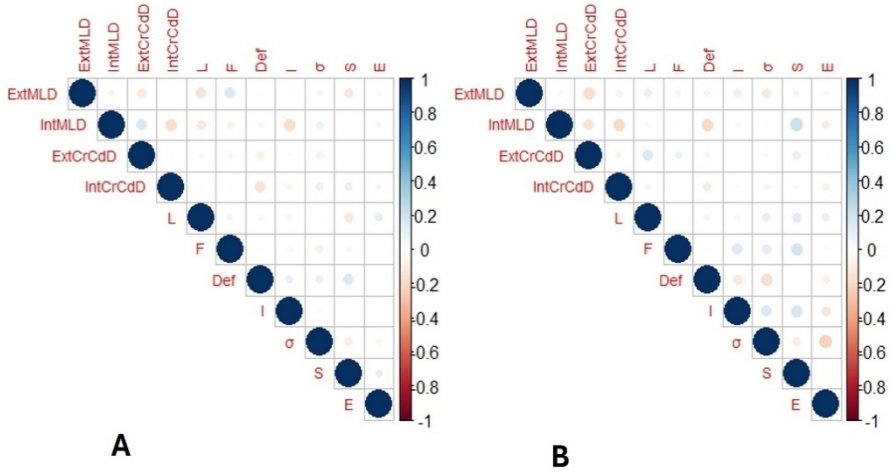

Figure 2. A Comparative Analysis of Correlation Patterns the Male Groups (A), Female Groups (B). Red Colours: negative correlation, Blue Colours: positive correlation. Abbreviations in Figure are as explained in Table 2.

moment of inertia values varied depending on gender, affecting the mechanical properties of the bone (Ciosek et al., 2021). Therefore, although no statistically significant difference was observed between the sexes, it was found that the elasticity parameters showed lower values in female individuals with high resistance values.

In addition, poultry has three types of bone tissue: compact, spongy and medulla bone. The Medulla bone meets the additional calcium (Ca) requirement for eggshell formation. The primary purpose of this bone type is to prevent skeletal defects that may occur due to decreased calcium supply during growth in laying hens (Korver et al., 2004). Since the materials used in our study were 35 days old and had not yet started laying eggs, it is thought that the amount of calcium required for eggshell formation was not used from the bone tissue. This situation led to higher values in female animals.

Although no statistically significant difference was observed between the groups, it was determined that F, I and S parameters showed higher values in the NZn group with high cortical thickness, like gender dimorphism. On the other hand, it was found that the elastic modulus and strength parameters reflecting the mechanical properties of the bone at the tissue level showed lower values in this group.

In conclusion, while nano zinc oxide supplementation appears to enhance periosteal growth, its limited impact on biomechanical properties underscores the complexity of zinc's role in bone development. The contrast between morphometric and biomechanical outcomes highlights the influence of factors such as zinc form, species, and experimental design. Future studies should investigate the long-term effects of nano zinc, histological changes in bone microstructure, and sex-specific metabolic pathways to elucidate its mechanisms. Additionally, comparative studies across poultry species could clarify whether the observed effects are unique to quails or broadly applicable.

Although the effects of nano zinc oxide supplementation on bone development were examined in detail in this study, there are some limitations. First, the lack of histological analyses limited the understanding of microstructural changes in bone

tissue. Finally, since the results were evaluated only on Japanese quails, the generalizability of the findings to other species is limited. Addressing these shortcomings may provide an important roadmap for future studies.

Conclusion

Nano zinc oxide (NZn) supplementation increased the outer mediolateral diameter (ExtMLD) of the tibiotarsus bone in Japanese quails and promoted periosteal development, but did not show a significant effect on biomechanical resistance (fracture strength, elastic modulus). The high fracture resistance observed in female individuals in the control and Zn groups may be attributed to the interactions between sex hormones and mineral metabolism. Further studies are required to elucidate the bone remodeling mechanisms and bioavailability of nanoparticles. These findings emphasize the morphometric adaptation potential of nanomineral use in poultry feeding but reveal that the biomechanical effect is limited.

Ethical Permission

This study was carried out with the permission of Aydın Adnan Menderes University, Animal Experiments Local Ethics Committee, number 64583101/2023/46.

Conflict Of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Founding Information

This research received no external funding.

Author Contributions

Conceptualization; investigation; writing – original draft; formal analysis. FTY

Project administration; writing – review and editing: İGY

Supervision; methodology; validation; visualization; software; data curation: FSK

Sımılarıty Rate

We declare that the similarity rate of the article is 12% as stated in the report uploaded to the system.

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Determination of Heavy Metals in the Liver of Dairy Cows and the Risk to their Health

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Abstract: Heavy metals are characterized by their high atomic mass and toxicity to living organisms. This study aimed to investigate the presence of heavy metals in the livers of slaughtered dairy cows and to discuss their possible effects on animal health. In the study, 50 Holstein dairy cows were used. After slaughtering, samples of 4 X 10 g were taken from the liver of the animals for heavy metal analysis. Samples prepared according to the wet burning method were analyzed for the presence of arsenic (As), aluminum (Al), lead (Pb), mercury (Hg), and nickel (Ni) using the ICP-OES device. In clinical examination, rumen acidosis and ovarian diseases were detected as the most common diseases in 20 and 13 animals, respectively. None of the samples contained Ni. On average, Al was detected at 4.60±8.71 ppm, As at 0.39±0.11 ppm, Hg at 0.41±0.29 ppm and Pb at 0.04±0.13 ppm. Based on the total number of animals, the following prevalence was calculated: 72% for Al, 100% for As, 88% for Hg, 0% for Ni, and 10% for Pb. The study showed that the average As content was in a toxic range. In 28% of the samples, the Al value was categorized as toxic to animal health. The average Hg values, on the other hand, were above the acceptable limits for human health. In summary, the prevalence of toxic heavy metals Al, As and Hg in Holstein livers was quite high.

Keywords: *Aluminium, Arsenic, Heavy metal, Lead, Liver, Mercury.*

Süt İneklerinin Karaciğerlerinde Ağır Metal Varlığı ve Sağlık Riski

Özet: Ağır metaller, yüksek atom ağırlıkları ve canlılar üzerine olan toksisiteleri ile bilinirler. Bu çalışma, kesilen süt sığırlarının karaciğerlerinde ağır metallerin varlığını ve bunların hayvan sağlığına olası zararlarını araştırmayı amaçlamıştır. Bu çalışmada 50 Holstein süt ineği kullanılmıştır. Ağır metal analizi için, kesimden sonra hayvanların karaciğerinden 4x10 g örnekler alındı. Yaş yakma yöntemine göre hazırlanan örnekler, ICP-OES cihazı kullanılarak arsenik (As), alüminyum (Al), kurşun (Pb), civa (Hg) ve nikel (Ni) varlığı açısından incelendi. Klinik muayenede sırasıyla 20 ve 13 hayvanda, rumen asidozu ve ovaryum hastalıkları en yaygın hastalıklar olarak tespit edildi. Karaciğer numunelerinin hiçbirinde Ni bulunmadı. Sırasıyla, Al 4.60±8.71 ppm, As 0.39±0.11 ppm, Hg 0.41±0.29 ppm ve Pb 0.04±0.13 ppm olarak tespit edildi. Yaygınlık yüzdeleri toplam hayvan sayısına göre hesaplandı: Al %72, As %100, Hg %88, Ni %0 ve Pb %10. Çalışmada ortalama As değerinin toksik aralıkta olduğu görülmüştür. Al numunelerin %28'i hayvan sağlığı açısından kabul edilebilir limitlerin üzerinde tespit edilmiştir. Hg ortalama değerleri ise insan sağlığı için kabul edilebilir limitlerin üzerindedir. Özet olarak, toksik ağır metaller Al, As ve Hg Holstein karaciğerlerindeki prevalansı oldukça yüksek bulunmuştur.

Anahtar Kelimeler: *Ağır metal, Alüminyum, Arsenik, Civa, Karaciğer, Kurşun.*

Introduction

Nowadays, all living organisms and their environment are constantly exposed to environmental pollutants. Industrial activities are increasing in parallel with the growing population, and air, soil, and water pollution are threatening life on Earth (Bilge and Cimrin, 2013; Kodrik et al., 2011). Heavy metals are dangerous agents that cause significant health problems in animals due to their toxicity. Many industrial areas, fertilizers, traffic, cars, chemicals, groundwater, and animal feeds are substantial sources of heavy metal contamination (Anjulo and Mersso, 2015). The organism absorbs heavy metals through the mouth, respiration, and skin, and most of them cannot be excreted through the body's excretory pathways (kidney, liver, intestine, lung, skin) without special assistance. Therefore, a large portion of heavy metals accumulates in biological organisms. As a result of accumulation, these metals, which concentrate on living organisms, can cause serious diseases (such as thyroid, neurological, autism, and infertility) and even death when they reach effective doses (Özbolat and Tuli, 2016). The liver is a vital storage organ for trace elements and heavy metals. Therefore, the concentrations of these elements in the liver reflect the liver's exposure to these substances (Suttle, 2010). While heavy metal toxicities such as arsenic (As), cadmium (Cd), and lead (Pb) are well known, others like zinc (Zn), copper (Cu), cobalt (Co), manganese (Mn), iron (Fe), magnesium (Mg), and selenium (Se) are necessary in trace amounts for basic physiological functions (Dutta et al., 2022; Pandey et al., 2014; Samy et al., 2022; Sevostyanova et al., 2020). Pb, Cd, As, and mercury (Hg) elements have no defined biological functions and are considered undesirable and potentially toxic contaminants in animal feed (Anjulo and Mersso, 2015; Underwood and Mertz, 1987), and due to their high toxicity, they pose a threat to public health even at very low exposure levels (Tchounwou et al., 2012). The excessive levels of these elements in cattle are generally caused by anthropogenic emissions and food contamination (Alonso, 2012). Pb and Cd negatively affect many biochemical and physiological processes when exposed to sub-lethal doses (Elarabany and El-Batrawy, 2019; Wang et al., 2022). Long-term exposure to heavy metals (Cd, Ni, As, Pb) can affect central nervous system functions and damage the kidneys, lungs, and liver. Among all metals, Pb, As, and Cd have more adverse effects on animal and human health (Patwa et al., 2022; Penticoff and Fortin, 2023). The absence of a reference Veterinary Poison Control Center reporting animal poisoning cases in Türkiye and the European Union makes it difficult to obtain information about clinical poisoning cases (Guitart et al., 2010).

Products used as animal feed may contain undesirable substances that may jeopardize animal health or pose a risk to human health or the environment due to their presence in animal products. It is impossible to avoid undesirable substances altogether, but reducing their quantity in animal feed products is essential. This way, unwanted and harmful effects such as acute toxicity, bioaccumulation, and biodegradability can be prevented. Suppose the products used as animal feed are reliable, free from adulteration, of marketable quality, and used correctly. In that case, they do not pose a risk to human, animal, or environmental health and do not harm livestock production. The presence of certain undesirable substances in complementary feedingstuffs should be limited by setting appropriate maximum levels (EUP Council Directive, 2002).

While there are many scientific studies on the presence of heavy metals in Türkiye, there are only a limited number of studies on the presence of heavy metals in the liver tissue of dairy cows. In the present study, we aimed to investigate the accumulation of heavy metals in the liver of slaughtered dairy Holsteins from the Muğla region and to discuss the potential effects of this accumulation on the health of dairy cows.

Materials and Methods

Ethical Consideration: The present study was approved by the Animal Experiments Local Ethics Committee of Muğla Sıtkı Koçman University (MUDEM-HADYEK) with the ethical approval number of 23.09.2021, 33/21.

Animal material: 50 Holstein dairy cows aged 3 to 13 years from 24 different villages in the province of Muğla/Türkiye were used. The study material was randomly selected from dairy Holsteins brought to the slaughterhouse from Milas, Menteşe, and Yatağan provinces of Muğla city according to the slaughter order.

Clinical examination: Animal materials were Holstein dairy cows brought to the slaughterhouse due to losing their breeding characteristics because of various diseases. All animals were subjected to detailed clinical examinations before slaughter. As a result of the clinical examination, liver samples were taken from 50 animals that had completed their productive lifespan after slaughter.

Liver material and processing: Immediately after the dairy cows were slaughtered, four (4x10 g) 10-gram liver tissue samples were taken from the *lobus caudatus* of the liver (Alonso et al., 2004) and placed in sealed bags. On each sample bag, the ear number of the animal, the name of the farm, its breed, and its age were written. These samples were transported in +4 °C containers to the laboratory. Liver samples not analyzed on the same day were frozen at -20 °C until analysis time. Liver tissue samples collected in sterile petri dishes were dried for 24 hours at 100 °C. Approximately 0.5 g of samples were examined in the ICP OES device after burning 6 ml of HNO_3 (nitric acid) + 2 ml of H_2O_2 (hydrogen peroxide) in the microwave.

Detection of heavy metal presence in samples: Elemental analysis was performed using an ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometer) device. The ICP-OES technique determines the elements in an aqueous solution based on the various wavelengths formed according to the optical properties of the light passed through the plasma. Samples were analyzed for the presence of As, Al, Hg, Ni, and Pb.

Statistical method: Using SPSS 13.0, one-way analysis of variance (ANOVA) was used to evaluate the amount of heavy metals, and the Tukey test was used to determine the source of differences. A value of $p<0.05$ will be considered statistically significant.

Results

Clinical examination: Rumen acidosis and ovarian diseases were identified as the most frequently detected, with 20 and 13 animals, respectively. Other diseases identified during the clinical examination were traumatic reticuloperitonitis, abomasum diseases, omasum constipation, mastitis, and old age, respectively. Clinical examination results are similar to the official health reports. The clinical examination showed no signs of heavy metal toxicity in the animals.

Heavy metals: The study analyzed the presence of 5 different heavy metals in liver samples from 50 Holstein dairy cows. The total and average values and the standard deviation of all heavy metals were presented in Table 1. None of the samples contained Ni. The presence of Pb was only detected in 5 samples, while Hg was found in 44 samples and Al in 36 samples. As was detected in all samples. Concerning the total number of animals, the prevalence was determined as follows: 72% for Al, 100% for As, 88% for Hg, 0% for Ni, and 10% for Pb (Table 2). All heavy metals,

Table 1. Mean and standard deviation and min-max value of heavy metals (dry weight) in the liver of 50 Holsteins.

Element	Mean±SD (ppm)	Min-Max (ppm)
Al	4.60±8.71^{a}	0.30-59.52
As	0.39±0.11^{b}	0.21-0.65
Hg	0.41±0.29^{c}	0.01-1.03
Ni	0±0	0.00-0.00
Pb	0.04±0.13^{d}	0.13-0.68

Al: Aluminium, **As:** Arsenic, **Hg:** Mercury, **Ni:** Nickel, **Pb**: Lead, **SD:** Standart deviation, ppm; in original sample mg/kg, a,b, c, d: $p<0.05$ (different letters indicate significant difference)

Table 2. Prevalence of heavy metals in livers of slaughtered dairy Holsteins.

	Aluminum	Arsenic	Mercury	Nickel	Lead
n total	50	50	50	50	50
positive	36	50	44	0	5
negative	14	0	6	100	45
Overall positive (%)	**72.0**	**100.0**	**88.0**	**0.0**	**10.0**
>0.5 ppm positive (%)	**70.0**	**20.0**	**34.0**	**0.0**	**4.0**
>1.0 ppm positive (%)	**60.0**	**0.0**	**2.0**	**0.0**	**0.0**

including Al, As, and Hg, were detected in 32 samples. The total ppm values of heavy metals were measured as Al 229.86 ppm, As 19.65 ppm, Hg 20.74 ppm, Ni 0.00 ppm, and Pb 1.79 ppm. The average value of aluminum was higher compared to other heavy metals. Al was not detected in 14 liver tissues. Al was detected in varying amounts in 36 samples. In the detected tissues, the lowest value of aluminum was measured at 0.30 ppm, while the highest value was 59.92 ppm. As was detected at different levels in all samples. The lowest As value detected was 0.21 ppm, while the highest was 0.65 ppm. Hg was detected at various levels in 44 of the samples. The lowest level of Hg detected was 0.01 ppm, while the highest level detected was 1.03 ppm. Ni was not detected in any of the samples. Pb was not detected in 45 of the samples. Pb was detected in 5 samples, with the lowest value measured at 0.22 ppm and the highest at 0.68 ppm. Among the heavy metals, Al was detected in the most significant quantity at 229.86 ppm. As was detected in every sample, making it the heavy metal with the highest percentage occurrence. Conversely, Ni was the only heavy metal not found in the analysis, as it was absent in all samples. The average amount of As was categorized as hazardous in the study. The amount of Al in 28% of the samples was classified as harmful. Conversely, the average Hg levels are higher than what is considered safe for human health.

Heavy metal accumulations between the districts (Milas, Yatağan, Menteşe) were compared, and no significant difference was found between the districts ($p>0.05$). Animals were divided into groups 1-6 years and 7 years and older. The heavy metal accumulations in cattle were compared according to their ages, but no significant differences were found between the age groups ($p>0.05$)

Additionally, the amounts of heavy metals detected in liver tissues from various studies conducted in Türkiye and worldwide are presented in Table 3.

Discussion and Conclusion

Health problems arising from poisoning in animals appear to be a rare health issue compared to other clinical diseases, such as infectious diseases, trauma, or neoplasia. One reason may be the lack of information about the most common toxins affecting veterinary species, resulting in very little information to guide and facilitate diagnosis. The European Union (EU) does not have a Veterinary Poison Control Center that centralizes and publishes poisoning information, and even outside the European Union, data is often managed by different institutions, mostly Veterinary Medicine or Science faculties, and is limited in scope. This leads to the inadequate and limited availability of toxicological epidemiological data, with very little or no

accessibility for veterinarians in other regions or countries. There are no annual reported poisoning case records, but there is region-specific case-based information in studies published in peer-reviewed journals (Guitart et al., 2010).

Table 3. The concentration of heavy metals reported by other studies in liver tissues of cattle.

Heavy metal	n	Dry/wet	Mean or median value (ppm)	Country	References
Pb	172	wet	0.1-1.0	Western Canada	(Cowan and Blakley, 2016)
Pb	56	wet	0.0475	Spain	(Alonso et al., 2000)
Pb	1254	wet	0.07 ± 0.05	Canada	(Salisbury et al., 1991)
Pb	3	wet	16.78	Austria	(Krametter-Froetscher et al., 2007)
Pb	10	dry	0.0079 ± 0.0015	Saudi Arabia	(Meligy et al., 2019)
Pb	120	wet	0.028	Spain	(Alonso et al., 2004)
Pb	180	wet	0.103 ± 0.130	Pakistan	(Mushtaq et al., 2024)
Pb	61	wet	0.12	Brasil	(Aranha et al., 1994)
Pb	87	wet	0.059	Germany	(Kreuzer et al., 1988)
Pb	30	wet	0.405	Italy	(Amodio-Cocchieri and Fiore, 1987)
Pb	290	wet	0.160	Poland	(Falandysz, 1993)
Pb	6	wet	0.465	Slovac Republic	(Kottferova and Koréneková, 1995)
Pb	21	wet	1.072	Slovac Republic	(Koréneková et al., 2002)
Pb	68	wet	0.10	Slovenia	(Doganoc, 1996)
Pb	112	wet	0.047	Zambia	(Yabe et al., 2012)
Pb	90	dry	0.059±0.020	Colombia	(Madero and Marrugo-Negrete, 2011)
Pb	50	dry	0.485	Türkiye	(Kocasari et al., 2016)
Pb	698	dry	0.3	Netherlands	(Counotte et al., 2019)
Pb	30	wet	0.05 ± 0.02	Spain	(Rodríguez-Marín et al., 2019)
Pb	30	wet	0.23 ± 0.26	Spain	(Rodríguez-Marín et al., 2019)
As	56	wet	0.0102	Spain	(Alonso et al., 2000)
As	351	wet	0.03 ± 0.01	Canada	(Salisbury et al., 1991)
As	120	wet	ND	Spain	(Alonso et al., 2004)
As	15	dry	0.14±0.03	Türkiye	(Oymak et al., 2017)
As	10	dry	0.0111 ± 0.025	Saudi Arabia	(Meligy et al., 2019)
As	156	dry	3.2-350	in the World	(Bertin et al., 2013)
As	180	wet	0.333 ± 0.200	Pakistan	(Mushtaq et al., 2024)
As	112	wet	0.002	Zambia	(Yabe et al., 2012)
As	571	wet	0.01	Norway	(Kluge-Berge et al., 1992)
As	50	dry	ND	Türkiye	(Kocasari et al., 2016)
Hg	624	wet	0.01 ±0.01	Canada	(Salisbury et al., 1991)
Hg	146	wet	0.003	Netherlands	(Vos et al., 1987)
Hg	112	wet	0.0003	Zambia	(Yabe et al., 2012)
Hg	3	wet	0.873/0.750/ <0.001	Austria	(Krametter-Froetscher et al., 2007)
Hg	120	wet	ND	Spain	(Alonso et al., 2004)
Hg	114	wet	<0.010-0.012	Finland	(Niemi et al., 1981)
Hg	340	wet	0.00765-0.00101	Spain	(Alonso et al., 2003)
Hg	1036	wet	0.002 ± 0.0022	Poland	(Nawrocka et al., 2020)
Hg	1096	wet	0.008	Poland	(Szprengier-Juszkiewicz, 1994)
Hg	33	wet	0.047	Sweden	(Jorhem et al., 1991)
Hg	470	wet	0.006	Poland	(Zmudzki et al., 1991)
Hg	72	wet	0.002	Iran	(Hashemi, 2018)
Hg	6	wet	0.0243	Czech Republic	(Čelechovská, 2008)
Hg	6	wet	0.0033	Czech Republic	(Čelechovská, 2008)
Hg	180	wet	0.425 ± 1.110	Pakistan	(Mushtaq et al., 2024)
Hg	90	dry	0.028±0.025	Colombia	(Madero and Marrugo-Negrete, 2011)
Al	15	dry	2.44±1.06	Türkiye	(Oymak et al., 2017)
Al	30	wet	55.3 ± 58.0	Spain	(Rodríguez-Marín et al., 2019)
Al	30	wet	8.65 ± 4.38	Spain	(Rodríguez-Marín et al., 2019)
Ni	112	wet	0.594	Zambia	(Yabe et al., 2012)
Ni	21	wet	0.231	Slovac Republic	(Koréneková et al., 2002)
Ni	694	dry	0.3	Netherlands	(Counotte et al., 2019)
Ni	120	wet	ND	Spain	(Alonso et al., 2004)

ND: not detected, **Pb:** lead, **As:** arsenic, **Hg:** mercury, **Al:** aluminum, **Ni:** nickel: **n:** animals number

The acute lethal oral dose for Pb is 200 to 400 mg/kg body weight (bw) for calves and 600 to 800 mg/kg bw for adult cattle (Payne and Livesey, 2010). The toxic dose of Pb for the liver is reported to be 33.5 mg/kg (Counotte et al., 2019; Cowan and Blakley, 2016). Radostits et al. (2002) reported that the toxic dose of Pb in the liver of ruminants is

between 10 and 20 ppm, and the toxic dose in the kidneys is 20 ppm. In another study, an acute toxicity value of over 10 ppm for Pb in the kidneys and liver was reported (Baker, 1987). Amodio-Cocchieri and Fiore (1987) and Cowan and Blakley (2016) reported the normal values in liver tissue as 0.405±0.365 and 0.1-1.0 ppm, respectively.

In the current study, the highest level detected in the liver was 0.68 ppm, which is not toxic for a cow. In our study, Pb was detected in the liver of only four animals, which are below harmful levels.

Barceloux (1999) reported very little evidence that Ni compounds accumulate in the food chain and that Ni is not a cumulative toxin in animals or humans. In line with this information, the absence of Ni in any of our samples supports Barceloux's views.

In a study conducted by Counotte et al. (2019) in the Netherlands between 2007 and 2018, 1544 cattle livers were examined and toxic levels of Pb were only observed in the youngest group of cattle. Cowan and Blakley (2016) categorise the age groups in 525 cases of acute Pb poisoning that occurred in northern Canada between 1998 and 2013, and no significant difference is found. In their study, Şimşek and Dinçel (2023) revealed that the concentrations of certain heavy metals change with age and that heavy metal accumulation is higher in adult cattle. The current study did not determine the relationship between heavy metal accumulation and age. In our study, no significant difference was found between age groups ($p>0.05$).

In acute toxicosis, Hg residues in the kidney exceed 10–15 ppm (Buck, 1975), while in the liver, they exceed 100 ppm (Hapke, 1988). In our study, the values detected as an average of 0.41 ppm and a maximum of 1.03 ppm are below the toxic dose for cattle. However, it has been detected above the tolerable level for human health, 0.1 ppm, as determined by the Austrian Ministry of Health. At the same time, in the Netherlands, the maximum acceptable concentration for human health in bovine liver tissue is 0.05 ppm (Vos et al., 1987). In line with these values, the values we obtained are seen to be above the acceptable levels for human health. In the study conducted by Alonso et al. (2003) in Spain, Hg was detected in the liver of 19.6% of 56 cattle. In contrast, in our study, different amounts of Hg were detected in 88% of them. This result is not consistent with our study. In another study conducted in Poland, various amounts of Hg were detected in 69% of 1036 cattle (Nawrocka et al., 2020). The results of this study appear to be more consistent with our study. In a study conducted on the liver of beef cattle in Colombia, the values of Hg and Pb were determined to be 0.028±0.025 and 0.059±0.020 ppm, respectively. The results were below the detected values for human and animal health (Madero and Marrugo-Negrete, 2011). The Pb value is consistent with the average value in our study, while the Hg value was much lower than the value we detected. In a study conducted on 180 cattle livers in Pakistan, levels of 0.333±0.200 ppm As, 0.103±0.130 ppm Pb, and 0.425±1.110 ppm Hg were detected, respectively (Mushtaq et al., 2024). As and Hg values are close to those in our study, while the Pb value is observed to be at a higher level than our study. In another study conducted on 50 cattle livers in Türkiye, As was detected in only one of the samples, and Hg was not detected in any of the samples. Pb was detected in 2 samples (Kocasari et al., 2016) in the current study, As was detected in all samples, Hg in 44 samples, and Pb in 5 samples.

Reference ranges for As, obtained from the Diagnostic Center for Population and Animal Health at Michigan State University, are 0.05–0.17 ppm in urine, <50 ppb in blood, 0.004–0.40 ppm in liver, and 0.018–0.40 ppm in kidney (Bertin et al., 2013). Animals can tolerate low levels of As; the normal level in cattle tissues is approximately 0.5 ppm. When As level in the liver exceeds 10 to 15 ppm and is accompanied by clinical symptoms, acute As poisoning is diagnostically considered (Bahri and Romdane, 1991; Fletcher, 1986; Monies, 1999). In humans, the toxic dose is 10-50 mg, and the lethal dose (LD50: lethal dose) is 60-200 mg (Aliyev, 2011; Dousova et al., 2003; Or, 1996). The As levels detected in the current study are not toxic to either animal or human health. According to Chopra et al. (2017), cattle are more vulnerable to As poisoning than other species. Bertin et al. (2013) compiled cases of As poisoning published in peer-reviewed journals between 1941 and 2012 in a study and identified 156 cases. This result indicates that As poisoning is not very common or that the studies are insufficient. A study conducted on 10 cattle livers in Saudi Arabia detected 0.0079 ppm Pb and 0.0111 ppm As levels (Meligy et al., 2019). According to our study, the detected values are pretty low. In a study conducted in Türkiye, As was detected in only 1 out of 50 cattle livers, while none of the samples contained Hg (Kocasari et al., 2016). In the current study, As was detected in all 50 samples, while Hg was detected in 44 samples

In cattle and sheep, aluminum concentrations of 6-11 ppm in the liver and 4-5 ppm in the kidney are considered toxic. A levels ≥1.2 ppm in dog liver are considered high (Gupta, 2012). In the current study, the average was 4.60 ppm, below the toxic level. In comparison, 14 animals had liver Al levels detected at six ppm and above, indicating they were within the toxic dose range. A very high level of 59.92 ppm was detected in only one animal. In a study conducted on 15 cattle in Türkiye in 2017, the average Al was determined to be 2.44±1.06 ppm, and the average As was determined to be 0.14±0.03 ppm (Oymak et al., 2017). The average values of Al and As were lower in our study.

In conclusion, the prevalence of the toxic heavy metals Al, As and Hg in the liver of Holsteins was high. Al and Hg levels in the liver can be categorised as hazardous to animal and human health even at trace levels. Toxic heavy metals, even at minimal levels, pose significant potential dangers to animal and human health. Animal feeds, waters, soil, and industrial areas can be serious sources of contamination for heavy metals. In this regard, it is very important to regularly identify the damage caused by relevant environmental contaminant risks to the health of animals and humans.

Conflict of Interest

Authors declare no conflicts of interest.

Ethical Approval

This study protocol was approved by the Ethics Committee of Muğla Sıtkı Koçman University with approval number (MUDEM-HADYEK), 23.09.2021, 33/21.

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Diagnostic Efficacy of Certain Physical Examination and Serum Biochemistry Parameters in Dogs with Tick Paralysis

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Abstract: Recognizing tick paralysis is crucial as it can be fatal, but no specific diagnostic test exists. Therefore, this study aimed to evaluate the diagnostic effectiveness of routine clinical examinations and selected laboratory analytes and to understand the impact of tick burden on the severity of symptoms in tick-paralyzed dogs. Fourteen dogs, whose diagnosis of tick paralysis was confirmed by the *ex juvantibus* method, were divided into Low Tick (1-4 ticks, n=7) and High Tick (5-60 ticks, n=7) subgroups based on the number of ticks. The healthy group consisted of dogs with no disease history (n=7). Physical examinations and certain serum biochemistry profile analyses of all the dogs were evaluated comparatively. The diagnostic efficiency of all the parameters was investigated by ROC-based diagnostic performance analysis. The respiratory rate of the dogs with tick paralysis was higher than that of the healthy dogs ($p<0.0001$). The body temperature and heart rate of the High Tick group dogs were higher than those in the other groups ($p<0.0001$). Capillary refill time (CRT) was shorter in the High Tick group than in the other groups ($p=0.018$). Total protein, blood urea nitrogen (BUN), and total bilirubin levels were higher in the High Tick group than in the other groups ($p=0.035$). It was concluded that as the number of ticks increases, the diagnostic performance of the investigated analytes improves potently due to the rate and volume of toxin secretion by the ticks. However, they should be evaluated together with other parameters due to their low specificity.

Keywords: *Acute flaccid paralysis, Diagnosis, Dog, Serum biochemistry, Tick paralysis.*

Kene Felci Olan Köpeklerde Bazı Fiziksel Muayene ve Serum Biyokimyası Parametrelerinin Tanısal Etkinliği

Özet: Kene felcini tanımak, ölümcül olabileceğinden çok önemlidir, ancak spesifik bir tanı testi bulunmamaktadır. Bu nedenle, bu çalışmada rutin klinik muayenelerin ve seçilmiş laboratuvar analizlerinin tanısal etkinliğinin değerlendirilmesi ve kene felci olan köpeklerde kene yükünün semptomların şiddeti üzerindeki etkisinin anlaşılması amaçlanmıştır. Kene felci tanısı *ex juvantibus* yöntemi ile doğrulanan 14 köpek, kene sayısına göre Düşük Kene (1-4 kene, n=7) ve Yüksek Kene (5-60 kene, n=7) alt gruplarına ayrıldı. Sağlıklı grup, hastalık geçmişi olmayan köpeklerden oluşturuldu (n=7). Tüm köpeklerin fiziksel muayeneleri ve bazı seçilmiş serum biyokimya profili analizleri karşılaştırmalı olarak değerlendirildi. Tüm parametrelerin tanısal etkinliği, ROC-tabanlı tanı performans analizi ile araştırıldı. Kene felci olan köpeklerin solunum hızı, sağlıklı köpeklere göre daha yüksekti ($p<0.0001$). Yüksek Kene grubundaki köpeklerin vücut sıcaklığı ve nabzı diğer gruplardaki köpeklere göre daha yüksekti ($p<0.0001$). Kapillar tekrar dolum zamanı (CRT) Yüksek Kene grubunda diğer gruplara göre daha kısaydı ($p=0.018$). Total protein, kan üre azotu (BUN) ve total bilirubin düzeyleri Yüksek Kene grubunda diğer gruplara göre daha yüksekti ($p=0.035$). Kene sayısı arttıkça, kenelerin toksin salgılama hızı ve hacmi nedeniyle araştırılan analitlerin tanı performansının güçlü bir şekilde arttığı kanısına varıldı. Fakat, özgüllüklerinin düşük olması nedeniyle bu parametrelerin diğer bulgularla birlikte değerlendirilmesi gerekmektedir.

Anahtar Kelimeler: Akut yumuşak paraliz, Kene paralizi, Köpek, Serum biyokimyası, Tanı.

Introduction

The clinical manifestation of acute flaccid paralysis (AFP) is a sudden onset of weakness, sometimes affecting swallowing and respiratory muscles. The lack of spasticity or other symptoms of abnormal motor tracts of the central nervous system, such as clonus, hyperreflexia, or extensor plantar reflexes, is referred to as flaccid (Bowley and Chad, 2019; Marx et al., 2000). Etiologies contributing to AFP encompass spinal cord injuries (infectious, inflammatory, compressive), neuromuscular junction disorders (myasthenia gravis, botulism, Lambert-Eaton Myasthenic Syndrome), muscle-related issues (muscular dystrophies, necrotizing myopathies, hypokalemia or severe hyperkalemia), and root or peripheral nerve disorders (Guillain-Barré Syndrome, polyneuropathy, tick paralysis) (Fadia et al., 2019). Tick paralysis, among the causes of AFP, is a rare neurological syndrome that is non-infectious and characterized by acute ataxia. Its clinical presentation often resembles Guillain-Barré syndrome, botulism, and acute idiopathic polyneuropathies, thereby complicating the diagnostic process (Malik and Farrow, 1991; Simon et al., 2023).

Tick paralysis develops due to neurotoxins secreted by the salivary glands of certain tick species. Rhipicephalus sanguineus, one of the most common tick species in dogs, is recognized as a principal vector associated with this syndrome (Padula, 2016; Ceylan et al., 2019; Gülersoy and Günal, 2022). In Turkey, due to geographic and climatic diversity, various tick species are encountered throughout the year, and tick infestations are widespread (Aydın and Bakırcı, 2007; İça and Çetin, 2016). Although tick paralysis is most commonly attributed to a single engorged tick, cases associated with multiple ticks have also been reported (Padula et al., 2020). The severity of the disease may correlate with the number of attached ticks and the total amount of neurotoxin released. Currently, there is no specific diagnostic test for tick paralysis. Therefore, diagnosis primarily relies on the identification of engorged ticks through physical examination and the exclusion of other similar clinical presentations, followed by an ex juvantibus approach. However, in cases where ticks are few or not visible, this diagnostic process becomes more challenging. Hence, investigating the diagnostic utility of routine clinical examination findings and selected serum biochemical parameters is essential for early detection and timely intervention (Atwell et al., 2001; Gülersoy et al., 2023). This study aimed to evaluate the relationship between tick burden and the severity of clinical symptoms in dogs diagnosed with tick paralysis-induced AFP and to assess the diagnostic performance of physical examination findings and selected serum biochemistry analytes in this context.

Materials and Methods

This study was approved by the Harran University Animal Experiments Local Ethics Committee on 09.05.2022 with session 2022/003 and decision number 01-06.

Animals: This study included 21 dogs, which will later be divided into Healthy, Low Tick and, High Tick groups, admitted to the Animal Hospital of the Faculty of Veterinary Medicine, Harran University. The affected dogs in the study presented neurological findings strongly indicative of AFP caused by tick paralysis. These findings included the sudden onset of weakness, difficulty in movement, hind limb incoordination, quadriplegia, and attached and engorged ticks. The control (Healthy) group comprised clinically healthy dogs with no known history of disease and were admitted to the animal hospital for vaccination and/or check-up purposes.

Physical Examinations

Within the scope of the physical examination, rectal body temperature, heart and respiratory rate, and gingival capillary refill time (CRT) were evaluated. In addition, a visual assessment of the neurological manifestations of the affected dogs was performed. Moreover, the body weight and body surface area (BSA) of each dog were calculated. By thumb counting anatomical body locations such as the head, neck, ears, thorax, abdomen, interdigital areas, fore and hind limbs, tail, axillary and, inguinal area, tick-paralyzed dogs were assessed for the presence of ticks. To minimize potential confounding effects on serum biochemical parameters, all dogs included in the study underwent a complete blood count (CBC) and microscopic examination of blood smears. Both buffy coat and peripheral blood smears were evaluated for the presence of *Anaplasma platys*, *Ehrlichia canis*, *Babesia* spp., and *Hepatozoon canis* inclusions. Smears were examined using a light microscope under 100× oil immersion magnification. As a result of clinical examinations, dogs with any blood parasites and findings such as thrombocytopenia and pancytopenia, which have been commonly reported in dogs previously infected with R. *sanguineus* (Otranto et al., 2012), were not included in the study.

Inclusion/Exclusion Criteria

Inclusion criteria for the study required that dogs had no prior history of illness, had not received any antiparasitic treatment within the preceding month, presented with an engorged tick at the time of examination, and exhibited clinical signs consistent with AFP. The present study classifies the following clinical findings as suggestive of AFP: an inability to contract due to motor pathway impairment extending from the cerebral cortex to muscle fibers; the absence of spasticity or other signs of disordered central nervous system motor tracts, such as hyperreflexia, clonus, or extensor plantar reflexes; and the sudden development and worsening of weakness within a few days, particularly affecting respiratory muscles and swallowing ability (Marx et al., 2000). The observed clinical presentation in the affected dogs prompts consideration of various potential causes of diffuse lower motor neuron diseases, including snake envenomation, botulism and, acute idiopathic polyneuropathy, among others (Malik and Farrow, 1991). In brief, botulism in dogs typically arises concurrently with the ingestion of spoiled food or carrion, a scenario not applicable to the dogs under consideration, as they were exclusively fed commercial food. Clinically, botulism manifests as challenges

in food prehension and swallowing, accompanied by hypersalivation and regurgitation (Shelton, 2002). It has been observed that dogs with a history of systemic disease or those exposed to raccoon saliva may develop acute idiopathic polyneuropathy, which is characterized by neurogenic muscle atrophy and hyperesthesia lasting more than five to seven days (Malik and Farrow, 1991). The breathing pattern in the aforementioned neuromuscular paralysis diseases is usually shallow and fast. However, in the present cases, it was marked by an expiratory effort, resembling the pattern observed in tick paralysis (Holland, 2008). As a result, the neurological symptoms listed here are more similar to those of tick paralysis than those linked to other prevalent possible causes of lower motor neuron disorders.

Dogs exhibiting clinical signs of AFP but without detectable tick infestations, those in which an alternative etiology for AFP was identified based on anamnesis, clinical evaluation, and laboratory findings, as well as dogs diagnosed with other causes of neurological disorders such as spinal cord compression, epidural abscesses, or exposure to plant or snake toxins were excluded from the study. Confirmation of AFP due to tick paralysis was achieved through the *ex juvantibus* method, as all dogs showed prompt and complete recovery (median: 24 hours, min: 16 h, max: 34 h) following acaricidal treatment and tick removal. Additionally, eight dogs were excluded from the study because they did not exhibit clinical improvement despite acaricidal treatment and tick removal. Based on the anamnesis, these cases were suspected to be related to clostridial neurotoxin (botulism). The collected ticks were stored individually in vials and later identified at the species level using morphological keys. Morphological identification was conducted using a light stereomicroscope, examining the following characteristics: idiosoma, dorsal scutum, basis capituli, angles of basis capituli, hypostomal dentition, female genital opening, dorsal tail of spiracular plates, lateral and postmedian grooves, internal and external cervical grooves, marginal lines, accessory plates, presence of a male caudal process and body color. All ticks were identified as R. sanguineus (Ceylan et al., 2019; Gülersoy and Günal, 2022; Soulsby, 2005). As a result, 14 dogs infested with R. *sanguineus*, with clinical findings compatible with tick paralysis, and with a confirmed tick paralysis diagnosis *ex juvantibus*, constituted the tick paralyzed group of the study. To understand the impact of tick burden on the severity of symptoms and guide treatment strategies accordingly, dogs with tick paralysis were divided into two subgroups based on tick count: dogs with 1–4 ticks comprised the Low Tick group, while those with 5–60 ticks comprised the High Tick group. Seven dogs with similar BSAs (p=0.714), which were admitted either for vaccination and/or check-up purposes, constituted the healthy Control Group.

In summary, tick paralysis was diagnosed by ruling out other conditions with similar presentations and confirmed using the *ex juvantibus* method, in addition to the presence of engorged ticks and clinical signs such as quadriplegia, facial paralysis, megaesophagus, vomiting, and regurgitation. As previously mentioned, ticks were identified morphologically as *R. sanguineus* (Soulsby, 2005).

Taking Venous Blood Samples and Serum Biochemistry Profiling

Venous blood samples were taken from all the dogs via vena cephalica (5–10 mL) venepuncture with minimal restraint in order not to cause stress. Tubes without anticoagulant were used for serum extraction (1500 x rpm for 15 minutes at 4 °C). Serum biochemistry profiling was performed on the serum samples obtained using an autoanalyzer (Arkray Spotchem EZ SP-4430 automatic dry biochemistry, Japan) in the central laboratory of the animal hospital.

Statistical Analysis

The statistical program SPSS 25.00 (SPSS for Windows®) was used to analyze all the data. The Kolmogorov-Smirnov test was performed on the sample data to ascertain whether the data were parametric. Non-parametric data were analyzed using the Mann-Whitney U and Kruskal-Wallis tests and reported as the median (min-max). Statistical significance between group means was evaluated via one-way ANOVA with post-hoc Tukey analysis. Utilizing Receiver Operating Characteristic (ROC) curve analysis, the diagnostic and/or prognostic effectiveness of the previously described biomarkers was examined. The diagnostic sensitivity and specificity (>70%), standard deviation (Std. error), area under the curve (AUC >0.600), and diagnostic sensitivity were among the metrics used to assess the diagnostic performance of biomarkers. It was accepted that an AUC of 0.5 indicates no discrimination (i.e., the test's capacity to distinguish between patients with and without the illness or condition), 0.6 to 0.8 was regarded as acceptable, 0.8 to 0.9 as excellent, and >0.9 as exceptional (Hosmer and Lemeshow, 2000). Additionally, Spearman correlation analyses of all the aforementioned parameters were also performed. Within this scope, it was accepted that 0.40 to 0.69 refers to a moderate correlation, 0.70 to 0.89 a strong correlation, 0.90 to 1.00 a very strong correlation (Schober et al., 2018). Statistical significance was accepted as $p<0.05$, CI=95% for all data.

Results

Animal Characteristics

All dogs of the present study were domestic dogs, unvaccinated, fed on commercial dry dog food, and taken outside for walks 2-3 times a day. Among the dogs, mostly of mixed breed, the ones with tick paralysis were 4 (2-7) months old and the healthy ones were 4.5 (3-6) months old (p=0.843). Anamnestic data revealed that the dogs had no previous history of disease. The symptom duration of tick-paralyzed dogs was 4 (2-7) days and it was learned that the owners attempted to remove a few ticks themselves before presenting them to the hospital. It was observed that the head of the tick remained in some dogs (2 out of 14 paralyzed dogs). During the clinical examination, further ticks were removed from the dogs, and to validate the *ex juvantibus* diagnostic approach, all of the dogs were treated with a spot-on formulation of Fipronil 10% / (S)-Methoprene

9% (Frontline Combo, Merial S.A.S., France). Serum biochemistry profiling was conducted on dogs suspected of tick paralysis and deemed appropriate for inclusion in the study. This profiling occurred during the initial assessment. Results that did not support tick paralysis based on the *ex juvantibus* method were excluded from the study. Consequently, in addition to the 14 dogs included, 8 dogs were excluded because they did not show clinical improvement despite acaricidal treatment and tick removal, as previously mentioned.

Physical Examination Findings

The abnormalities detected as a result of visual assessment of neurological manifestations were hind limb incoordination (6 dogs, 43%), quadriplegia (3 dogs, 21%), ataxia (2 dogs, 14%), paresis (2 dogs, 14%), and absence of reflexes (1 dog, 7%). The respiratory rate of the tick-paralyzed dogs was higher than the healthy dogs ($p<0.0001$) but no statistical difference was determined between the Low Tick and High Tick groups. The heart rate of the High Tick group dogs was higher and statistically different from those in the other groups ($p<0.0001$). CRT was shorter in the High Tick group than in the other groups ($p=0.018$). While rectal body temperature was not statistically different between the Low Tick and the healthy groups, it was higher in the High Tick group than the others ($p<0.0001$). Physical examination findings are presented in Table 1.

Table 1. Physical examination findings.

Parameters	Healthy Group n:7 median (min-max)	Low Tick Group n:7 median (min-max)	High Tick Group n:7 median (min-max)	p value
Respiratory rate (breaths/min)	**39 (24-46)^{b}**	**84 (72-88)^{a}**	**76 (68-87)^{a}**	**0.0001**
Heart rate (beats/min)	**79 (73-96)^{b}**	**96 (90-108)^{b}**	**120 (104-164)a**	**0.0001**
Capillary refill time (sec)	**3 (2-3)^{a}**	**2.5 (2-3)^{ab}**	**2 (1-3)^{b}**	**0.018**
Body temperature (°C)	**38.1 (37.7-38.5)^{b}**	**38.8 (38.1-39.5)^{b}**	**40.1 (39.2-40.5)^{a}**	**0.0001**
Body weight (kg)	6 (5-7)	6.5 (5-8)	6 (5-8)	0.706
Body surface area (m^2)	0.33 (0.30-0.37)	0.35 (0.30-0.40)	0.33 (0.30-0.40)	0.714

The formula used to estimate BSA from body weight in dogs is $BSA=10.\ W^{2/3}$, where W=body weight in grams then converted into kilograms. $^{a, b}$: Indicates statistical differences.

Table 2. Serum biochemistry profiling results.

Parameters	Healthy Group n:7 median (min-max)	Low Tick Group n:7 median (min-max)	High Tick Group n:7 median (min-max)	p value
Total protein (g/dL)	**6.1 (5.3-7.5)^{ab}**	**5.1 (4.2-5.5)^{b}**	**7.4 (6.1-10)^{a}**	**0.002**
Albumin (g/dL)	3.1 (2.8-3.6)	3 (2.1-3.8)	3.3 (2.1-5)	0.728
Total bilirubin (mg/dL)	**0.3 (0.2-0.7)^{b}**	**0.35 (0.2-0.8)^{b}**	**1 (0.2-1.6)^{a}**	**0.002**
AST (U/L)	25 (15-45)	94 (10-112)	41 (17-467)	0.274
ALT (U/L)	66 (43-74)	36 (10-88)	31 (10-280)	0.781
LDH (U/L)	389 (236-401)	192.5 (100-665)	500 (102-2059)	0.281
BUN (mg/dL)	**7.4 (5.7-11)^{b}**	**18.4 (8.3-26.4)^{ab}**	**22.8 (6.7-43.1)^{a}**	**0.035**
Creatinine (mg/dL)	1.3 (0.7-1.6)	0.8 (0.3-1.1)	1.5 (1-26.2)	0.335

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen, $^{a, b}$: Indicates statistical differences.

Table 3. Diagnostic efficacies of clinical parameters of the tick paralyzed dogs in the Low Tick group.

Parameter	AUC	Std. Error	Asymp. Sig.	Asymptotic 95% Confidence Interval		Cut-off value	Sensitivity	Specificity
				Lower Bound	Upper Bound			
Respiratory rate	0.863	0.084	0.012	0.698	1.000	70	100%	57.1%
Heart rate	0.482	0.129	0.902	0.230	0.734	89	100%	42.9%
Capillary refill time	0.589	0.132	0.536	0.330	0.848	1.5	100%	21.4%
Body temperature	0.435	0.129	0.650	0.182	0.687	38.55	66.7%	50%

AUC: Area under curve, Std: Standard, Asymp. Sig.: Asymptotic Significance.

Table 4. Diagnostic efficacies of serum biochemistry parameters of the tick paralyzed dogs in the Low Tick group.

Parameters	AUC	Std. Error	Asymp. Sig.	Asymptotic 95% Confidence Interval		Cut-off value	Sensitivity	Specificity
				Lower Bound	Upper Bound			
Total protein	0.036	0.037	0.001	0.000	0.109	5.45	16.7%	7.1%
Albumin	0.440	0.166	0.680	0.116	0.765	3.35	50%	64.3%
Total bilirubin	0.369	0.125	0.364	0.124	0.614	0.35	50%	42.9%
AST	0.714	0.153	0.138	0.415	1.000	68	83.3%	85.7%
ALT	0.399	0.146	0.483	0.113	0.685	32.5	66.7%	28.6%
LDH	0.286	0.136	0.138	0.019	0.553	192.5	50%	21.7%
BUN	0.732	0.112	0.108	0.513	0.951	7.95	100%	50%
Creatinine	0.083	0.062	0.004	0.000	0.205	0.8	50%	7.1%

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen, AUC: Area under curve, Std: Standard, Asymp. Sig.: Asymptotic Significance

Table 5. Diagnostic efficacies of clinical parameters of the tick paralyzed dogs in the High Tick group.

Parameter	AUC	Std. Error	Asymp. Sig	Asymptotic 95% Confidence Interval		Cut-off value	Sensitivity	Specificity
				Lower Bound	Upper Bound			
Respiratory rate	0.665	0.123	0.235	0.425	0.905	57	100%	53.8%
Heart rate	0.984	0.023	0.000	0.939	1.000	101	100%	99.3%
Capillary refill time	0.181	0.107	0.022	0.000	0.391	2.5	14.3%	38.5%
Body temperature	0.973	0.033	0.001	0.909	1.000	39.1	100%	99.3%

AUC: Area under curve, Std: Standard, Asymp. Sig.: Asymptotic Significance

Table 6. Diagnostic efficacies of serum biochemistry parameters of the tick paralyzed dogs in the High Tick group.

Parameter	AUC	Std. Error	Asymp. Sig	Asymptotic 95% Confidence Interval		Cut-off value	Sensitivity	Specificity
				Lower Bound	Upper Bound			
Total protein	0.874	0.078	0.007	0.721	1.000	5.95	100%	69.2%
Albumin	0.533	0.147	0.812	0.245	0.821	3.25	57.1%	61.5%
Total bilirubin	0.879	0.109	0.006	0.665	1.000	0.75	85.7%	99.3%
AST	0.593	0.139	0.501	0.321	0.866	35.5	71.4%	53.8%
ALT	0.319	0.139	0.191	0.045	0.592	44	42.9%	38.5%
LDH	0.582	0.171	0.552	0.246	0.918	450.5	57.1%	99.3%
BUN	0.654	0.143	0.267	0.374	0.933	11.9	71.4%	69.2%
Creatinine	0.852	0.087	0.011	0.682	1.000	1.15	85.7%	69.2%

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen, AUC: Area under curve, Std: Standard, Asymp. Sig.: Asymptotic Significance

Serum Biochemistry Findings

Statistical differences were found in total protein, bilirubin, and BUN levels ($p<0.05$) among the parameters evaluated in the serum biochemistry profile. However, no differences were detected in the other parameters ($p>0.05$). Total protein level was higher in the High Tick group than in the Low Tick group ($p<0.002$). Total bilirubin level was statistically different and higher in the High Tick group compared to the other groups ($p<0.002$). Although the BUN level was numerically different between the dogs with tick paralysis, no statistical difference was determined. However, the BUN level was statistically different and higher in the High Tick group than in the Healthy group ($p<0.035$). Serum biochemistry profiling results are presented in Table 2.

ROC Analysis Results

As a result of the ROC analysis of the dogs with fewer than 5 ticks detected, it was determined that only respiratory rate, one of the clinical examination parameters, had diagnostic efficacy in making the clinical distinction of the disease (AUC=0.863; excellent diagnostic performance). ROC analysis and ROC curves of the clinical examination parameters investigated in the present study as a result of the grouping based on the number of ticks detected on the body (Low Tick Group, number of ticks < 5) are presented in Table 3 and Fig. 1.

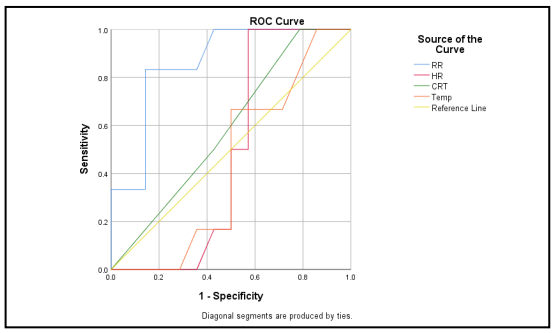

Figure 1. ROC curves of clinical examination parameters of the tick paralyzed dogs in the Low Tick group. RR: Respiratory rate, HR: Heart rate, CRT: Capillary refill time, Temp: Temperature

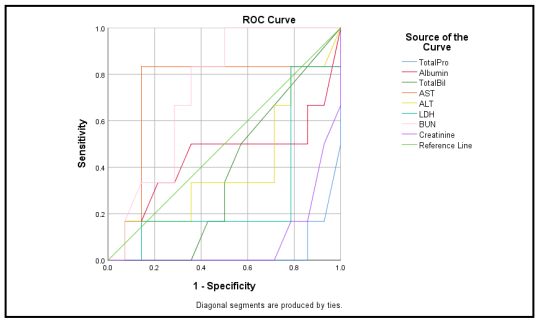

Figure 2. ROC curves of serum biochemistry parameters of the tick paralyzed dogs in the Low Tick group. TotalPro: Total protein, TotalBil: Total bilirubin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen

As a result of the ROC analysis of serum biochemistry parameters of the dogs with fewer than 5 ticks detected, it was determined that the diagnostic efficacies of aspartate aminotransferase (AST) and BUN levels for clinical differentiation of the disease were acceptable (AUC=0.714 and AUC=0.732; acceptable diagnostic performance). ROC analysis and ROC curves of the serum biochemistry parameters investigated in the present study as a result of grouping based on the number of ticks detected on the body (Low Tick Group, number of ticks<5) are presented in Table 4 and Figure 2. As a result of the ROC analysis of the dogs with more than 5 ticks detected, it was determined that respiratory rate had acceptable (AUC=0.665) and heart rate and body temperature had outstanding (AUC=0.984, AUC=0.973) diagnostic efficacies in clinically distinguishing the disease among the clinical examination parameters. ROC analysis and ROC curves of the clinical examination parameters investigated in the present study as a result of the grouping based on the number of ticks detected on the body (High Tick Group, number of ticks>5) are presented in Table 5 and Figure 3.

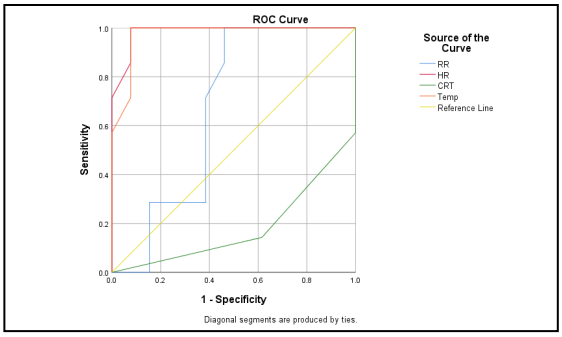

Figure 3. ROC curves of clinical examination parameters of the tick paralyzed dogs in the High Tick group.

As a result of the ROC analysis of serum biochemistry parameters of the dogs with more than 5 ticks detected, it was determined that the diagnostic efficacies of total protein, total bilirubin and creatinine levels in clinical differentiation of the disease were excellent (AUC=0.874, AUC=0.879 and AUC=0.852). The diagnostic efficacy of BUN level in distinguishing the disease was acceptable (AUC=0.654). ROC analysis and ROC curves of the serum biochemistry parameters investigated in the present study as a result of grouping based on the number of ticks detected on the body (High Tick Group, number of ticks>5) are presented in Table 6 and Figure 4.

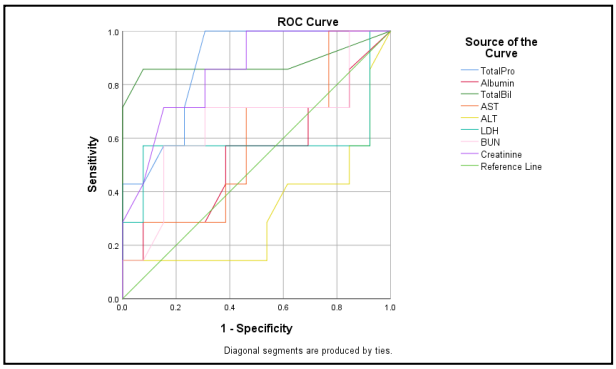

Figure 4. ROC curves of serum biochemistry parameters of the tick paralyzed dogs in the High Tick group

As a result of the Spearman correlation analysis of the parameters investigated within the scope of the present study, a strong negative correlation (Lim, 2020) was determined between albumin level and body weight (r=-0.713) and albumin and BSA (r=-0.713). A very strong positive correlation (Lim, 2020) was determined between body weight and BSA (r=1.000). The result of the Spearman correlation analysis is presented in Supplementary file.

Discussion

In the present study, routine examination parameters were comparatively evaluated and the diagnostic efficacies of these analytes were investigated for tick paralysis. An increase in tick burden was associated with corresponding elevations in pulse rate and body temperature, as evidenced by physical examination findings. Additionally, elevated levels of BUN and total bilirubin were detected in serum biochemistry parameters, potentially attributable to the rate and volume of toxin secretion by the ticks. As a result of the ROC-based diagnostic performance analysis of the investigated parameters, it was observed that when the number of ticks was less than 5, the respiratory rate showed excellent, while BUN and AST evaluation showed acceptable diagnostic performance. As the number of ticks increased, the diagnostic performance of respiratory rate, heart rate and body temperature increased. Among the serum biochemistry parameters, it was determined that total protein, total bilirubin and creatinine had excellent, and BUN had acceptable diagnostic performance. As a result, it was determined that the diagnostic performances of the investigated routine examination parameters increased as the number of ticks increased, contrary to previous reports (Padula, 2016). However, since these parameters have low specificity in atypical cases where ticks cannot be detected (Gülersoy and Günal, 2022), it was concluded that their diagnostic use is controversial and specific diagnostic tests should be developed by combining them with other parameters.

The clinical signs of tick paralysis, such as ascending flaccid paralysis, typically emerge 5-9 days post-tick attachment. The progression starts with hind limb weakness and advances to quadriplegia within the subsequent 24-72 hours. Failure to remove ticks may lead to respiratory paralysis and subsequent death within 1-5 days. However, removing all ticks generally leads to improvement in clinical manifestations within 24 hours and complete recovery within 72 hours (Atwell et al., 2001; Soulsby, 2005). According to previous reports, tick removal and supportive care alone can often lead to a full recovery in cases of tick paralysis when diagnosed early and treated promptly. On the other hand, if neglected, it can worsen and lead to respiratory failure and even death. These characteristic timeframes were also observed in the present study, where the diagnosis was confirmed using the *ex juvantibus* approach—clinical recovery following tick removal and acaricidal treatment. This underscores the critical importance of prompt tick identification and removal. While supportive care alone may lead to recovery, the reliance on *ex juvantibus* diagnosis in clinical settings where no specific test exists further highlights the need for objective diagnostic criteria (McGee, 2012).

Although physical examination findings provide limited information compared to specific tests, they can be useful in increasing the clinical suspicion of diseases (Ilkiw and Turner, 1987). In dogs with tick paralysis, in addition to neurological findings, clinical examinations reveal signs such as fever, altered mental status, and increased respiratory and heart rates (Atwell et al., 2001). In severe cases of tick paralysis, the main clinical anomaly and most likely the primary cause of mortality is respiratory failure (Shaffran, 2008). Adverse effects on the cardiovascular system include elevated blood pressure and heart rate, arrhythmias, and coagulopathies (Eyer and Zilker, 2007). The respiratory rate of the tick-paralyzed dogs in the present study was higher than that of the healthy dogs ($p<0.0001$), but no statistical difference was detected between the Low Tick and High Tick groups. The heart rate of the dogs in the High Tick group was higher and statistically different from the dogs in the other groups ($p<0.000$). Capillary refill time was shorter in the High Tick group than in the other groups ($p<0.018$). While rectal body temperature was not statistically different between the Low Tick group and healthy group, it was higher in the High Tick group than the others ($p<0.000$). In addition, in the ROC-based diagnostic performance analysis of the clinical examination parameters of dogs with fewer than 5 ticks detected, it was determined that only respiratory rate had a diagnostic efficacy in the diagnosis of the disease and this was excellent (AUC=0.863). When the number of ticks detected was more than 5, heart rate and body temperature were among the parameters with diagnostic efficacy (AUC=0.984, AUC=0.973; outstanding diagnostic performance) and the efficacy of respiratory rate decreased (acceptable diagnostic performance; AUC=0.665). In the present study, abnormal cardiovascular, respiratory, catabolic and metabolic rates, which are exacerbated by the increasing tick numbers, may be related to tick neurotoxin, which increases in conjunction with the increase in tick numbers (Padula, 2016). In addition, body temperature, whose diagnostic performance improves with increasing tick numbers, may be associated with intense skeletal muscle hypermetabolic reaction, which correlates with higher tick neurotoxin levels (Barrett and Topol, 2016).

The perception that physical examination holds limited value has become prevalent, despite ample literature data showcasing the diagnostic utility of many of its components. Physical examination remains essential to diagnosis, with its omission risking clinical errors. Despite criticism over its variability and calls for more sensitive tests like serum biochemistry, it remains fundamental to patient care and the therapeutic relationship (Nathwani et al., 2005). For this reason, not only the respiratory rate, previously highlighted as a prominent physical examination finding during the initial triage assessment of dogs with tick paralysis (Fadia et al., 2019; Shaffran, 2008), but also heart rate and body temperature can offer valuable clinical insights, enhancing the diagnostic suspicion index for the disease. Neuroimaging in tick paralysis typically reveals no abnormalities unless an

undetected tick is visualized. Cerebrospinal fluid and leukocyte (WBC) counts are usually normal. In cases with respiratory compromise, blood gas and pulmonary function tests assist in evaluating the need for intubation. Electromyography often shows reduced compound muscle action potential amplitudes, while repetitive nerve stimulation results are generally normal (McGee, 2012). Previously, hematochemical analysis findings in dogs with tick paralysis have revealed increases in hemoglobin concentration, red blood cell (RBC) and WBC counts, elevated BUN and creatinine concentrations attributed to dehydration, and elevated glucose and cholesterol levels due to sympathetic stimulation of the adrenal medulla (Shaffran, 2008). These previous data may highlight that the efficacies of routine tests including CBC and serum biochemistry should be investigated comparatively (Padula et al., 2020). In the serum biochemistry profiling of the present study, the total protein level exhibited a significant increase in the High Tick group compared to the Low Tick group ($p=0.002$). Moreover, the total bilirubin level was found to be statistically higher in the High Tick group than in the other groups ($p<0.002$). Additionally, BUN levels showed a significant elevation in the High Tick group compared to the Healthy group ($p<0.035$). Furthermore, upon conducting ROC based diagnostic performance analysis of serum biochemistry parameters in the dogs with fewer than 5 ticks detected, it was determined that the diagnostic efficacy of AST and BUN levels in clinically distinguishing the disease was deemed acceptable (AUC=0.714 and AUC=0.732, respectively; acceptable diagnostic performance). As a result of the ROC based diagnostic performance analysis of serum biochemistry parameters of the dogs with more than 5 ticks detected, it was determined that the diagnostic efficacies of total protein, total bilirubin and creatinine levels in clinical differentiation of the disease were excellent (AUC=0.874, AUC=0.879 and AUC=0.852, respectively). The diagnostic efficacy of BUN level in distinguishing the disease was acceptable (AUC=0.654). It was previously thought that in situations of tick paralysis, there would be minimal changes to biochemical parameters. However, given that these indices have not been previously measured, it was deemed necessary to conduct a detailed investigation to ascertain any potential changes. While many of these changes may be difficult to interpret in isolation, taken as a whole, they might represent the adrenal medulla's biochemical reaction to sympathetic stimulation. This stimulation can lead to the release of adrenaline and nor-adrenaline or the release of adrenocorticotrophic hormone, subsequently stimulating the adrenal cortex to secrete corticosteroids (Shaffran, 2008). None of these alterations, it was noted, is specific to tick paralysis and don't signify the severity or prognosis (Atwell et al., 2001; Padula et al., 2020). In the present study, findings such as elevated BUN and creatinine levels observed due to dehydration were consistent with previous reports (Atwell et al., 2001; Shaffran, 2008). Additionally, increased AST levels could be associated with recumbency-related rhabdomyolysis. It was reported that increased ALT levels could also be associated with rhabdomyolysis since it was reported (Guiloff et al., 1980) that along with AST, ALT levels also increase in case of acute muscular damage. Dehydration might be anticipated in animals exhibiting signs of reduced water and food intake, accompanied by vomiting. However, it is crucial to emphasize that if dehydration occurs in tick paralysis cases, it tends to be mild (Shaffran, 2008). Thus, the higher total protein level of serum samples of the dogs with more than 5 ticks on their bodies in the present study may be associated with dehydration and prolonged recumbency period (Lim, 2020). Although there was no statistical difference, the AST level, which has acceptable diagnostic performance in the diagnosis of the disease, was interpreted as a result of muscle damage (Guo et al., 2021). Furthermore, creatinine has been identified as an independent predictive biomarker for muscle damage; as a sign of malnutrition, it rises in the early stages of the damage and falls in the later stages (Boffey and Paterson, 1973). Although the creatinine level was not statistically different in the intergroup comparison similar to the AST level, its efficacy in the diagnosis of the disease was excellent and this finding was associated with muscle damage and dehydration (Atwell et al., 2001; Padula et al., 2020; Shaffran, 2008). Findings related to muscle damage were associated with tick toxin caused muscle damage by interference with cellular energy metabolic pathways (Terrault et al., 2018). In the present study, ALT concentrations of tick paralyzed dogs, which were statistically insignificant in the intergroup comparison but numerically lower than the healthy dogs, may be associated with higher mortality prediction (Bradbury, 2017). Nevertheless, studies on survival probability in tick paralyzed dogs may further elucidate the efficacies of these parameters.

Hyperbilirubinemia refers to the excessive accumulation of bilirubin in the bloodstream, typically stemming from impaired bilirubin metabolism. Bilirubin, a byproduct of hemoglobin breakdown, results from the degradation of red blood cells by the mononuclear phagocytic system. Initially, water-insoluble, unconjugated bilirubin binds to albumin for transport to the liver, where it undergoes conjugation via glucuronidation within hepatocytes. Subsequently, bilirubin glucuronides are actively transported into bile canaliculi, stored in the gall bladder, and eventually excreted primarily in feces. Hyperbilirubinemia manifests as the accumulation of bilirubin pigment in the blood and tissues, leading to jaundice (icterus), and can be categorized as pre-hepatic, hepatic, or post-hepatic (Bhutani and Johnson, 2009). During the mid-twentieth century, the impact of bilirubin on the central nervous system (CNS) remained largely unexplored. Physicians noted notable motor deficits in neonates with severe hyperbilirubinemia, prompting investigations into how bilirubin affected motor systems in the developing brain. The CNS was implicated, suggesting that bilirubin might cross the blood-brain barrier (BBB) and damage the neurons linked to movement (Terrault et al., 2018). The globus pallidus and subthalamic nuclei are two brain locations where bilirubin has been shown to have a neurotoxic effect. This effect causes motor-related sequelae that can range from severe movement abnormalities to loss of coordination (Lim, 2020; Shapiro, 2012). The higher total

bilirubin level of the High Tick group may be related to reduced hepatocyte function, intrahepatic cholestasis and accumulation of conjugated and unconjugated bilirubin (Bhutani and Johnson, 2009). At physiological levels, bilirubin plays a crucial role in brain function as a potent antioxidant, protecting neural tissues from oxidative damage by neutralizing reactive oxygen species (ROS). Furthermore, it contributes to immune regulation by modulating microglial activation, cytokine release, complement system activity, Fc receptor function, and MHC II expression, thereby reducing the risk of inflammatory and autoimmune reactions in the CNS (Kaur et al., 2025). Given these roles, monitoring total bilirubin levels—which exhibit excellent diagnostic performance based on the present ROC analysis—may help predict CNS damage in dogs with tick paralysis, clarify motor pathway dysfunction, and aid in prognosis assessment.

The fact that the CBC results evaluated in the present study were used only as inclusion/exclusion criteria and selected parameters were evaluated within the context of serum biochemistry profiling can be considered a limitation. A key limitation of the present study is the relatively small number of animals included, which may restrict the generalizability of the findings. Therefore, future studies should include a larger sample size and incorporate a broader range of clinical and laboratory parameters for a more comprehensive evaluation.

Conclusions

In this study assessing the diagnostic efficacy of routine physical and selected serum biochemistry parameters in tick paralysis, notable considerations emerged regarding heart rate, body temperature, and respiratory rate in the assessment of clinical manifestations. Furthermore, total protein, total bilirubin, and creatinine levels demonstrated excellent diagnostic performance, while BUN levels exhibited acceptable performance. Consequently, the findings suggest that heightened exposure to tick neurotoxins, associated with an increased tick burden, may exacerbate the disease advanced stages, thereby amplifying the diagnostic efficacy of the parameters above through tissue and organ damage.

Conflict of Interest

The authors stated that they did not have any real, potential or perceived conflict of interest.

Similarity Rate

We declare that the similarity rate of the article is 17% as stated in the report uploaded to the system.

Ethical Approval

This study was approved by the Harran University Animal Experiments Local Ethics Committee (09.05.2022, 2021/003 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

Author Contributions

Motivation / Concept: EG, CB
Design: EG, CB
Control/Supervision: EG, CB
Data Collection and / or Processing: EG, CB, AŞ, İG, EK
Analysis and / or Interpretation: EG, CB, AŞ, İG, EK
Literature Review: EG, CB, AŞ, İG, EK
Writing the Article: EG, CB, AŞ, İG, EK
Critical Review: EG, CB, AŞ, İG , EK

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

A Prospective View of Oral and Dental Examination and Dental Diseases in Horses in İzmit and Karacabey Pension Hara of Turkey Jockey Club

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Abstract: This study, the aim was to prospectively examine the oral cavities and teeth of 300 horses of various age groups housed at the İzmit and Karacabey Pension Hara of the Turkish Jockey Club, focusing on dental disorders and dental diseases. Within the scope of the study, the oral cavities of the examined 300 horses were inspected, including the color of the oral mucosa, their appetite, whether they finished their feed, body condition scores, the type of feed they were fed, and whether salivation occurred during feeding. Following clinical examinations of the 300 horses, endoscopic and radiological examinations were performed, revealing dental disease and disorders, particularly in the molar teeth, in 59 horses. Among the group of 59 horses, consisting of stallions, broodmares, and yearlings aged 7-25 years, tooth extraction of deciduous teeth, tooth displacement, mobile teeth, caries, sharp-edged teeth, odontoclastic tooth resorption, and conditions such as equine odontoclastic tooth resorption and hypercementosis (EOTRH), fistula formation, gum abscess, gingivitis, and periodontitis were identified. Clinically, 54 out of 59 horses had their molar teeth rasped to correct sharp edges, resulting in the normalization of chewing movements and the ability of horses to consume their feed comfortably. Following treatment, the horses were monitored, and efforts were made to improve their nutrition and body condition scores. This study emphasizes the significance of oral health in horses and the impact of regular dental care on their overall well-being.
Keywords: *Equine, Periodontal disease, Teeth.*

Türkiye Jokey Kulübü İzmit ve Karacabey Pansiyon Hara Müdürlüklerinde Bulunan Atlarda Ağız Diş Muayenesi ve Diş Hastalıklarına Prospektif Bir Bakış

Özet: Atlarda diş hastalıkları veteriner hekimlik açısından oldukça önemlidir. Ancak Ülkemizde yakın zamana kadar oldukça ihmal edilmiştir, birçok atın teşhis edilmemiş, ağrılı diş hastalıklarından muzdarip olduğu gözlenmiştir. Ağız padanı(spekulum) kullanılarak yapılan detaylı klinik muayenede, ön koşul tüm dişlerin muayenesini gerçekleştirmektir. Bu tez çalışmasında Türkiye Jokey Kulübünün İzmit ve Karacabey Pansiyon Hara Müdürlüklerinde 2012-2013 yıllarında barınmakta olan çeşitli yaş gruplarındaki 300 atın ağız boşluğu ve dişleri muayene edilerek, diş bozuklukları ve diş hastalıkları yönünden incelenerek prospektif olarak ele alınması amaçlandı. Çalışma kapsamında muayene edilen 300 atın nabız, vücut sıcaklığı, solunum sayısı ve kalp atım sayısı gibi hayati fonksiyonları not edildi. Ayrıca tüm atların ağız boşluğu muayene edilerek; ağız mukozasının rengi, iştahları, yemlerini bitirip bitirmedikleri, vücut kondüsyon skorları (VKS), hangi tür yemle beslendikleri, ağızlarından yem tüketimi sırasında salivasyon olup olmadığına bakıldı. Bunların yanısıra yem tüketimiyle ilişkili olarak herhangi bir sağlık sorunu geçirip geçirmedikleri araştırıldı. Klinik olarak muayenesi yapılan 300 atın endoskopik ve radyolojik olarak da muayenelerinin yapılmasından sonra 59 atta özellikle molar dişlerde (MD) diş hastalığı ve diş bozukluğu olduğu tespit edildi. 59 adet at grubunu oluşturan, 7-25 yaş aralığındaki damızlık aygır, kısraklar ve 1 yaşlı taylarda; süt dişi çekimi, dişlerde yer değiştirme, mobil dişler, karies, keskin kenarlı dişler, odontoklastik diş rezorpsiyonu ve hipersementozis (EOTRH), fistül oluşumu, diş eti apsesi, gingivitis ve periodontitis tespit edildi. Diş hastalıkları ve diş bozukluklarının modern teknikler kullanılarak tedavi edilmeleri sağlandı. Özellikle beşeri diş hekimliğinde Ülkemizde kullanılan dolgu materyallaleri denendi ancak ağız boşluğunun aşırı ıslak oluşu ve atların kuvvetli çiğneme haretketleri nedeni ile bir başarı elde edilemedi. Karies olgularında diş çekiminde, gingivitis ve periodontitis vakalarında dişler başarıyla tedavi edildi. Klinik olarak ele alınan 59 attan 54' ünün MD' i törpülenerek dişlerin keskin kenarları düzeltilerek atların çiğneme hareketlerinin normale döndüğü ve yemlerinin tamamını rahatlıkla tüketebildikleri görüldü. Tedavi sonrası atlar takip edilerek beslenmelerinin ve VKS' nın yükseltilmesi sağlandı.
Anahtar Kelimeler: *At, Diş, Periodontal Hastalık.*

Introduction

Dental abnormalities are an important problem in equine medicine practice (Dixon et al., 1999; Dixon and Dacre, 2005; Simhofer et al., 2008). Dental abnormalities in horses can cause weight loss, poor performance, pain, behavioral abnormalities and disease (Kirkland, 1994). Equine dental disease is common, but there are few reports documenting the prevalence of dental disease in the general equine population (Dixon et al., 1999; Kilic et al., 1997; Kirkland et al., 1994). Dental problems are the third most common medical problem in large animal practice in the USA (Traub-Dargatz et al., 1991). Most equine dental disorders have not been adequately studied, and as a result, it is unclear how abnormalities progress (Dixon et al., 1999; Kilic et al., 1997). In addition, many autopsy studies have reported significant levels of undiagnosed dental disease in horses (Brigham and Duncanson, 2000; Jasiński et al., 2025; Kirkland et al., 1994; Wafa, 1988). With the development of diagnostic techniques in recent years, it is now recognized that equine dental disease has a significant impact on the welfare of domestic horses (Gorski et al., 2022; Kennedy and Dixon, 2018).

Especially in recent years, horse owners' knowledge about the welfare of their horses to dental care has improved (Rebecca et al., 2024). The majority of oral problems in horses are associated with dental diseases and routine oral cavity examinations should be performed (de Melo and Ferreira, 2023). Horse breeders typically allocate up to 10% of their expenditure to oral cavity-related issues (Hain et al., 2025; Samad et al., 2020).

The most common equine dental problem is overgrowth of the buccal margin of the maxillary cheek teeth (CT) and the lingual margin of the mandibular CT (O'neill et al., 2010). A common symptom of periodontal disease and dental caries is bad breath (Dixon et al., 2000). Dental problems are less likely to cause weight loss because horses with these conditions spend more time chewing food. 'Diastema' describes a gap between the cheek teeth and predisposes to periodontal disease (Easley, 2009). Successful results may not be achieved in all cases, but good treatment can be achieved with anti-inflammatory, antibiotic, and restorative agents. Otherwise, the traumatized tooth cannot be saved, and tooth extraction will be necessary (Buonavoglia, 2021).

The results of this study may help horse owners and veterinarians horses understand the prevalence of dental problems among different horse groups and thus pay more attention to susceptible groups.

Material and Methods

Animal Material

The study material consisted of a total of 300 Thoroughbred British horses, including breeding mares (adult 1-year-old in females), breeding stallions (adult males), and 1-year-old foals between the ages of 7 and 25 years, housed at the Izmit and Karacabey Pension Hara of the Turkish Jockey Club.

Clinical Examination

The body temperature, respiratory rate, and heart rate of all horses included in the study were determined. In addition, the color of the mucous membrane of the mouth, appetite, weight loss, hair cover, dehydration, the way of eating feed, whether they left feed during feeding, what kind of feed they eat, the way the feed was given, chronic diseases and whether they had gastrointestinal symptoms related to feed were examined in detail and recorded.

Oral Cavity Examination

A dental examination card was prepared for each horse to be examined, and the defects observed during the oral cavity examination and recorded separately. Before the oral cavity examination, the oral cavity was washed to remove feed residues, foreign bodies and grasses covering the teeth. After the clinical examinations of the traumatized horses were completed, oral cavity examination was started. For oral cavity examination, during the examination of stallions, mares and 1 year old foals, McPherson (Austria) model mouth speculum or padan was used to open the mouth. The intraoral area of the horse opened with the padan was illuminated with a head lamp. The oral cavity was examined for the presence of foreign bodies, mucous membranes of the upper and lower lips, mucous membranes of the cheeks, and possible anatomical abnormalities of the tongue. Teeth were visually examined using a dental mirror. The areas where the cheek blocked the view, especially the posterior molars of the upper jaw, were examined using a dental mirror. The oral cavity was examined for anatomical arrangement of the teeth, color, pain, mobility, spikes, sharp edges, decayed teeth, broken teeth and gingival dental disorders. After completion of the oral cavity examination, endoscopic and radiologic examinations were performed.

Endoscopic Examination

After inspecting and palpating the oral cavity, the cheeks and teeth were examined for dental and periodontal disease using endoscopic techniques. Endoscopic examination was performed with a 40 cm long, 5 mm - 8 mm diameter device with a 60-degree viewing angle (Richard Wolf, Germany). The endoscopy device was used for observation of ulcers on the cheek and tongue, detection of tooth elongation, detection of sharp-pointed teeth, detection of gaps between teeth, periodontitis, cementum abnormalities, observation of parasite formations in the gums and examination of calculus fractures (Figure 1).

Radiography

Intraoral radiographs were taken at doses of 50 kVp and 0.5 mAs to reveal the periodontium of the incisor, canine and molar teeth and 60 kVp and 0.6 mAs for the molar teeth.

Treatment Protocols for Dental Diseases

Treatment Protocol for Horses Diagnosed with Periodontal Disease

In horses diagnosed with periodontitis, the first goal was to balance the occlusal surfaces mutually. Occlusal problems, or in other words, malocclusional areas, were

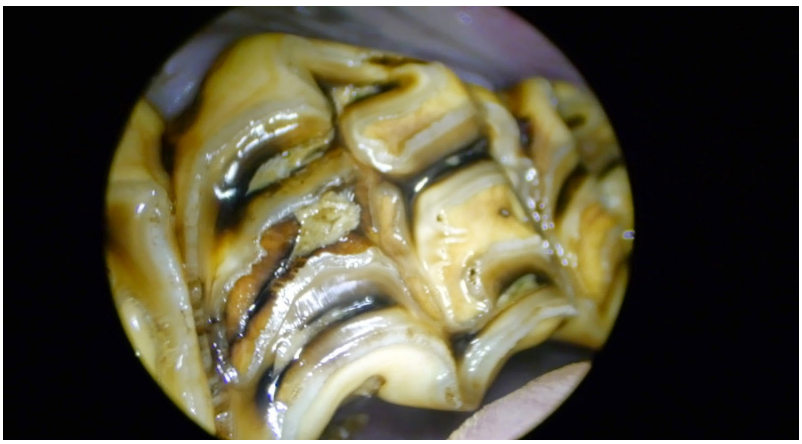

Figure 1. Endoscopic examination of a molar tooth.

corrected by simple filing, and occlusal balance was achieved. Swabs were taken from periodontal pockets for culture and antibiogram. These areas were washed with chlorhexidine to mitigate the risk of bacterial infection. Samples taken with swabs were analyzed in the Laboratory of Istanbul Veliefendi Hippodrome Racehorse Hospital. After cleaning the periodontal pockets, the remaining area was filled with aluminum oxide powders for period repair, and tooth repair was performed.

Treatment Protocol for Horses with Odontoclastic Tooth Resorption and Hypersementosis (EOTRH)

The affected tooth was extracted as treatment in horses with Eorth. After extraction, the area was disinfected with chlorhexidine solution for 3 days and the gingiva and alvelolar clots were removed for healing of the tooth cavity and gingiva. In addition, the nonsteroidal anti-inflammatory agent Flumed (Alke, Istanbul, Turkey) was administered at a dose of 2.2 mg/kg by IV injection every other day. In addition, Danilon powder (Esteve Pharmaceutıcals, Barcelona, Spain) 2.2 mg/kg was administered orally for 7 days post-operatively.

Treatment Protocol for Horses with Dental Calculus (Calculus, Tartar)

During the oral cavity examination, tartarage was performed on the teeth of horses with tartar formation in their teeth. Feed residues around the teeth were cleaned and the area around the teeth was cleaned with oral antiseptic.

Treatment Protocol for Horses Diagnosed with Caries (Dental Caries, Caries)

First, the carious tooth was diagnosed radiologically. The superficially affected teeth were firstly restored with filling material. Teeth that could not be restored were extracted after applying a maxillary nerve block and local infiltration anesthesia around the tooth.

Treatment Protocol for Horses with Mobile Teeth

The treatment protocol was determined according to the mobility of the tooth.

-Stage 1 mobile tooth: Sharp edges were corrected after simple routine filing. Gingiva was cleaned with chlorhexidine solution.

-Stage 2 mobile tooth: The problem was solved by reducing the height of the opposing tooth and cleaning the periodontal formation around the affected tooth with chlorhexidine solution.

-Stage 3 mobile tooth: The tooth was found to be stage 3 mobile due to periodontal pocket formations measuring up to 3 mm, accompanied by occlusal wear. These teeth were extracted completely. In addition, the alveolar pocket was washed with chlorhexidine solution, and antibiotic treatment was applied.

Results

It was learned that oral cavity examinations and dental controls of the purebred British breed stallions, breeding mares, and foals used in the study were not performed recently, and the majority of them were last examined or controlled for oral cavity and dental health 2 years ago.

It was determined that the horses evaluated were in the age range of 1-25 years (13.70 ± 6.3 years). The body weights of the horses constituting the study material were found to be in breeding stallions (578.13±26.33 kg), mares (561.81±23.94 kg), 1-year-old racehorses (327.14±9.75 kg) and foals separated from their mothers (257.33±67.57 kg).

The treatment protocol and the results of the treatment of horses with dental disorders and diseases were recorded. The information about the horses with dental disease or disorder as a result of oral cavity examination within the scope of the study is presented in Table 1.

Out of a total of 300 horses whose oral cavity was examined, 59 horses were found to have tooth and gum disorders. 45 breeding stallions, 9 breeding mares and 5 1-year-old foals were treated.

Within the scope of the study, sharp edges, pointed tips and abnormal length were detected in the molar teeth of a total of 31 horses, including 23 breeding stallions, 7 breeding mares and 1 1-year-old foal. It was observed that periodontitis was formed due to the accumulation of feed and similar residues between the teeth, especially due to occlusal imbalance, and then the feed and similar residues accumulated between the teeth due to excessively long teeth or wavy teeth could not be removed and where they accumulated; they damaged the teeth and gums, caused bacterial accumulation and infection, and in this way, gingivitis, periodontal pocket formation, weakness in the

Table 1. Dental Disorders Observed in Horses as a Result of Dental Examination.

	Breeding Stallion	Breeding Mare	1 Old Foal	Total	Total Percentage(%)
Gingivitis	14			14	(%10,5)
Peridontitis	8			8	(%6)
Gum abscess	2	1		3	(%2,25)
Fistula formation	1			1	(%0,75)
Eotrh	5	2		7	(%5,25)
Sharp-edged tooth	23	7	1	31	(%23,25)
Karies	5			5	(%3,75)
Mobile tooth	3			3	(%2,25)
Relocation	1		1	2	(%1,50)
Milk tooth extraction			1	1	(%0,75)

periodontal ligament and damage to the alveolar bone occured.Among the horses examined, gingivitis was diagnosed in 13 breeding stallions and periodontitis in 8 breeding stallions. In addition, in the clinical examination of the horses with occlusal imbalance in the teeth, it was noted that there were complaints of feed refusal, bad odor in the mouth, ulcerations on the cheeks and increased salivation (Figure 2, Figure 3, Figure 4).

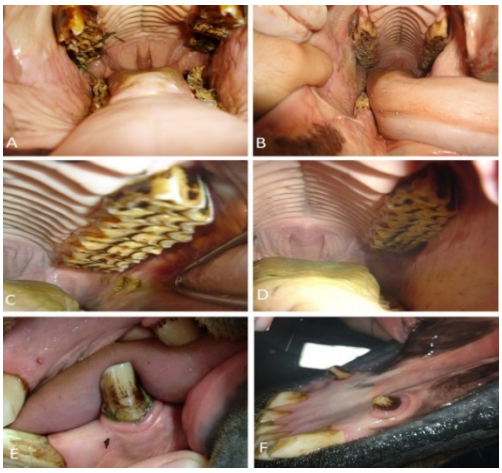

Figure 2. A) Excessive growth of teeth, B) Appearance after rasping, C) Ulceration formation in the left cheek mucosa, D) Appearance of the ulcer after treatment, E) Gingivitis with dental plaque on canine tooth number 204, F) Appearance after treatment.

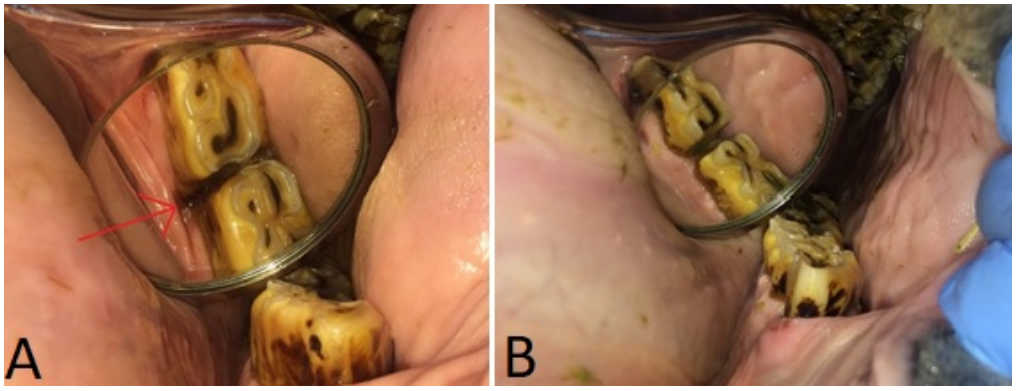

Figure 4. A) Diastema and periodontal pocket formation, B) Post-treatment appearance.

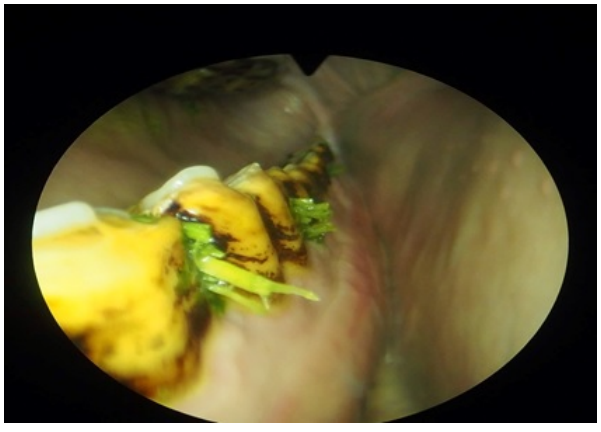

Figure 3. Endoscopic visualization of diastema and periodontal pocket.

In mild and moderate periodontal disease, occlusal balancing and cleaning of feed residues between the teeth and disinfection with chlorhexidine were mostly sufficient to prevent periodontitis. In 4 of 59 horses with grade 3 periodontitis; severe inflammation, edema, gingival bleeding, pustular discharge, bone tissue loss and 25-50% loss of periodontal support were observed. In 4 of 8 horses with grade 4 periodontitis, severe inflammation, edema, gingival bleeding, pustular discharge, severe mobility in the affected tooth, and more than 50% loss of periodontal ligament support were found (Figure 5, Figure 6).

After examination of the teeth, EORTRH was detected in a total of 7 horses, 5 stallions and 2 mares. In clinical examinations, horses with EOTRH had difficulty in eating, difficulty in tearing even soft grass, increased salivation, drainage canals and ulcer formations in the gums. For this reason, chewing movements were painful and limited. Radiographic examinations showed resorption, fracture or hypersementosis of the tooth roots. EOTRH was detected mostly in the incisive teeth of older horses brought with the complaint of difficulty in eating grass.

Within the scope of the study, caries was detected in the teeth of 5 breeding stallions. In horses with caries, symptoms included a bad odor in the mouth, excessive salivation, difficulty in feeding, feed withdrawal, and head

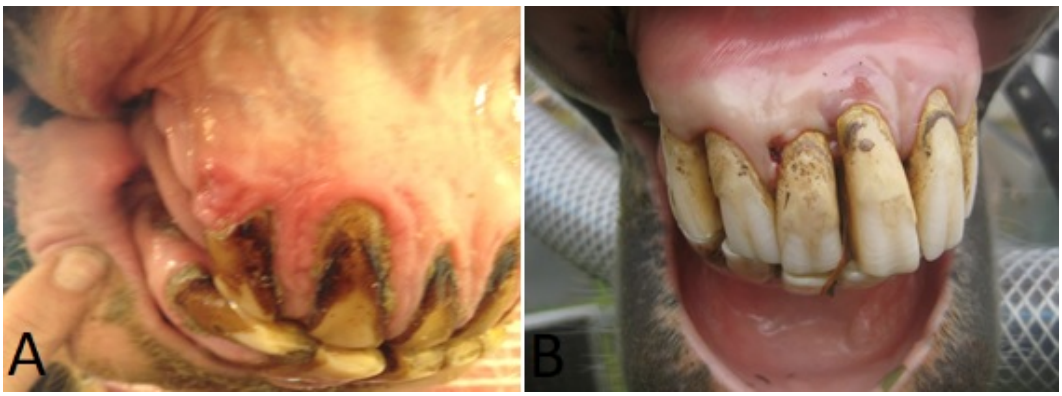

Figure 5. A. Appearance of EOTRH before dental care, B. Appearance after dental care.

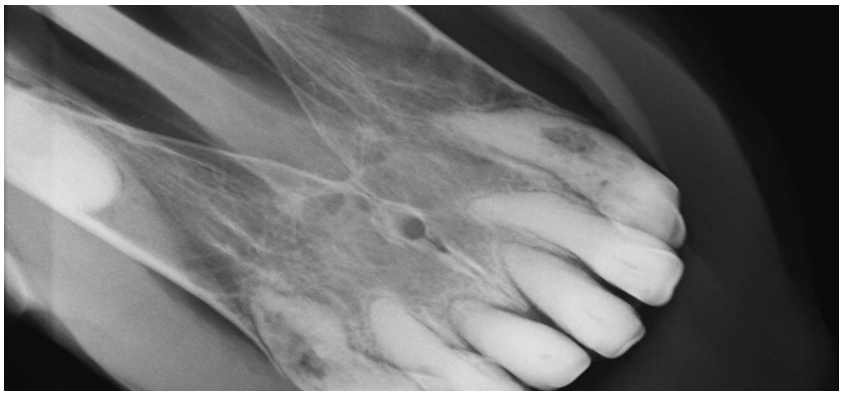

Figure 6. EORTH in incisor teeth numbered 303-401-402-403.

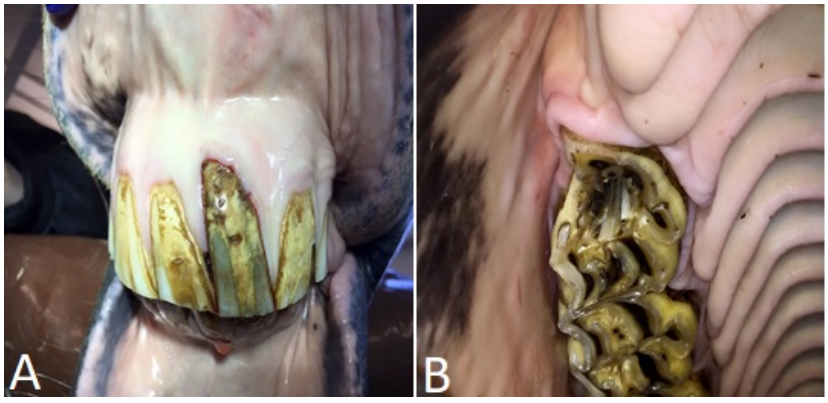

Figure 6. A) Caries in tooth number 201 B) Caries in tooth number 106.

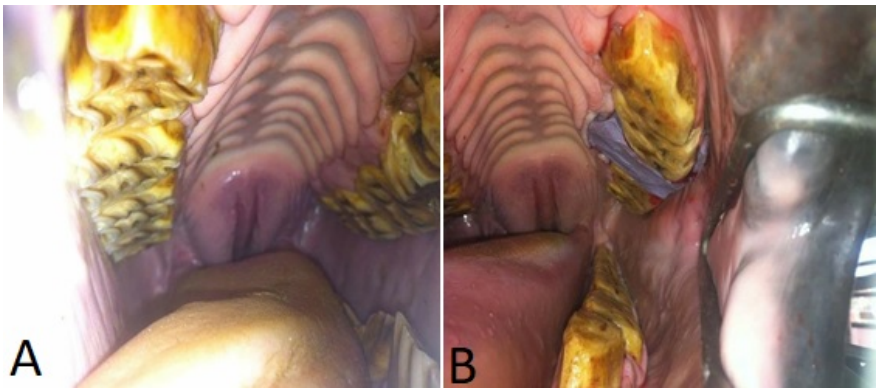

Figure 7. A) Corrected views of the pointed ends, ramps and wavy appearance. B) Filling the space of the rotated (left) molar tooth number 208 with filling material (aluminum oxide powder) after extraction.

shaking in painful cases. In cases of caries without severe destruction, treatment was provided with filling material (3 cases). In some cases, caries was too advanced to allow restoration (2 cases). In cases with advanced caries, the tooth was extracted.

Bacteria were not isolated in 34 (63%) of 54 horses from which swabs were taken, while bacteria were isolated in 20 (37%) of the remaining horses. Among the isolated bacterial species; 10% Bacteriodes spp.(2 horses), 10% Fusabacterium spp.(2 horses), 50% Streptococcus spp.(10 horses), 15% Bacillus spp.(3 horses) and 15% Corynebacterium spp.(3 horses) were grown.

The treatment protocol was determined based on the mobility of the tooth in horses with mobile tooth cases identified during the examinations. Stage 1 mobile teeth were generally observed in 8 horses used in the study, aged between 14 and 25 years. It was observed that the sharp-edged teeth affected the gingiva, resulting in abrasion of the soft tissues of the mouth. In horses with stage 2 mobile teeth, the occlusal surface of the tooth was also affected (4 cases). The presence of premolar stage 3 mobile teeth was detected in 3 horses with periodontal pocket formations up to 3 mm with occlusal abrasion (Figure 7).

Discussion and Conclusion

Endoscopic and radiological examinations of 300 horses were found to have various dental diseases and disorders. Oral examinations and dental care for these horses were provided using modern techniques and treatment options, resulting in improved nutrition and performance.

In many of the adult horses used in the study, sharp-edged teeth, pointed corners, teeth called steps, and ramps with a wavy appearance were observed. In such teeth, the sharp edges were corrected, the pointed corners were filed to ovalize them, and the tooth took its normal shape. The step and ramp appearance was gradually corrected by filing to allow the horse to eat, and the wavy appearance was minimized. Especially in cases with hook-shaped elongation, teeth numbered 106 and 206 were shortened gradually, and the worn occlusal surfaces of the opposing teeth were corrected. Opposite teeth were followed up for gingivitis and periodontal disease.

Malocclusions, or overgrowth, contribute significantly to the development of periodontal disease. Therefore, they should be identified and addressed as a part of many treatments. Regular care and filing of teeth help eliminate such malocclusions and prevent the development of diseases (Weijs and Dantuma, 1975).

In this study, gingivitis and periodontitis were identified as important pathological diseases affecting horses. Periodontitis was classified according to the periodontium of the tooth and the support of the periodontal ligament. It was also evaluated according to the mobility of the teeth. Teeth should be carefully examined one by one by hand for mobility. This procedure can be performed easily in horses with calm and quiet temperaments, but it is important for the safety of the practitioner to give mild sedation to the horse when there is a feeling of pain (Barone, 1997). The most basic treatment principle in periodontal disease is early diagnosis, controlling the bacteria is the main goal (Richardson et al., 1995). If the bacteria are controlled, enzyme production can be stopped, thus ensuring the continuity of periodontal support and preventing the accumulation of food around the teeth. Treatment starts with diagnosis and the treatment option may vary depending on the location in the mouth (Muylle et al., 1998). When the problem is detected in the canine and incisor teeth, it may be possible to treat the disease without tooth extraction by cleaning the teeth and removing food residues trapped between them at regular intervals, depending on the stage of the periodontal disease. However, in cases of gingivitis and periodontitis formed on the cheek teeth, the treatment protocol is determined according to gingivitis, periodontal pocket depth, tooth mobility and pain status. Especially for the treatment of ulcerations with edema and inflammation in the gingiva, oral disinfection with chlorhexidine solution was performed, yielding effective results by spraying the chlorhexidine solution directly onto the affected gingiva and tooth with the aid of a syringe.

Malocclusions, or overgrowth, contribute significantly to the development of periodontal disease. Therefore, they should be identified and addressed as a part of many treatments. Regular care and filing of teeth help eliminate such malocclusions and prevent the development of diseases (Leue, 1941). Therefore, prevention of the disease with early diagnosis should be the main goal in periodontal disease. In all cases, occlusal surfaces were exposed in the first intervention. Gingivas were checked more accurately. Tooth defects were corrected through routine filing, addressing sharp edges, hooks, pointed tips, wavy appearance, steps, and ramps. Jaw movements were balanced by adjusting the opposing teeth in the presence of existing teeth.

In general, two factors contribute to the development of periodontitis. The first of these is periodontal pocket formation and the second is diastema. Both teeth with diastema should be intervened with special diastema files and the space should be carefully widened (Ten Cate, 1998). The prognosis is generally favorable in diastemas, but a disciplined dental cleaning should be performed at regular intervals after the gap is widened. In addition, diastema areas can be resealed with dental sealant, and thus, healing of gingivitis can be accelerated. Surgical and non-surgical treatment methods were tried in patients with periodontal disease. In non-surgical methods, the main goal was oral hygiene and oral disinfection to stop the progression of the disease. Malocclusions and periodontal diseases remain problems that cannot be completely cured, although they have been a focus of equine dentistry for years. For this reason, it is argued that prophylactic treatment is more important than cure. Based on the depth of the periodontal pocket, non-surgical and easier-to-apply oral hygiene and oral disinfection, swabs were taken from the cases before isolation of the causative agent, and after the diagnosis of the source of infection, parenteral and local treatment options were applied with appropriate antibiotic selection.

Advanced periodontitis cases that could be detected with dental probes or revealed endoscopically in detail were surgically intervened. Restorative treatment and extraction of mobile teeth are mostly recommended as surgical treatment (Ramzan et al., 2001). Surgical intervention was performed according to the percentage of gingivitis and periodontal attachment loss. In cases with periodontal disease, it was found that the depth of perioceps increased due to advanced age and lack of dental care for years, and restorative treatment was mostly not possible. Restorative treatment could only be used in two cases due to the lack of sufficient dental instruments and restorative composite materials. However, in two cases, it was observed that it fell off after the chewing movements and grass consumption of the horses.

In cases with gingivitis and periodontal ligament support of more than 50%, extraction was performed as the tooth could not be saved. In these cases, agent identification and by performing antibiograms, periodontitis and infection were prevented from affecting healthy teeth, and it was noted that the horses' feeding activities were regulated. In addition, the risk of infection in tooth extraction and tooth cavities was reduced.

High numbers of streptococci, micrococci, starch hydrolyzers are isolated as normal bacterial flora in the oral cavity of horses. Moderate anaerobic bacteria, villanelle species, and hydrogen sulfide producers are isolated. At low level, lactobacillus, fusabacterium species and coliforms are isolated (Baker, 1979). In our study, bacterial identification was achieved in accordance with the literature. After isolation and identification of the agent, antibiogram tests were performed to determine to which antibiotics the agent was resistant and susceptible. Of the 300 horses used in the study, 59 horses were intervened in terms of dental disorders and dental diseases. Bacteria (*Bacteroides spp., Fusabacterium spp., Streptococcus spp., Bacillus spp., and Corynebacterium spp.*) were isolated from the gums and pockets of 18 of these 59 horses. Although the majority of the patients were elderly, the progression of non-severe periodontal disease was halted with effective antibiotics and oral hygiene.

In cases of dental trauma, the use of sedation and analgesics is necessary to achieve a good result and a detailed examination (Colyer, 1931). Loose, loose or fragmented tooth fragments should be carefully removed from the area. In cases of severe fractures, total tooth extraction is performed, and in less severe fractures, the teeth are treated conservatively. In all cases, careful tetanus prophylaxis and antibiotic treatment should be used. Accordingly, dental cracks were examined radiographically. Total tooth extraction was performed in teeth with high mobility and lost functionality. The bleeding around the teeth with low mobility was stopped and the area around the tooth was cleaned. Antibiotic treatment appropriate for the horse's age group with a tooth fracture and a diet consisting of feeds available in the facilities was applied. It was noted that all of the horses treated in this way regained their dental health.

Pulp stones, also known as calcified dentin, give the impression of a second pulp around the tooth (Huidekoper, 1891). The presence of these calculi causes pulp irrigation or inflammation and thus pulpitis is formed. They damage the pulp layer due to microcirculation. Therefore, they negatively affect dentin production and the production rate, causing tooth weakening and tooth loss, which can lead to caries in the future (Hayward, 1981). Cleaning of these stones is usually neglected by clinicians (Tomeck, 1994). Therefore, tartar formations resembling a pulp layer on the incisors, especially on the canines, were cleaned with a dental curette in all cases during the examination. During cleaning, bleeding occurred in many cases. It was noted that the tartar formation formed inflammation and edema in the gingiva. After removal of tartar formations, i.e. detartarage, the teeth were disinfected with chlorhexidine solution for 3 days. After the use of disinfectant, it was observed that tartar formations completely disappeared, inflammation and edema in the gingiva improved, and feed residues did not accumulate between the gingiva and teeth.

In cases of caries, caries were classified. The grading was decided according to the extent to which the cementum layer, enamel layer and dentin layer were affected. Mobile and painful teeth were extracted under sedation, analgesics and anesthetics. The bleeding was stopped, and antibiotic treatment was administered. Such cases resulted in prophylactic cleaning of the tooth, removal of the affected gingiva, disinfection of the oral cavity and antibiotic treatment to try to save the affected tooth. As in human dentistry, broken teeth and partially decayed teeth can be saved by filling and repairing with light-curing epoxy materials, but these are very costly applications (Hiiemae, 1978). The average age of the horses used in the study was 13.7 years, and since they were mostly older horses, positive results could not be obtained, especially in terms of odontoclastic tooth resorption and hypersementosis. In these horses, tooth root lysis due to odontoclastic tooth resorption and related dental caries resulted in the total extraction of teeth.

As in periodontal disease, it causes the formation of drainage canals and ulcers in the gingiva and therefore chewing movements become painful and limited (Weijs, 1975). Definitive diagnosis of EOTRH is possible with radiography. The disease is recognized when resorption, fracture or hypersementosis is observed in the tooth roots (Bonin, 2001). As a treatment, the affected teeth are extracted; however, after tooth extraction, it is essential to check whether any remaining fragments are present using radiography (Leue, 1941). In our study, EOTRH cases were observed in very old stallions. General oral care and dental cleaning were performed. There was a serious difficulty in eating feed in EOTRH. It was detected that the biting resistance was very weakened and the horses in this condition could not pluck soft dry grass. The affected teeth were extracted in cases where EOTRH was detected in the study. In some cases of advanced age, adequate intervention could not be performed because the number of affected teeth was high and multiple extractions were required. However, in general, tooth extraction and radiographic

examination were performed in cases where EOTRH was detected.

Extraction is generally recommended for wolf teeth and canine teeth that have not erupted completely and are found to be embedded under the mucosa (Richardson et al., 1994). The rudimentary wolf teeth embedded under the mucosa (wolf teeth numbered 105 and 205) were removed by applying sedation Domosedan (Detomidine HCL, 0.01 mg/kg) and injecting the local anesthetic agent directly into the mucosa where these teeth were located.

In conclusion, routine oral and dental care of horses at intervals of 6 months or 1 year at the latest would be beneficial in preventing dental disorders and, thus, dental diseases. It was concluded that the use of antibiotics based on bacterial identification and antibiogram testing can successfully treat many dental diseases.

Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

Ethical Approval

This study was approved by the Erciyes University Animal Experiments Local Ethics Committee (11.01.2021, 12/03 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

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Similarity Rate

We declare that the similarity rate of the article is 4% as stated in the report uploaded to the system.

Author Contributions

Motivation / Concept: GA
Design: GA
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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Isolation of *Staphylococcus* Species from Some Clinical and Food Samples and Investigation of Their Biofilm Formation Abilities

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Abstract: This study aimed to identify *Staphylococcus* species from clinical and food samples and investigate the biofilm formation ability of the isolates using various methods. Thirty clinical samples were brought to the diagnostic laboratory of the Microbiology Department at the Veterinary Faculty of Harran University, and 100 food products obtained from food outlets operating in Şanlıurfa province were designated for examination. Isolations from clinical samples were performed using bacterial culture techniques. ISO standards were followed for the analysis of food samples. All isolates were identified at the species level through verification with a VITEK-2 device. The biofilm formation ability of the isolates was explored using three different methods: Congo red agar, tube, and microplate. Fisher's Exact Test was employed for statistical analyses. As a result, 32 *Staphylococci* belonging to seven different species were isolated, with 11 from clinical samples and 21 from food samples. *Staphylococcus aureus* and *Staphylococcus pseudintermedius* were the most frequently identified species. Most of the isolates (81.25%) could form biofilms at varying levels, and the results from the methods used to detect biofilm formation were consistent. Statistical evaluation of the relationship between the biofilm-forming abilities of the isolates revealed no significant relationship between clinical and food isolates. However, a substantial relationship was found between coagulase-positive and coagulase-negative isolates. This study highlighted the ongoing potential threat of *Staphylococcus* species to human and animal health and concluded that rational control methods should be implemented to guard against bacterial contamination in products, particularly to prevent biofilm formation by taking necessary precautions during the production and marketing of foods.

Keywords: *Biofilm, Food, Infection, Isolation, Staphylococcus.*

Bazı klinik örneklerden ve gıda numunelerinden *Staphylococcus* türlerinin izolasyonu ve biyofilm oluşturma yeteneklerinin araştırılması

Özet: Bu çalışmayla klinik ve gıda örneklerinden *Staphylococcus* türlerinin tanımlanması ve bu etkenlerin biyofilm oluşturma yeteneklerinin farklı yöntemlerle araştırılması amaçlandı. Harran Üniversitesi Veteriner Fakültesi Mikrobiyoloji Ana Bilim Dalı Tanı Laboratuvarına getirilen 30 adet klinik örnek ile Şanlıurfa'da faaliyet gösteren gıda satış yerlerinden temin edilen 100 adet gıda numunesi çalışmada inceleme örneği olarak kullanıldı. Klinik örneklerden izolasyonlar bakteriyel izolasyon yöntemleriyle gerçekleştirilirken gıda numunelerinden izolasyonlar için ISO standartları kullanıldı. Tüm izolatlar VİTEK-2 cihazıyla doğrulanarak tür seviyesinde tanımlandı. İzolatların biyofilm oluşturma yetenekleri, kongo kırmızısı agar, tüp ve mikropleyt olmak üzere üç farklı yöntemle araştırıldı. İstatisiksel analizler için Fisherin Exact Testi uygulandı. Sonuç olarak klinik örneklerden 11, gıda numunelerinden 21, toplamda 32 adet Stafilokok izolasyonu gerçekleştirildi. Bu etkenlerin yedi farklı türe ait olduğu tespit edildi. *Staphylococcus aureus* ve *Staphylococcus pseudintermedius* en çok tanımlanan türler oldu. İzolatların büyük bir bölümünün çeşitli seviyelerde olmak üzere biyofilm oluşturabildikleri görüldü. Biyofilm oluşumunun tespiti için kullanılan yöntem sonuçlarının birbirleriyle uyumlu olduğu görüldü. İzolatların biyofilm oluşturma yetenekleri arasındaki ilişkinin araştırıldığı istatistiksel değerlendirmede, klinik ve gıda izolatlarının arasında anlamlı bir ilişki tespit edilemezken, koagulaz pozitif ve negatif izolatlar arasında anlamlı bir ilişkinin var olduğu belirlendi. Bu çalışma ile *Staphylococcus* türlerinin insan ve hayvan sağlığına yönelik potaniyel tehditlerinin devam ettiği görüldü. Özellikle gıdaların üretiminden pazarlanmasına geçen sürede gerekli önlemlerin alınarak ürünlerin bakteri kontaminasyonlarından uzak tutulması ve özellikle biyofilm oluşumlarının engellenmesi için akılcı mücadele yöntemlerinin uygulanması gerektiği kanaatine varıldı.

Anahtar Kelimeler: *Biyofilm, enfeksiyon, gıda, izolasyon, Stafilokok.*

Introduction

The genus *Staphylococcus*, which is widespread in the environment (Heo et al., 2020), is taxonomically classified within the *Eubacteria* kingdom, *Firmicutes* phylum, *Bacilli* class, *Bacillales* order, and *Staphylococcaceae* family (Yüksekdağ and Baltacı, 2013). As of 2019, *Staphylococci*, encompassing 53 species and 27 subspecies (Heo et al., 2020), are divided into two main groups: coagulase-positive and coagulase-negative *Staphylococci*, based on their ability to produce coagulase. While coagulase production was once linked to pathogenicity, this understanding has evolved with the realization that many coagulase-negative *Staphylococci* are responsible for infections. Consequently, coagulase production is no longer considered a reliable marker for pathogenicity (Songer and Post, 2012). However, coagulase production can indicate high virulence (Quinne et al., 2011). It has been reported that 40 species and 24 subspecies of *Staphylococci* include coagulase-negative members. Except for opportunistic-pathogenic species, coagulase-negative *Staphylococci* are harmless and do not cause disease. They can be found in fermented foods and utilized as starter cultures. Coagulase-positive *Staphylococci* exhibit the highest virulence within the genus (Heo et al., 2020).

In 1881, *Staphylococci* were identified as a causative agent of infection and developed resistance to penicillin and later to methicillin over time. *Staphylococcus aureus* has become the most significant health issue as a nosocomial pathogen worldwide (Kireçci, 2009). *Staphylococci* can be found as commensals in the normal skin and mucosal flora of living organisms. Therefore, human intervention has been the primary cause of food contamination. In addition to many food products, particularly ready-to-eat foods, raw meat and meat products, raw milk, and dairy products pose risks concerning *Staphylococcus aureus* enterotoxins (Muratoğlu et al., 2015). Besides local purulent infections, they can also cause mastitis in cattle, pyemia in sheep, and botryomycosis in horses. *Staphylococci* lead to exudative epidermitis in pigs, as well as ear infections, conjunctivitis, skin inflammations, urinary tract infections, bone infections, and wound infections in dogs and cats. They can also cause joint, tendon sheath, bursa disorders, endocarditis, and yolk sac infections in poultry (Songer and Post, 2012; Quinn et al., 2011). In humans, *Staphylococci* induce similar infections and sepsis. In particular, *Staphylococcus aureus* is often responsible for infections and poisoning due to the enterotoxins it produces in foods (Aygen et al., 1997; Mubarak, 2021; Xiying et al., 2024). Enterotoxigenic *Staphylococci* can especially be isolated from protein-rich foods of animal origin (Erol and İseri, 2004).

Biofilm formation is recognized as one of the most important virulence factors of microorganisms (Temel and Eraç, 2018). A biofilm is a complex structure of bacterial colonies embedded in an exopolysaccharide matrix that adheres to foreign surfaces in living organisms (Sharma et al., 2023). Its structure includes intricate molecules such as proteins, polysaccharides, extracellular DNA, water, and ions. Bacteria can adhere to both living and non-living surfaces and colonize them. These colonies may also contain mixed species (Temel and Eraç, 2018). Biofilms increase antimicrobial resistance in the host and can trigger inflammatory responses, potentially leading to chronic inflammation (Aydemir, 2018). In a comprehensive review study on this subject, researchers have reported that biofilms cause approximately 70% of all human microbial infections and lead to various diseases, including non-healing chronic wounds, endocarditis, periodontitis, cystic rhinosinusitis, fibrosis, meningitis, osteomyelitis, kidney infections, and infections related to prostheses and implantable devices (Sharma et al., 2023). In food contamination, microorganisms may also produce biofilms (Öksüztepe and Demir, 2019). Deficiencies in sanitation procedures at food establishments can lead to biofilm formation on various surfaces (Sharma et al., 2023). Biofilms formed in production facilities promote bacterial colonization and protect these bacteria from many unfavorable conditions (Temel and Eraç, 2018).

This study aimed to investigate the presence of *Staphylococcus* species in samples from various clinical cases and food materials offered for sale in Şanlıurfa region, as well as to explore the biofilm formation ability of isolated *Staphylococci* using different methods.

Material and Methods

Samples

Thirty clinical samples from various clinical cases were brought to the diagnostic laboratory of Harran University, Faculty of Veterinary Medicine, Department of Microbiology between June 2023 and July 2024. Additionally, 100 food samples obtained from Şanlıurfa province during the same period were used as examination samples for agent isolation (Table 1 and 2).

Table 1. Clinical samples and sample numbers.

Origin of the clinical samples (From..)	Sample numbers according to the species of the animals				Total
	Cat	Dog	Cattle	Chicken	
Wound infection	4	4	-	-	8
Eye infection	3	1	-	-	4
Ear infection	2	3	-	-	5
Mastitis	-	-	12	-	12
Beak infection	-	-	-	1	1
Total	**9**	**8**	**12**	**1**	**30**

Isolation of *Staphylococcus* species from clinical and food samples

Clinical specimens were inoculated directly onto mannitol salt phenol red agar (MSA) (Merck, Germany), and the Petri dishes were incubated under aerobic conditions at 37 °C for 24 hours (Quinn et al., 2004; Quinn et al., 2011).

Table 2. Food samples and sample numbers.

Food samples	Numbers of food materials
Adana kebab	3
Cake	12
Frozen cake	9
Rice pilaf with vermicelli	3
Melt cheese	4
Cheese	12
Doner is made of chicken meat	13
Butter	2
Salad	13
Chocolate cake	8
Stuffed meatballs	2
Rice pilaf	14
Kebab	5
Total	**100**

Twenty-five grams of the food samples were weighed and added to 225 ml of Buffered Peptone Water (BPW) (Merck, Germany) using a sterile spatula. After homogenizing with a stomacher (Smasher-Biomerieux, France) for 20 seconds, the medium was incubated in an incubator (Memmert, Germany) under aerobic conditions at 37 °C for 24 hours. Following this pre-enrichment, subcultures were made by inoculating 100 µl of the incubated and non-diluted BPW onto Baird-Parker Agar (BPA), a selective-differential medium prepared by adding egg yolk-tellurite emulsion (Merck, Germany) to the agar base of Baird-Parker (Merck, Germany). Petri dishes were incubated under aerobic conditions at 37 °C for 24 hours, and the incubation period was extended to 48 hours for suspected colonies. Typical colonies on BPA were evaluated based on their black color and halo formation (ISO 6888-1:2021).

Colonies from clinical and food sources were subcultured onto tryptic soy agar (TSA) (Merck, Germany) for purification and incubated under aerobic conditions at 37 °C for 24 hours. The pure colonies were examined using Gram staining (Merck, Germany), and Gram-positive cocci underwent a catalase test (Bactident, Merck, Germany). Subsequently, suspected *Staphylococcus* species were identified, and a coagulase test was performed (ISO 6888-1:2021; Quinn et al., 2004; Quinn et al., 2011).

Identification of colonies at the species level

Confirmation and species-level identification of the isolated colonies suspected to be *Staphylococci* were performed using a VITEK-2 device (Biomerieux, France).

Biofilm determination by the CRA method

To evaluate the phenotypic biofilm-forming properties of the isolates, staphylococcal strains, along with positive and negative control strains, were obtained from the TSA using a quarter of a loop and transferred into sterile glass tubes containing 10 ml of tryptic soy broth (TSB) (Merck, Germany). The tubes were incubated for 24 hours at 37 °C under aerobic conditions without shaking. After incubation, cultures were inoculated onto CRA, which was prepared by adding 50 g of sucrose, 37 g of brain heart infusion agar, and 0.8 g of Congo red agar (Merck, Germany) per liter. Biofilm formation was assessed using two different methods: the single colony method (smear plate technique) and the dropping method. A loopful of culture was inoculated onto CRA plates for the single colony method. In the dropping method, 0.1 ml of liquid culture was pipetted onto five different points of the CRA plates using an automatic pipette. CRA plates were incubated at 37 °C for 24 hours under aerobic conditions. After incubation, isolates forming dry, crystalline black colonies were categorized as strong or moderate biofilm producers based on the intensity of color formation. Isolates forming red or pink colonies were considered weak or no-biofilm producers. The CRA method was performed in triplicate for each isolate (Gündoğ et al., 2023).

Determination of biofilm by tube method

In the Tube Adherence Method, staphylococcal isolates were transferred from TSA petri dishes into sterile glass tubes containing 10 ml TSB and incubated at 37 °C under aerobic conditions for 24 hours. After incubation, *Staphylococcus* strains that formed or did not form biofilms on the walls of the glass tubes were washed twice with phosphate-buffered saline (PBS) (Merck) (pH: 7.3), and the tubes were stained with 0.1% crystal violet for 1 hour. Following the staining, the tubes were washed twice with PBS to remove excess dye and air-dried. All tubes were evaluated by comparing the staining results to the reference strains used as controls. Biofilm formation was considered positive if a visible film was observed on the walls or bottom of the tubes. Biofilm production was graded as follows: biofilm negative (-), weak biofilm (+), moderate biofilm (++), and strong biofilm (+++) formation. The studies were repeated three times for each isolate (Christensen et al., 1982).

Determination of biofilm by microplate method

The isolates were transferred to a TSB liquid medium and incubated at 37 °C under aerobic conditions for 24 hours. Twenty microliters of each culture were added to microplate test wells (Greiner BioOne, Austria), which contained 230 µl of TSB in triplicate, and then incubated in an aerobic incubator at 37 °C for 24 hours. After incubation, the wells were discarded and washed three times with 350 µl of sterile distilled water. To fix the cells, 250 µl of methanol was added to each well and held for 15 minutes. The microplates were subsequently discarded and allowed to dry in an inverted position at room temperature for approximately 12 hours. The biofilm layer was stained by adding 250 µl of crystalline violet solution to each well for 5 minutes at room temperature. The wells were then rewashed under running water. After thoroughly removing the excess dye, the microplates were dried at room temperature. The dye bound to the cells was solubilized by adding 33% glacial acetic acid (Merck, Germany) to each well, and the optical density (OD) was measured at 570 nm using a microplate reader (VersaMax, USA). The cut-off OD (ODC) value was determined based on the wells containing only medium and served as a negative control. The results were evaluated

according to Table 3, based on average OD values from three replicates (Stepanović et al., 2004).

Table 3. Criteria for evaluation of the biofilm-forming abilities level.

Biofilm-forming abilities level	Calculation of ODC
No Biofilm	OD ≤ ODC
Weak	ODC
Medium	2× ODC < OD ≤ 4× ODC
Strong	OD > 4X ODC

Statistical analysis: The effect of the clinical or food origin of *Staphylococcus* isolates on their ability to form biofilm, specifically, whether the relationship between the origin of the isolates and their biofilm-forming ability was statistically significant, was analyzed using Fisher's Exact Test method, creating a 2x2 contingency table. The significance level was set at $\alpha = 0.05$. Additionally, in a separate statistical study, the effect of the isolates being coagulase positive or negative on their biofilm-forming ability, specifically, whether the relationship between coagulase enzyme production and biofilm formation was significant, was also analyzed using Fisher's Exact Test method, creating a 2x2 contingency table. The significance level was again accepted as $\alpha = 0.05$.

Reference strains: In all tests, *Staphylococcus aureus* ATCC 25923 served as the reference control strain for coagulase-positive *Staphylococci*, while *Staphylococcus epidermidis* ATCC 35984 was utilized for coagulase-negative *Staphylococci*.

Results

Regarding the isolation and identification of staphylococcal species revealed that thirty-two isolates were identified as suspected *Staphylococci* based on their Gram staining and biochemical characteristics, with 11 isolates derived from 30 clinical samples and 21 from 100 food samples. The isolates were confirmed and identified at the species level using VITEK 2. All suspected isolates were verified (see Tables 4 and 5). The isolation rates of the species-level identified isolates are detailed in Table 6.

Table 4. Test results of isolates from clinical samples.

No	Orgine	Mannitol fermentation in MSA	Gram staining	Catalase	Coagulase	VITEK
1	A wound infection of a dog	+	+	+	+	*Staphylococcus aureus*
2	An eye infection of a dog	+	+	+	-	*Staphylococcus sciuri*
3	A wound infection of a cat	-	+	+	+	*Staphylococcus pseudintermedius*
4	An ear infection of a dog	-	+	+	+	*Staphylococcus pseudintermedius*
5	A skin infection of a cat	-	+	+	+	*Staphylococcus pseudintermedius*
6	An ear infection of a cat	-	+	+	+	*Staphylococcus pseudintemedius*
7	An eye infection of a cat	-	+	+	+	*Staphylococcus pseudintemedius*
8	A case of mastitis in a cow	+	+	+	+	*Staphylococcus aureus*
9	A case of mastitis in a cow	+	+	+	+	*Staphylococcus aureus*
10	The beak infection of a chicken	-	+	+	-	*Staphylococcus epidermidis*
11	An ear infection of a dog	-	+	+	-	*Staphylococcus epidermidis*

Table 5. Test results of isolates from food samples.

No	Origin of the food samples	Growth in BPA	Gram stain	Catalase	Coagulase	VITEK
1	Adana kebab	black	+	+	-	*Staphylococcus lentus*
2	Cake	black	+	+	-	*Staphylococcus lentus*
3	Rice pilaf with vermicelli	black with a halo	+	+	+	*Staphylococcus pseudintermedius*
4	Melt cheese	black with a halo	+	+	+	*Staphylococcus pseudintermedius*
5	The meat of the chicken	black with a halo	+	+	+	*Staphylococcus aureus*
6	Cake	black with a halo	+	+	+	*Staphylococcus aureus*
7	Cake	black	+	+	-	*Staphylococcus xylosus*
8	Frozen cake	black	+	+	-	*Staphylococcus warneri*
9	Butter	black	+	+	-	*Staphylococcus sciuri*
10	Cake	black	+	+	-	*Staphylococcus xylosus*
11	Salad	black	+	+	-	*Staphylococcus xylosus*
12	Chocolate cake	black	+	+	-	*Staphylococcus xylosus*
13	Doner is made of chicken meat	black	+	+	-	*Staphylococcus xylosus*
14	Cheese	black with a halo	+	+	+	*Staphylococcus aureus*
15	Stuffed meatballs	black	+	+	-	*Staphylococcus warneri*
16	Doner is made of chicken meat	black with a halo	+	+	+	*Staphylococcus aureus*
17	Rice pilaf	black with a halo	+	+	+	*Staphylococcus pseudintermedius*
18	Kebab	black	+	+	-	*Staphylococcus lentus*
19	Chocolate cake	black	+	+	-	*Staphylococcus xylosus*
20	Cheese	black	+	+	-	*Staphylococcus warneri*
21	Cake	black with a halo	+	+	+	*Staphylococcus aureus*

Table 6. Isolation rates of *Staphylococcus* species.

Species	Clinical samples (n=30)	Food samples (n=100)	Total (n=130)
Staphylococcus pseudintermedius	5 (16.6%)	3 (3%)	8 (6.15%)
Staphylococcus aureus	3 (10%)	5 (5%)	8 (6.15%)
Staphylococcus xylosus	0 (0%)	6 (6%)	6 (4.61%)
Staphylococcus lentus	0 (0%)	3 (3%)	3 (2.3%)
Staphylococcus warneri	0 (0%)	3 (3%)	3 (2.3%)
Staphylococcus sciuri	1 (3.33%)	1 (1%)	2 (1.53%)
Staphylococcus epidermidis	2 (6.66%)	0 (0%)	2 (1.53%)
Total	11 (36.6%)	21 (21%)	32 (24.6%)

Table 7. Comparative results of biofilm-forming abilities of the isolates.

No	Origin of the samples (From..)	Isolated species	Biofilm-forming abilities of the isolates		
			CRA method	Tube method	Micropleyt method
1	A wound infection of a dog	*Staphylococcus aureus*	Strong	Strong	Strong
2	An eye infection of a dog	*Staphylococcus sciuri*	Moderate	Moderate	Medium
3	A wound infection of a cat	*Staphylococcus pseudintermedius*	Moderate	Weak	Weak
4	An ear infection of a dog	*Staphylococcus pseudintermedius*	Low	Weak	Weak
5	A skin infection of a cat	*Staphylococcus pseudintermedius*	Low	Moderate	Medium
6	An ear infection of a cat	*Staphylococcus pseudintermedius*	Moderate	Moderate	Medium
7	An eye infection of a cat	*Staphylococcus pseudintermedius*	Low	Weak	Medium
8	A mastitis case of a cattle	*Staphylococcus aureus*	Strong	Strong	Strong
9	A mastitis case of a cattle	*Staphylococcus aureus*	Strong	Strong	Strong
10	A beak of a chicken	*Staphylococcus epidermidis*	Strong	Moderate	Strong
11	An ear infection of a dog	*Staphylococcus epidermidis*	Strong	Weak	Strong
12	An Adana kebab	*Staphylococcus lentus*	No biofilm	Negative	No biofilm
13	A cake	*Staphylococcus lentus*	No biofilm	Negative	No biofilm
14	A rice pilaf with vermicelli	*Staphylococcus Pseudintermedius*	Low	Moderate	Weak
15	A melt cheese	*Staphylococcus pseudintermedius*	Low	Moderate	Weak
16	A meat of chicken	*Staphylcoccus aureus*	Strong	Strong	Strong
17	A cake	*Staphylococcus aureus*	Strong	Strong	Strong
18	A cake	*Staphylococcus xylosus*	Moderate	Strong	Weak
19	A frozen cake	*Staphylococcus warneri*	No biofilm	Negative	No biofilm
20	A butter	*Staphylococcus sciuri*	Moderate	Moderate	Medium
21	A cake	*Staphylococcus xylosus*	Moderate	Weak	Medium
22	A salad	*Staphylococcus xylosus*	Moderate	Weak	Weak
23	A chocolate cake	*Staphylococcus xylosus*	Moderate	Moderate	Weak
24	A doner made of chicken meat	*Staphylococcus xylosus*	Moderate	Moderate	Weak
25	A cheese	*Staphylococcus aureus*	Strong	Strong	Strong
26	A stuffed meatball	*Staphylococcus warneri*	No biofilm	Negative	No biofilm
27	A doner made of chicken meat	*Staphylococcus aureus*	Strong	Strong	Strong
28	A rice pilaf	*Staphylococcus pseudintermedius*	Moderate	Weak	Weak
29	A kebab	*Staphylococcus lentus*	No biofilm	Negative	No biofilm
30	A chocolate cake	*Staphylococcus xylosus*	Moderate	Weak	Weak
31	A cheese	*Staphylococcus warneri*	No biofilm	Negative	No biofilm
32	A cake	*Staphylococcus aureus*	Strong	Moderate	Strong
R1	*Staphylococcus aureus* ATCC 25923		Strong	Strong	Strong
R2	*Staphylococcus epidermidis ATCC* 35984		Strong	Strong	Strong

R: Reference

Biofilm test results: The comparative test results of the methods used to determine the biofilm-forming abilities of the isolates are presented in Table 7.

Statistical analysis findings

There was no statistically significant relationship between the food or clinical origin of the isolates and their ability to form biofilms (P>0.05). A statistically significant relationship was found between the production of the coagulase enzyme by the isolates and their ability to form biofilms (P<0.05).

Discussion

Many studies have been conducted to isolate *Staphylococcus* spp. from clinical samples over time. Uysal and Kırkan (2012) isolated 42 staphylococcal agents from 60 wound swab samples, 30 of which were coagulase-positive and 12 were coagulase-negative. Among the coagulase-positive isolates, 22 were *S. aureus*, eight *S. intermedius*, and among the coagulase-negative isolates, seven were *S. hyicus*, two *S. sciuri*, two S. *haemolyticus*, and one *S. cohnii* subsp.

cohnii. Molnar et al. (1994) reported that some species, such as *S. hominis* and *S. epidermidis*, can adapt to and establish significant dominance on human skin and mucosa. Öcal et al. (2022) reported that *S. hominis* and *S. epidermidis* were the most frequent isolates among *Staphylococci* in their study. In another study, *S. aureus* was isolated from 28, *Streptococcus uberis* from 21, and *Streptococcus dysgalactiae* from 8 of 100 milk samples obtained from cattle with subclinical mastitis. No bacterial growth was detected in 43 samples (Genç and Kaya, 2015). In another study, 75 *S. aureus* strains were isolated from 512 samples of horses with skin infections (Chiers et al., 2003). In a study examining 158 milk samples from 7 dairy cow herds in East and West Azerbaijan regions of Iran using bacteriological and molecular methods, the isolation of many *Staphylococcus* species from 113 samples was reported. The researchers identified five of the 113 isolates as *S. aureus* and 108 as coagulase-negative *Staphylococci* (CoNS). They identified 44 of the 108 CoNS species as *S. haemolyticus*, 17 as *S. chromogenes*, 11 as *S. epidermidis*, *S. arneri*, and *S. cohnii*, six as *S. simulans*, four as *S. hominis*, three as *S. capitis*, and one as *S. xylosus*. They reported that only *S. haemolyticus*, *S. warneri*, and *S. chromogenes* species were isolated from clinical mastitis cases (Hosseinzadeh and Saei, 2014). In another study by Göçmen et al. (2018), researchers used 7% sheep blood agar and BPA to isolate staphylococcal species from various clinical materials of 67 animals. They applied catalase and coagulase tests for the Gram-positive cocci derived from pure bacterial colonies and performed species-level identification using the VITEK 2 device.

In this study, the MSA medium was utilized as a selective medium for isolating agents from clinical specimens, similar to approaches taken by other researchers (Tanış and Gülseren, 2020). The MSA medium, which provides a high-density salt environment, also displayed high selectivity. However, mannitol fermentation was positive only for *S. aureus* and varied for other coagulase-positive isolates. This result indicates that mannitol fermentation is not exclusive to the presence of coagulase. In veterinary medicine, *S. aureus* and *S. pseudintermedius*, which are the main coagulase-positive *Staphylococci* species, and *S. chromogenes* and *S. epidermidis*, which are coagulase-negative, are reported to cause significant diseases (Göçmen et al., 2018). In this study, four different species were isolated from clinical samples: *S. pseudintermedius* 5 (16.6%), *S. aureus* 3 (10%), *S. sciuri* 1 (3.33%), and *S. epidermidis* 2 (6.66%) (Table 4). The differences among the isolation rates of staphylococcal species reported in similar studies may have resulted from factors such as the location where the examination samples were collected, the number of samples, geographical diversity, the type of examination samples, storage conditions, processing methods, and methodological variations in the analyses (Akyol et al., 2023). In this study, *S. pseudintermedius* and *S. aureus* exhibited the highest isolation rates of 6.15%. When the characteristics of these species were analyzed, they differed from other species by being coagulase-positive. Although this suggests that coagulase is an important factor that increases the presence of these microorganisms as dominant species compared to other coagulase-negative species, some researchers reported that biofilm-producing CoNS strains can frequently be isolated from infections (Keskin et al., 2003).

Numerous studies have been conducted over the years to isolate *Staphylococci* from food samples. In these studies, a wide variety of *Staphylococci* types were isolated and identified from both animal and non-animal origin food samples. These isolates were classified according to their coagulase properties (Akyol et al., 2023; Güngören et al., 2022; Resch et al., 2008; Tanış and Gülseren, 2020). In this study, the BPA medium, which provides selectivity and discrimination, was used to isolate *Staphylococci* from food samples. *Staphylococci* were isolated from 21 out of 100 food samples. Compared to previous studies, this isolation rate is lower than that from cheese samples (Güngören et al., 2022; Tanış and Gülseren, 2020). This situation may be attributed to several factors, including the active role of human elements in cheese production and marketing processes, as well as non-compliance with cheese storage and transportation conditions. Furthermore, it is believed that these differences in staphylococcal species isolation rates may be analogous to the previously mentioned reasons for the disparities in isolation rates in clinical materials.

In this study, black-colored colonies grown on the BPA medium, which is utilized to isolate staphylococcal species from food samples, were classified based on the presence or absence of white-bright halos. Additional tests confirmed that colonies with white-bright halos were coagulase-positive, while those without halos were coagulase-negative staphylococcal species (Table 5). Although the manufacturer did not highlight the white-bright halo as a distinguishing feature for detecting coagulase in staphylococcal species, a previous study (Tanış and Gülseren, 2020) suggests that this halo may offer preliminary information about the coagulase activity of the isolates. This implies a possible correlation between lipase (forming a bright ring) and lecithinase (forming turbidity) activities of staphylococcal isolates detectable in the BPA medium and the coagulase activities of these isolates. In a study conducted in Istanbul, this feature of BPA medium was utilized to investigate the microbiological quality of cooked chicken doners. Colonies grown on the medium were classified into black-colored typical colonies with a transparent halo and atypical colonies. The presence of coagulase-positive *Staphylococci* was confirmed by the coagulase test (Alçay, 2019).

In several studies, researchers reported varying rates of isolation for different *Staphylococci* species. Resch et al. (2008) isolated 330 coagulase-negative *Staphylococci* from foods including fermented fish, meat, cheese, and sausages. It was reported that 137 isolates were *S. xylosus*, 106 were *S. carnosus*, 64 were *S. equorum*, 11 were *S. piscifermantans*, 10 were *S. succinus*, and two were *S. condimenti*. In another study of minced meat samples, it was noted that six of the 56 isolates were identified as *S. aureus*, while 50 were classified as CoNS. Of the CoNS species, 36 were *S. xylosus*, seven were *S. hominis*, three were *S. capitis*, two were *S. epidermidis*, and two were *S. cohnii*. The same researchers obtained a total of 41 isolates from chicken meat samples,

all classified as CoNS species: 13 of these were *S. simulans*, 10 were *S. cohnii*, nine were *S. capitis*, six were *S. hominis*, two were *S. auricularis*, and one was *S. haemolyticus* (Gündoğan and Ataol, 2012). In this study, six different species were isolated from food samples and identified as follows: *S. xylosus* 6 (6%), *S. aureus* 5 (5%), *S. pseudintermedius* 3 (3%), *S. lentus* 3 (3%), S. *warneri* 3 (3%), and *S. sciuri* 1 (1%) (Table 5). Although staphylococcal agents were obtained from various species in these studies, the isolation rates varied significantly.

Since staphylococcal species can be found in nearly every environment that negatively impacts human and animal health, researchers have frequently investigated the virulence properties of these agents. The ability of staphylococcal species to form biofilms has also been a focus of many studies, as this enables the agent to survive and maintain its activity. Öcal et al. (2022) examined the ability of staphylococcal isolates to form biofilms on CRA media. They reported no difference in effectiveness between smear and drip methods for detecting biofilm formation; however, the drip method made the results easier to interpret. They also compared the methods used to detect biofilm formation and determined that the microplate method detected significantly more biofilm than the CRA method. A study conducted at Erciyes University researchers reported that 35% of *S. aureus* isolates could form biofilm in CRA, 36% in microplate, and 94.4% in both methods (Gündoğ et al., 2023). Similarly, a study conducted in India found that 79% of 84 *S. aureus* isolates analyzed in studies comparing biofilm diagnostic results were able to produce biofilms using the microplate method and 75% with CRA methods (Jain and Agarwal, 2009). Mathur et al. (2006) investigated the biofilm formation properties of 152 CoNS isolates using CRA and microplate methods. They observed biofilm formation in 8 (5.2%) with CRA and in 82 (53.9%) with the microplate method, stating it was more sensitive. They also isolated *Staphylococci* from blood, infected vehicles, and skin surfaces, reporting high rates of biofilm-forming ability.

Manandhar et al. (2021) stated they could detect biofilm at a higher rate with the microplate method (42.1%) compared to the tube method (31.8%) and CRA method (20.1%). Kord et al. (2018) found biofilm formation in 53.6% of 41 *S. epidermidis* isolates by tube and microplate and 24.4% by CRA. Cafiso et al. (2004) also explored the biofilm formation ability of coagulase-negative staphylococcal isolates isolated from infections by the CRA method and showed that 83% could form biofilm. Demir and Battaloğlu İnanç (2015) evaluated 65 coagulase-negative *Staphylococci* and 127 *S. aureus* isolates from clinical samples using three detection methods concurrently and reported that the results were comparable concerning biofilm detection, with no statistically significant difference between the methods. Some studies in the literature include similar comparisons, along with studies indicating that the microplate method has a sensitivity comparable to that of other methods (Demir and Battaloğlu İnanç, 2015; Gündoğ et al., 2023; Jain and Agarwal, 2009); there are also studies suggesting it may be more sensitive (Manandhar et al., 2021; Mathur et al., 2006). This study investigated the biofilm formation abilities of 32 staphylococcal isolates from various species derived from food and clinical samples using three different methods. The levels of biofilm formation determined by the methods employed were quite similar across all isolates (Table 7). Considering the laboratory infrastructure, it was concluded that any of these methods could be preferred. Researchers attribute differences in biofilm formation to several factors. It has been reported that various factors, such as medium composition (Dhanawade et al., 2010), glucose availability and concentration, hydrogen ion concentration, and the presence of H_2O_2, may influence biofilm formation (Nostro et al., 2014). In this study, no statistically significant relationship was found between clinical and foodborne isolates regarding their ability to form biofilms. However, a statistically significant relationship was identified between the production of the coagulase enzyme by staphylococcal isolates and their ability to form biofilms. The literature does not establish a definite causal relationship between coagulase production and biofilm formation. Nonetheless, there is strong belief that coagulase-positive species generally possess a greater capacity for biofilm production. There are instances where coagulase-negative species also exhibit significant biofilm production. In both groups, biofilm production is determined by a wide range of phenotypic and genotypic factors (Nostro et al., 2014), making it difficult to interpret the dynamics of biofilm formation.

Conclusion

As a result, it was observed that the isolation of *Staphylococcus* spp. from food and clinical samples can be performed easily and frequently. This finding reinforces the importance of maintaining strict hygiene practices throughout all stages of producing and marketing food products from farm to table to prevent microbial contamination. Biofilms can play a significant role in the persistence, chronicity, and recurrence of infections. One of the important challenges in treating such infections is the increasing resistance of biofilm-forming microorganisms to host immune defenses and antimicrobial agents. Regardless of their origins, the biofilm-producing potential of most food and clinical staphylococcal isolates emphasizes the necessity of rational practices in the fight against these agents. Although the general characteristics of biofilm formation mechanisms are similar across many microorganisms, species-specific traits necessitate tailored evaluation and intervention approaches. The ability of these agents to survive in diverse environments contributes to the risk of food contamination and results in significant economic consequences due to antibiotic resistance, ultimately posing a broad threat to public health.

Ethical Approval

This study was approved by the Harran University Animal Experiments Local Ethics Committee (21.12.2023, 2023/008/06 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

Conflict of Interest

The authors stated they had no real, potential, or perceived conflict of interest.

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

Ruminantlarda Rumenin Önemi

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Özet: Çalışmada ruminantların rumenlerinin bu hayvanlar için ne kadar önemli olduğunu ortaya koymak amaçlanmıştır. Ruminantlardan elde edilen süt ve et insanların beslenme kaynakları açısından oldukça önemlidir. Ruminantların ön midelerinden olan rumen ruminantlar için çok önemlidir. Fakat ruminantların doğduklarında rumenleri faaliyet gösterememektedir. Rumen içerisindeki mikroorganizmalar ile birlikte ruminantlar selüloz gibi maddeleri sindirebilirler. Rumen mikroorganizmaları tüm gıdaların sindirim faaliyetleri neticesinde ruminantların büyük ölçüde enerji ihtiyacını sağlayan Uçucu Yağ Asitlerine (UYA) dönüşürler. Ayrıca azot kaynakları da rumen mikroorganizmalarının faaliyetleri sonrasında mikrobiyel proteinlere dönüştürülmektedir. Rumen mikroorganizmaları B grubu vitaminleri de sentezleyerek ruminantların faydalanmasını sağlamaktadır.
Rumen mikroorganizmaların yasayabilmeleri için rumenin içerisinin dengede kalması gerekir. Rumenin içerisinin sıcaklık, besin, pH gibi değerliliklerinin standart aralıklarda tutulmalıdır. Rasyonlarda bulunan yemlere ek olarak rasyona canlı mikroorganizmalar ve aromatik yağlar gibi katkı maddeleri eklemek rumen standartlarını sağlamaya yardımcı olur. Rumen içerisine yerleştirilen sensörlerle rumenin pH, sıcaklık gibi değerlerin anlık ölçümleri yapılabilmektedir. Böylece hayvanlar sürekli gözlem altında tutularak daha sağlıklı ve verimli yetiştirilmektedir.
Anahtar Kelimeler: *Besleme, Mikroorganizma, Rumen, Ruminant.*

The Importance of the Rumen in Ruminants

Abstract: In this study, it is aimed to reveal how important the rumens of the ruminants are for these animals. Milk and meat obtained from ruminants are very important sources of nutrition. The rumen, which is the fore stomach of ruminants, is very important for ruminants. However, when ruminants are born, their rumens cannot function. Ruminants, along with the microorganisms in their rumen, can digest substances such as cellulose. As a result of the digestive activities of rumen microorganisms, all foods are transformed into Volatile Fatty Acids (VFA), which provide a large part of the energy needs of ruminants. In addition, nitrogen sources are converted into microbial proteins after the activities of rumen microorganisms. Rumen microorganisms also synthesize B group vitamins, enabling ruminants to benefit from them.
In order for rumen microorganisms to survive, the inside of the rumen must remain in balance. The rumen's internal values such as temperature, nutrients and pH should be kept within standard ranges. In addition to the feeds in the rations, adding additives such as live microorganisms and aromatic oils to the ration helps to ensure rumen standards. Instant measurements of values such as rumen pH and temperature can be made with sensors placed inside the rumen. In this way, animals are kept under constant observation and are raised healthier and more efficiently.
Keywords: *Microorganism, Nutrition, Rumen, Ruminant.*

Giriş

Ruminantlardan elde edilen et, süt, yün ve deri gibi ekonomik yönden değerli verim özellikleri ile hayvancılık sektörünün vazgeçilmez unsurlarıdır (Öztürk, 2008). Geviş getiren hayvanlar yapısal karbonhidratları kullanabilecekleri enerjiye dönüştürmekten sorumlu mikroorganizmaları barındıran muhteşem rumen yapıları nedeniyle benzersiz çiftlik hayvanı türleridir (Jami ve ark., 2014).

Bitkilerdeki yapısal karbonhidratlar olarak ifade edilen sindirimi zor karbonhidrat kaynakları; ruminantlar tarafından sahip oldukları çok bölmeli mideleri ve içerisindeki ortam sayesinde enerji kaynağı olarak kullanabilmektedir. Tüketilen sindirimi zor karbonhidratlar rumen içindeki çeşitli mikroorganizmalar tarafından parçalanmaktadır (Xu ve ark., 2021).

Böylelikle insanlar tarafından sindirilemeyen selüloz vb. yapısal karbonhidratları rumen ortamında sindirerek insanların tüketebileceği yüksek kaliteli proteinlere dönüştürebilmektedir (Moe ve Tyrrell, 1979).

Dünya nüfusu arttıkça ve sürdürülebilir şekilde üretilen gıdaya olan ihtiyaç daha da önemli hale geldikçe, geviş getiren hayvanların bir gıda kaynağı olarak kullanılmasındaki önem daha da dikkat çekici olmaktadır (Liebe ve ark., 2020).

Ülkemizdeki 2004-2023 yılları arasındaki TÜİK (2023) verilerine göre ruminant sayıları ve ruminantlardan elde edilen hayvansal ürünlere bakıldığında yıllara göre değişim Tablo 1 ve Tablo 2'de görülebilmektedir.

TÜİK (2023) verilerine bakıldığında Türkiye içerisindeki son 20 yıl içerisindeki hem ruminant sayısındaki hem de ruminantlardan elde edilen hayvansal ürünlerin arttığı görülebilmektedir. Bu durum ruminantların beslenmesini daha da önemli kılmaktadır. Hayvansal kaynakların üretiminin arttırılması ve insanların tüketimine daha fazla sunulması için hayvan başına verimin arttırılması adına daha etkili besleme yapmak olumlu sonuçlar doğuracaktır. Bunun için ülkemizde büyük sayılarda yetiştiriciliği yapılan ruminantların beslenmesinde besin madde gereksinimlerinin iyi bir şekilde öğrenilmesi gerekmektedir. Bunun sağlanabilmesi için ağızdan giren her türlü besinin ilk muamele edildiği rumenin öneminin anlaşılabilmesini daha da önemli kılmaktadır.

Tablo 1. 2004-2023 yılları arasında Türkiye' deki ruminant varlığı (TÜİK, 2023).

Yıl	Toplam	Sığır	Manda	Koyun	Keçi
2004	41 984 338	10 069 346	103 900	25 201 155	6 609 937
2005	42 453 194	10 526 440	104 965	25 304 325	6 517 464
2006	43 232 086	10 871 364	100 516	25 616 912	6 643 294
2007	42 870 109	11 036 753	84 705	25 462 293	6 286 358
2008	40 514 391	10 859 942	86 297	23 974 591	5 593 561
2009	37 688 958	10 723 958	87 207	21 749 508	5 128 285
2010	40 837 450	11 369 800	84 726	23 089 691	6 293 233
2011	44 793 487	12 386 337	97 632	25 031 565	7 277 953
2012	49 804 866	13 914 912	107 435	27 425 233	8 357 286
2013	53 042 643	14 415 257	117 591	29 284 247	9 225 548
2014	55 830 403	14 223 109	122 114	31 140 244	10 344 936
2015	56 051 937	13 994 071	133 766	31 507 934	10 416 166
2016	55 551 460	14 080 155	142 073	30 983 933	10 345 299
2017	60 417 333	15 943 586	161 439	33 677 636	10 634 672
2018	63 338 302	17 042 506	178 397	35 194 972	10 922 427
2019	66 353 810	17 688 139	184 192	37 276 050	11 205 429
2020	72 270 597	17 965 482	192 489	42 126 781	11 985 845
2021	75 555 321	17 850 543	185 574	45 177 690	12 341 514
2022	73 289 541	16 851 956	171 835	44 687 888	11 577 862
2023	68 946 415	16 421 256	161 749	42 060 470	10 302 940

Rumen Gelişimi: Tek mideli hayvanlardan farklı olarak ruminantlarda rumen, retikulum, omasum ve abomasum

olmak üzere 4 mide bulunmaktadır (Patterson, 1992). Bu mideler bezsiz mideler olan rumen, retikulum ve omasum ile bezli mide bölümü olan abomasumdur. Ruminantların tüketmiş oldukları gıdalar rumene geldiklerinde rumen içerisinde bulunan mikroorganizmalar tarafından fermentasyona uğratıldığı ifade edilmiştir (Cunningham ve Klein, 2008).

Yeni doğan buzağıların rumen gelişimleri sütten kesim öncesinde hayvanlara verilen gıdaların kalite ve türüne bağlı olarak şekillenmektedir. Süt ve süt ikame yemlerinin rumen gelişimine faydaları yeterli ve kaliteli kuru yemin alımına kıyasla daha sınırlı olduğu ve kuru yemin rumen gelişimini desteklediği belirtilmiştir. Konsantre yemlerin propiyonik asit ve bütirik asit nedeniyle rumen epitel gelişimini desteklediği, kaba yemlerin ise asetik asit nedeniyle rumen kas gelişimi, motilitesi, rumen hacminin artması, ruminasyonun uyarılması ve salyanın ön midelere aktarılmasında olumlu sonuçlar doğurduğu ifade edilmiştir (Gümüş ve Küçükersan, 2018).

Tablo 2. 2004-2023 yılları arasında ruminantlardan elde edilen hayvansal ürünler (TÜİK, 2023).

Yıl	Kırmızı et(Ton)	Çiğ süt(Ton)	Yapağı(Ton)	Kıl (Ton)	Tiftik (Ton)
2004	736 074	10 679 406	45 972	2 716	304
2005	737 220	11 107 897	46 176	2 654	302
2006	754 625	11 952 099	46 776	2 728	274
2007	796 000	12 329 789	46 752	2 536	237
2008	828 527	12 243 040	44 166	2 238	194
2009	846 939	12 542 186	40 270	2 002	174
2010	879 819	13 543 674	42 823	2 607	200
2011	969 443	15 056 211	46 586	3 062	194
2012	1 067 553	17 401 262	51 180	3 570	200
2013	1 099 081	18 223 713	54 784	4 902	260
2014	1 123 059	18 630 859	58 402	5 460	280
2015	1 187 018	18 654 682	59 196	5 569	325
2016	1 303 648	18 489 161	58 168	5 518	340
2017	1 440 327	20 699 893	63 315	5 797	356
2018	1 661 767	22 120 716	66 428	5 999	371
2019	1 740 616	22 960 379	70 588	6 162	380
2020	1 785 952	23 503 790	79 754	6 401	463
2021	1 952 038	23 200 306	85 916	6 700	468
2022	2 191 625	21 563 492	84 885	6 393	417
2023	2 384 047	21 481 567	80 195	5 684	347

Buzağılar yaşamlarının ilk birkaç haftasında sütten elde edilen besin maddelerine bağlı olarak beslenmektedir. Rumen gelişimini tetiklenerek rumen içerisindeki anaerobik mikrobiyal ekosistem oluşturularak fermentasyon mekanizmasının oluşturulmasına ihtiyaç olmaktadır (Baldwin ve ark., 2004).

Doğumdan sonra; rumen içeresinde bulunması gereken mikroorganizmalardan bazıları doğumdan en az 24 saat sonra yeni doğan buzağıların rumeninde tespit edilir. Rumen mikrobiyotasının çoğunluğu doğumdan sonraki birkaç gün içinde aeroblardan veya fakültatif anaeroblardan zorunlu anaeroblara mikroorganizmalara doğru değişir (Jami ve ark., 2013). Tespit edilen mikroorganizmalar; ruminantların gastrointestinel sistem kanalındaki ilk mikroorganizma kolonizasyonu çevre ile annenin dışkısı, salyası, tükürüğü, derisi ve vajinal kanalından kaynaklandığı ileri sürülmektedir (Meale ve ark., 2016).

O'Hara ve ark. (2020) çalışmalarında rumenin doğumdan sütten kesim sonrasına kadar bakteri kolonizasyonunu izlemiş ve rumen mikroorganizmalarının bileşiminin doğumdan 21 gün sonra sabitlendiğini gözlemlemişlerdir.

Ayrıca; Górka ve ark. (2011) 5 günlük buzağılar üzerinde yaptıkları çalışmada bir grup buzağıya tam süt diğer grup buzağılara ise soya proteini içeren süt ikamesi verilerek 21 gün süren bir çalışma gerçekleştirmişlerdir. Elde ettikleri verilerde tam yağlı süt ile beslenen buzağılarda soya proteini içeren ikame yemi ile beslenen buzağılara göre rumen ve

retikulum papillaları ile rumen kas kalınlığı ve ağırlığının arttığını belirtmişlerdir.

Rumen Ortamı: Erişkin bir sığırdaki rumen abdominal boşluğun hemen hemen %75' ini kaplamaktadır ve vücut ağırlığının neredeyse %6' sına denk gelen bir ağırlığa sahiptir. Sığır rumeni ortalama olarak 150-200 litre arasında bir hacme sahiptir (Cunningham ve Klein, 2008).

Rumen iç yüzeyinde rumen papilları bulunmaktadır. Toplam rumen papilla yüzey alanı doğrudan UYA emilim kapasitesiyle ilişkili olduğundan rumen performansında önemli bir faktör olarak kabul edilir (Bannink ve ark., 2008). Rumen papillaları rumen mikroorganizmalarının yapısal karbonhidratları parçaladıktan sonra açığa çıkan UYA emilmesi, taşınması ve metabolize edilmesinde merkezi bir rol oynamaktadır (Ji ve ark., 2021).

Rumen bol miktarda bakteri, protozoa ve mantar barındıran karmaşık bir mikrobiyal ekosistemdir (Zhou ve ark., 2017). Islam ve Lee (2019) çalışmalarında; rumendeki mikroflorada bakteriler, mantarlar, arkler, siliyalı protozoalar ve virüsler gibi çok çeşitli türler vardır ve bu türler rumen içerisinde anaerobik şartlar altında birbirleriyle sıkı bir bağ halinde yaşamlarını sürdürmektedir.

Rumen içerisinde var olan mikroorganizmaların yaşamlarına devam edebilmeleri ve ruminal sindirimin düzgün bir şekilde sürmesi için rumen ortamındaki standartlar önemlidir. Bu standartlar bazıları**;** rumen iç sıcaklığının 38-41 °C ve rumen pH' sının ise 5,5-7,5 arasında olmasıdır (Murphy ve ark., 1982; Lederberg, 1992). Rumen pH' sının dengesi oldukça önemlidir. Rumen pH' sındaki değişimler hayvanlarda sindirimde aksaklıkların olmasına ve hayvanın genel sağlık durumunun bozulmasına neden olabilmektedir (Özel ve Sarıçiçek, 2009).

Rumendeki mikroorganizmalar tarafından üretilen UYA (asetik asit, propiyonik asit ve bütirik asit) üretilmeye devam edebilmeleri için rumen pH seviyesinin standartlar dahilinde olmadır. Bunun içinde rumen pH' sının tamponlanarak standart aralıklarda tutulması gerekmektedir (Steele ve ark., 2011).

Rumendeki mikroorganizmaların yaşayabilmesi için gerekli olan pH' nın stabil kalabilmesinde tükürük salgısının sahip olduğu bikarbonat önemli rol oynamaktadır ve devamlı olarak rumen pH' sını tamponlanmaktadır. Böylelikle rumende yaşayan mikroorganizmalar için ideal bir ortam sağlanmış olmaktadır (Garipoğlu ve Sarıçiçek, 2000).

Salya rumeni tamponlamak için önemlidir ve bu yüzden çiğneme gıdaların mikrobiyal sindirimi için optimum rumen pH sının sürdürülmesinde anahtar rol oynar. Ayrıca çiğneme sırasında yemin fiziksel olarak parçalanması, mikrobiyal kolonizasyonu ve küçük partiküllerin rumenden alt gastrointestinal sisteme geçişini kolaylaştırır (De Boever ve ark., 1990). Çiğneme; tükürük salgısını, partikül boyutunu küçültmek, mikrobiyal sindirimi ve sindirilmemiş materyalin rumenden geçişini teşvik etmek için kritiktir (Beauchemin, 2018).

Çiğnemenin sağlamış olduğu bu özelliklerin devamlılığının sağlanabilmesi için; ruminant rasyonlarında çiğneme aktivitesini arttırıcı yapısal karbonhidrat içeriği olan yemler olmalıdır. Böylelikle çiğnemeyle birlikte tükürük salgısı tetiklenerek rumeni tamponlanması sağlanacaktır. Ayrıca rumen hareketliliğini ve rumen içerisindeki besinlerin birbirleriyle ve mikroorganizmalarla karışmasını sağlayarak rumen ekosisteminin stabil olabilmesine katkı sağlayacağı ifade edilmiştir (Zebeli ve ark., 2012).

Rumen Mikroorganizmaları ve Fermantasyon: Rumen içerisinde var olan mikroorganizma sayıları ve türleri ile alakalı olarak; Cunningham ve Klein (2008), rumen sıvısındaki mikroorganizmalar 10^{10}-10^{11}/ml civarında bakteri ve 10^{5}-10^{6}/ml kadar da protozoa bulunmaktadır. Rumendeki bu mikroorganizmaların büyük kısmı selülolitik, amilolitik, preotolitik ve lipolitiktir. Ruminantların rumenlerinde baskın olan mikroorganizmalar eurobakterler (10^{9}-10^{11}/ml hücre), arkler (10^{5}-10^{8}/ml), siliatalar (10^{4}-10^{6}/ml) ve bakteriyofaj (10^{8}-10^{9}/ml) gibi birçok mikroorganizma ile oluşan mikrobiyal flora vardır (Millen ve ark., 2016). Rumen içerisindeki rumen sıvısının 1 ml' de en az 30 baskın tür bakteri bulunmaktadır. Bu bakteriler 10^{10}-10^{11}/ml sayısı kadar bulunmaktadır. Yine rumen sıvısının mililitresinde 40 kadar baskın protozoa türü bulunmaktadır. Bu protozoa türlerinin sayısı 10^{5}-10^{7}/ml kadardır. Ayrıca rumen sıvısında 5 baskın mantar türü de vardır sayısı ise $<10^{5}$/ml kadardır (Orpin ve ark., 1997).

Buzağıların yaşamlarının ilk gününde fibrolitik bakterilerden *Fibrobacter succinogenes* ve *Ruminococcus flavefaciens* buzağıların rumenlerine kolonize olduğu tespit edilmiştir (Guzman ve ark., 2015).

Ruminant rasyonlarında kaba yem önemli bir yer teşkil eder. Ruminant rasyonlarındaki kaba yem içerisindeki selülozu sindiren bakteriler rumen içerisindeki mikrofloranın önemli bileşenleridirler. Bu selülolitik bakterilerden en önemli 3 tanesi *Fibrobacter succinogenes, Ruminococcus albus* ve *Ruminococcus flavefaciens* bakterileridir (Regensbogenova ve ark., 2004). Selülozu parçalayan diğer bazı bakteriler şu şekildedir; *Fibrobacter succinogenes*, *Ruminococcus albus, Ruminococcus flavefaciens, Clostridium longisporum* ve *Eubacterium celluloselvens* (*Cillobacterium cellulosolvens*) (Theodorou ve France, 1993).

Rumende bulunan mikroorganizmaların yoğunluğu, çeşitliliği ve fonksiyonları rasyon içeriği, doğum, mevsim, coğrafi konum, besleme stratejisi, yem katkı maddeleri, yemin alım seviyesi, büyüme evresi, ruminant türü ve ruminantın fizyolojik durumu gibi birçok faktörden etkilenebilir (Lan ve Yang, 2019). Buna bağlı olarak; Henderson ve ark. (2015) geniş bir coğrafi aralıkta çoklu faktörlerin rumen mikrobiyal topluluk kompozisyonu üzerinde etkili olduğunu tespit etmişlerdir.

Miron ve ark. (2001) yapmış oldukları çalışmalarında rumende bulunan baskın selülolitik bakteriler olan Fibrobacter succinogenes, Ruminococcus flavefaciens ve Ruminococcus albus bakterilerin rumen papillarına adhezyon süreçlerini 4 aşamaya ayırmışlar; 1. aşama hareketsiz bakterilerin substrata taşınması, 2. Aşama bakteriyel glikokaliksin yapıcı elemanlarının hakim olduğu substratın korunmasız bölgelerine bakterinin başlangıçta spesifik olmayan adhezyonu, 3.aşama substrat oluşumunun ligandlar yada adhezinler üzerinden bakterilerdeki bazı yapılar kompleks yapılı sellulozomlar, fimbria bağlantıları, selüloz bağlayıcı proteinlerin, glikokaliks ve selüloz bağlayıcı alan enzimleri tarafından özel adhezyon ve 4.aşama

substrattaki sindirilecek doku üzerinde çoğalmış bakterilerin tutunması olarak 4 aşama halinde açıklamışlardır.

Ruminantlar yemlerde bulunan kompleks yapılı lignoselülozik karbonhidratları ve selülozu parçalayabilecek olan enzimleri kendi vücutları tarafından üretemezler (Gruninger ve ark., 2019). Bahsi geçen enzimleri üretemedikleri için; ruminantlar yedikleri yapısal karbonhidratları (selüloz, hemiselüloz gibi karbonhidratları) bağırsaklardaki enzimler ile sindiremez. Bunun yerine rumende bulunan mikroorganizmalar olan (protozoonlar, bakteriler ve mantarlar) tarafından fermentasyona uğratarak bu yapısal karbonhidratları sindirebilir (Tekce ve Gül, 2014; Gharechahi ve ark., 2021); rumende bulunan mikroorganizmalar sayesinde gerçekleştirilen sindirim sonucunda mikrobiyal protein sentezi ve UYA' ni üretebilmektedir (Phakachoed ve ark., 2012; Liu ve ark., 2019).

Rumen mikroorganizmaları bitki hücre duvarında bulunan ve kolay sindirilmeyen bileşenleri parçalamak için ürettikleri çeşitli hidroliz enzimleri ile birliklerdeki hücre duvarı unsurlarını parçalayabilmektedirler (Wang ve McAllister, 2002). Rumendeki mikroorganizmalar bitkilerdeki polisakkaritleri parçalayabilmek için çeşitli kompleks enzim kokteylleri üretirler, glukozid hidralaz, karbonhidrat esteraz ve polisakkarit liaz bunlarla birlikte ek olarak katalitik olmayan karbonhidrat bağlama modülleri lignoselülozik yapılara bağlanılarak mikrobiyel sindirimde rol alırlar (Gharechahi ve ark., 2021).

Kompleks yapıda olan selülozların sindirimi rumendeki mikrobiyal sindirim ile olur. Rumendeki selülozun mikroorganizmalar tarafından sindirilmesi ile birlikte UYA' leri (Garipoğlu ve Sarıçiçek, 2000; Argov-Argaman ve ark., 2012); vitaminler (Russell ve Rychlik, 2001); karbondioksit, metan, amonyak ve mikrobiyel proteinler oluşur (Dijkstra ve ark., 1998).

Rasyondaki kaba/konsantre yem oranı ve formu UYA ile rumen pH üzerinde etkili olduğu gibi rumen mikroorganizmaları üzerine de etkilidir (Maia ve ark., 2007).

Rasyonda yeterli düzeyde amonyak bulunduğunda selülolitik bakteriler çoğalabilirken, pH 6.0' ın altına indiğinde hem selülolitik bakterilerin sayısında bir azalma olduğu görülmekte hem de selüloz parçalanmasında bir azalma meydana gelmektedir. Böylelikle bitkisel yemler olan kaba yemlerdeki sindirilebilme oranı düşmektedir (Orskov, 1992).

Alataş ve Umucalılar (2011) sindirim sistemi açısından tek ve çok mideliler arasındaki en önemli farkın çok midelilerin rumeni içerisinde bulunan anaerobik mikroorganizmalar olduğunu ifade etmişlerdir. Bu mikroorganizmalar uygun rumen koşullarında sindirime fayda sağlamakta ve B kompleks vitaminler ile tüm esansiyel olan aminoasitleri de mikrobiyel protein olarak üretmektedirler.

Rumen Ortamını Anlamaya Yönelik Bazı Uygulamalar: Ruminantlardaki genel durumu stabil hale getirmek, tedavi etmek ve verimi iyileştirmek için rumeni konu alan birçok uygulama yapılmış ve yapılmaya da devam etmektedir. Rumeni konu alan bu uygulamalardan bazıları ise şu şekildedir;

Rasyona canlı maya kültürü ilave edilmesi: Çiftlik hayvanlarının verimliliklerini arttırmak için rasyonlarına ilave edilen canlı mayalar yem katkı maddesi olarak maya kültürleri *Saccharomyces cerevisiae* kullanılmıştır (Gümüş ve Şehu, 2016). *Saccharomyces cerevisiae* fermantasyon ürünleri dahil olmak üzere birçok farklı yem katkı maddesi süt buzağılarında ölüm oranını azaltmak, sağlık ve büyümeyi iyileştirmek için kullanılmış. Saccharomyces cerevisiae mayası anaerobik olarak fermente edildiğinde amino asitler, lipitler, nükleotidler, B vitaminleri ve organik asitler ürettiği ifade edilmiş (Deters ve ark., 2018).

Canlı maya kültürlerinin rumende asetik asit ve bütirik asit miktarını arttıran propiyonik asit miktarını azaltan etkisi yaptığı anlaşılmıştır (Kumar ve ark., 1997). Rasyonlara ilave edilen canlı maya kültürleri rumendeki selulolitik bakteri sayısını arttıracağı ve rumende amonyak seviyesini düşüreceği ifade edilmiştir (Chaucheyras-Durand ve Fonty, 2001).

Maya kültürü ürünlerinin süt sığırlarına verilmesi ile rumen ortamını değiştirerek ham selüloz sindirimiyle ilişkili mikroorganizma popülasyonunu artırabilir (Mullins ark., 2013). Ruminantlarda rasyona ilave edilen canlı maya kültürleri rumen içerisindeki amonyağı kullanan mikroorganizmaların sayısını arttırarak rumen içerisindeki toplam amonyak miktarını azaltmakta ve mikrobiyel proteinlerin miktarını da arttırmaktadır (Erasmus ark., 1992). *Saccharomyces cerevisiae*' nin fermantasyon ürünlerinin rumen mikrobiyotasını olumlu yönde etkilediği ve rumen morfolojisini iyileştirdiği görülmüştür (Kumar ve ark., 1997). *Saccharomyces cerevisiae* fermantasyon ürünleri dahil olmak üzere çeşitli maya ürünlerinin rumen fermantasyonunu ve süt üretimini iyileştirdiği gösterilmiştir (Acharya ve ark., 2017).

Özellikle *Bacillus licheniformis*, *Saccharomyces cerevisiae* ve bunların bileşiklerinin etkileri rumen mikrobiyal topluluğunun azot kullanımını arttıracağı ve besi kuzularının büyümesi için faydalı olduğu, rumen fermantasyon modelini etkileyebileceği ifade edilmiştir (Jia ve ark., 2018). Kuzulardaki çalışmalara benzer olarak buzağılar üzerindeki bir çalışmada da; maya ürünlerinin süt buzağılarında ve emziren ineklerde oksidatif durumu ve bağışıklık tepkisini modüle ettiği de gösterilmiştir (Alugongo ve ark., 2017).

Rasyona aromatik yağ ve ekstrakların ilave edilmesi: Geviş getiren hayvanlarda rasyona ilave edilen aromatik yağ asitleri; nişasta ve protein sindirimi, amonyak ve UYA üretimi üzerine etkili olduğu ve ayrıca metan emisyonlarını azalttığı ifade edilmiştir (Cobellis ve ark., 2016).

Ruminant beslemede rasyona ilave edilecek olan kekik yağı ile birlikte rumendeki amonyak miktarı azalarak by-pass aminoasit miktarının artmasına neden olacağı ifade edilmektedir. Ayrıca ilave dilen kekik yağı rumende üretilen metan ve karbondioksit üretimini azaltabileceği ve böylelikle sera gazı salınımını azaltabileceği ifade edilmiştir (Canbolat ve ark., 2010).

Yapılan bir çalışmada üzüm çekirdeği ekstraktının verilmesinin rumen fermentasyonu üzerine etkisini inceledikleri çalışmada; üzüm çekirdeği ekstraktlarının farklı dozlarda uygulanmasında ruminal pH, propiyonat üretimi, asetatın propiyonata oranı, toplam protozoon sayısı,

amonyak azotu konsantrasyonu ve yemlerin kuru madde sindirilebilirlik düzeyinde istatistiksel açıdan herhangi bir farklılık olmadığını fakat deneme grubu ile kontrol grubu kıyaslandığında toplam UYA, asetat ve bütüratın günlük üretim miktarlarındaki artışların olduğu anlaşılmış ve bu durum istatiksel olarak önemli bulunmuştur (Öztürk ve ark., 2011). Üzüm çekirdeği ile ilgili yapılan başka bir çalışmada ise üzüm çekirdeğinin geniş spektrumlu olarak birçok gram negatif ve gram pozitif bakterilere karşı antibakteriyel özellik gösterdiği tespit edilmiştir. (Jayaprakasha ve ark., 2003).

Ruminantlarda rumen ortamı üzerine yapılan diğer bir araştırmada; rasyona ilave edilen kekik yağı, nane yağı, portakal yağı, karanfil yağı ve tarçın yağının toplam UYA, asetik asit ve propiyonik asit üzerine önemli düzeyde düşürücü etki gösterdiği tespit edilmiştir. Aynı çalışmada en etkili sonucu kekik yağı vermiştir ve UYA miktarındaki azalma rumen mikroorganizmaları üzerine antimikrobiyal etki göstererek fermentasyonun sınırlandırılmasının aromatik yağlar ile ilgili olduğu ifade edilmiştir (Canbolat, 2012). Curabay ve ark. (2019) yaptıkları benzer bir çalışmada ruminant rasyonlarına sarımsak yağı, nane yağı, kekik yağı ve portakal yağının ilave edilmesinin rumen pH' sı dışında rumen sıvısı metabolitleri ile rumendeki fermentasyonu azaltıcı yönde etki ettiği görülmüştür. Bu çalışmada araştırıcıların elde ettikleri bulgulara göre en fazla azaltıcı etkiye sahip olan aromatik yağlar sırasıyla kekik, nane, sarımsak ve portakal yağları olmuştur. Aromatik yağların en etkili olduğu doz 1200 mg L^{-1} RS olarak bulunmuş ve bunun sonucunda da ruminant rasyonlarına düşük dozlarda aromatik yağ ilave edilmesinin ruminantlarda verim performansını düşürücü etki göstermeyeceği kanısına varılmıştır.

Canbolat ve ark. (2011) yapmış oldukları çalışmada 3 adet kıvırcık ırkı koç kullanılmış olup bu koçlara rumen kanülü uygulaması yapılmıştır. Bu hayvanlardan elde edilen rumen sıvılarına nane yağı, kekik yağı ve portakal yağı ilave edilerek çalışmayı gerçekleştirmişler ve yapılan analizlere göre asetik asit ve propiyonik asit oranındaki en yüksek oran kontrol grubunda en düşük oran ise kekik yağında bulunmuştur.

Busquet ve ark. (2005) yapmış oldukları çalışmaya göre karvakrol maddesinin rasyonda 300 mg/lt rumen sıvısındaki düzeyin artması rumendeki pH ve bütirik asit seviyesini arttırdığı fakat toplam UYA miktarını ve asetik asit İle propiyonik asit oranını ise düşürdüğünü tespit etmişlerdir.

Benchaar ark. (2007) Holstein süt inekleri üzerinde yaptıkları çalışmaya göre; aromatik yağlar ve aromatik yağ bileşiklerinin sadece fenolik bileşikleri olan karvakrol, timol ve eugenol ruminal fermentasyonu etkilediğini göstermiştir. Aromatik yağlar ve aromatik yağların bileşikleri rumen mikrobiyal fermentasyonu üzerinde yararlı etki göstermemiştir. Fenolik bileşikler temel olarak rasyon fermente edilebilirliğini azaltarak ve UYA modelini daha az propiyonat ve daha fazla bütirata kaydırarak antimikrobiyal aktiviteler sergilemiştir. Aynı etkilerin in vivo ifade edilmesi durumunda, fenolik bileşikler, yem kullanımının etkinliğini artırmak için süt ineği beslenmesinde kullanım için faydalı olmayabileceği ifade edilmiştir.

Yu ve ark. (2020) Crossbreed Boer cinsi keçilerden elde ettikleri rumen sıvıları üzerine timol ilave edilerek yapılan in vitro çalışmada timol ilavesinin bakteriler, arkeler ve protozoa dahil olmak üzere rumen mikroorganizmaları üzerine etkilerine bakılmış. Rumendeki üretimin sürdürülmesi ve rumen mikroorganizmalarındaki genel değişiklikler dikkate alındığında metan üretiminin azaltılması için etkili timol dozu 200 mg/L olarak belirlenmiş. Ayrıca timol ilavesinin; arkea ve protozoaya kıyasla rumen bakterileri üzerinde daha güçlü bir etki yaptığı ifade edilmiştir.

Ruminantların yaşadığı coğrafyadaki deniz seviyesine göre yüksekliğin rumende yaşayan mikroorganizmalar üzerindeki etkilerini araştırmak üzere Fan ve ark. (2020) tarafından yapılmış olup bu çalışmaya göre**;** 2800 metre, 3700 metre ve 4700 metre yükseklikte beslenen Yak sığırlarında Christensenellaceae R-7 group, Ruminococcus 1, Romboutsia, Alloprevotella, E. coprostanoligenes ve Clostridium dahil olmak üzere bazı potansiyel probiyotiklerin, yüksek seviyelerde yaşayan yakların rumeninde zenginleştiğini göstermiştir. Bu farklılığın soğuk havalar, hipoksi ve yüksek lifli ot üretimi ile karakterize edilen yüksek rakımlı bir ortamdan kaynaklandığı ifade edilmiştir (Fan ve ark., 2020).

Canbolat (2012) araştırması ve daha önceki çalışmalar ile yaptığı kıyaslamaya göre; aromatik yağlar ruminant beslemede yemden yararlanmayı düşürerek verim performansını azaltabileceğini ifade etmiştir. Bu nedenle ruminant beslemede aromatik yağların kullanılmasında önce yemden yararlanma, verim düzeyi ve çevresel etkileri göz önünde bulundurularak hangi aromatik yağın ne kadar kullanılması gerektiği tespit edilmelidir.

Rumen ortamını anlamaya yönelik uygulanan teknolojik yenilikler: Çok farklı firma tarafından üretilmiş olan ve rumen içerisine yerleştirilen birçok aparatlar ile rumen içerisinde var olan sıcaklık, pH vb. rumen içi değerlerin ölçümüne olanak sağlanmaktadır. Böylelikle rumen içerisinde anlık olarak alınan verilere göre hayvanın genel sağlık durumu hakkında bilgiler edinilmiş olup bunlara göre de kararlar alınabilmektedir. Hayvan besleme açısından Subakut Rumen Asidozu (SARA) gibi birçok beslenme hastalığına daha yakalanmadan müdahale edilmesine beslenme hastalıkları gibi birçok hastalığın olumsuz sonuçlarının kısmi ya da tamamen ortadan kaldırılmasına yardımcı olabilmektedir.

Geliştirilen bir ekipman ile hayvanların rumen içerisine yerleştirilen bir aparat ile birlikte hayvanın rumen pH ve sıcaklık ölçümleri anlık olarak yapılmakta ve veri olarak 15 dakikada bir olmak kaydı ile sistem üzerinden gösterilmekte olduğu ifade edilmiştir. Ayrıca cihazın rumen içerisinde herhangi bir zararı olmadan 5 ay boyunca veri toplayabileceği de aktarılmıştır (Mottram ve ark., 2010). Zhang ve ark. (2016) rumen içi pH değerinin gerçek zamanlı olarak ölçülebilmesi ve verilerin aktarılmasını sağlayabilen bir cihaz geliştirmişler (Zhang ve ark., 2016).

Kim ve ark. (2019) süt sığırlarının mastitis hastalığı sebebiyle yaşadıkları sağlık sorunları ve oluşan ekonomik kayıplara dikkat çekmek istemişler. Mastitisi erken olarak tespit edebilmek için rumen içerisinde sindirilebilir gerçek zamanlı ölçüm yapabilen bir biyosensörü rumen içerisine yerleştirmişler. Çalışma 50 adet inekte gerçekleştirilmiş.

Çalışma 6 ay süreyle uygulanmış ve çalışma kapsamında normal rumen sıcaklık aralık olarak 38-39.4 ^{o}C ifade edilmiştir. Ortalama olarak kabul edilen rumen sıcaklık değerlerinden yüksek olan sıcaklık değerleri yerleştirilen biyosensör tarafından algılanarak uyarı sinyali gönderilmiş. Gelen yüksek sıcaklık değerlerinin mastitis ile alakalı olup olmadığını doğrulamak için hayvanlara California Mastitis Test (CMT) uygulaması yapılarak mastitis varlığı doğrulanmış. Çalışma kapsamında ifade edildiğine göre rumende zamanla sindirilerek kaybolan bu biyosensörlerle hem insan gücünün kullanımını azaltan hem de hayvanlarda meydana gelebilecek olan stresi azaltabilen bu uygulama sayesinde mastitisi erken tespit etmenin mümkün olabileceği ifade edilmiş.

Sonuç

Sonuç olarak anlaşılmaktadır ki; rumen ruminantların sindirim sistemi içerisinde yer alan en önemli bileşenlerden bir tanesidir. Yeni doğan buzağılarda gelişmemiş olan rumenin gelişiminin sağlanabilmesi için hayvanın daha buzağı döneminde dikkatli bir şekilde beslenmesi gerekmektedir. Rumen gelişim aşamalarının devamında da rumen ortamında yer alan mikroorganizmaların sindirim üzerinden direkt etkisinin bilinmesinden dolayı rumen ortam standartlarının korunmasına önem gösterilmelidir.

Rumen ortam standartlarının devamlılığının sağlanabilmesi için hayvanların rasyonlarına çok farklı yem katkı maddeleri ilave edildiği görülmüştür. Aynı zamanda rumen içerisindeki sıcaklık, pH gibi değerlerin sürekli ve güncel olarak takip edilebilmesi için rumen içerisine sensörlerin yerleştirildiği ve bu sayede teknolojik imkânlar ile birlikte rumen sağlığının kontrol altında tutulmasına çalışıldığı fark edilmektedir.

Tüm bu bilgiler ışığında rumen içerisindeki ortam koşullarının istenilen ölçülerde kalması için ileride yapılacak olan çalışmalar ile teknolojik gelişmelere bağlı olarak daha doğru ve daha uzun süreli sonuçlar veren uygulamalarda yapılabilecektir. Bu sayede sağlıklı bir rumen ortamı ile sağlıklı ve yüksek verimli hayvanlar üretilebilecektir.

Çıkar çatışması

Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Benzerlik Oranı

Makalenin benzerlik oranının sisteme yüklenen raporda belirtildiği gibi % 3 olduğunu beyan ederiz.

Yazar Katkıları

Fikir/Kavram: SH, İSÇ
Tasarım: SH, İSÇ
Denetleme/Danışmanlık: SH, İSÇ
Veri Toplama ve/veya İşleme: SH, İSÇ
Analiz ve/veya Yorum: SH, İSÇ
Kaynak Taraması: SH, İSÇ
Makalenin Yazımı: SH, İSÇ
Eleştirel İnceleme: SH, İSÇ

Kaynaklar

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ

The Use of Melatonin Implants as a Method for Estrus Suppression in Cats

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Abstract: Melatonin implants have gained attention as an effective and reversible method for estrus suppression in female cats. As seasonally polyestrous animals, cats exhibit cyclic reproductive behavior influenced by photoperiod, with melatonin playing a crucial role in regulating the hypothalamic-pituitary-gonadal axis. Subcutaneous melatonin implants mimic short daylight conditions, leading to a temporary suppression of ovarian activity and estrus-related behaviors. Studies have demonstrated that melatonin implants extend the interest interest interval for 2–4 months with minimal adverse effects. However, variations in estrus suppression duration and occasional induction of ovulation and pseudopregnancy highlight individual variability in response to treatment. Compared to traditional hormonal contraceptives, melatonin offers a safer alternative without the severe side effects associated with progestins and androgens. Despite these advantages, concerns regarding application challenges, cost, and temporary efficacy persist. Further research is needed to refine dosage protocols, evaluate long-term effects, and address individual response differences. Melatonin implants represent a promising tool for feline reproductive management, particularly for cat breeders and owners seeking a non-surgical alternative to sterilization.

Keywords: *Contraceptive implant, Estrus suppression, Feline reproduction, Melatonin, Non-surgical contraception, Ovarian activity, Reproductive cycle.*

Kedilerde Östrus Baskılama Yöntemi Olarak Melatonin İmplantlarının Kullanımı

Özet: Melatonin implantları, dişi kedilerde östrus baskılanması için etkili ve geri döndürülebilir bir yöntem olarak kullanılmaktadır. Mevsimsel poliöstrik hayvanlar olan kediler, üreme döngülerini ışık periyoduna bağlı olarak düzenler ve bu süreçte melatonin, hipotalamik-hipofizer-gonadal aksın kontrolünde kritik bir rol oynar. Subkutan melatonin implantları, kısa gün uzunluğu koşullarını taklit ederek ovaryum aktivitesinin ve östrusla ilişkili davranışların geçici olarak baskılanmasını sağlar. Yapılan çalışmalar, melatonin implantlarının interöstrus aralığını 2–4 ay boyunca uzattığını ve minimal yan etkilere sahip olduğunu göstermektedir. Ancak, östrus baskılanma süresinde görülen bireysel farklılıklar ve bazen ovulasyon ile psödogebelik oluşumu, değişken bireysel yanıtları ortaya koymaktadır. Geleneksel hormonal kontraseptiflerle karşılaştırıldığında, melatonin, progestinler ve androjenlerle ilişkili ciddi yan etkiler olmadan daha güvenli bir alternatif sunmaktadır. Bununla birlikte, uygulama zorlukları, maliyet ve geçici etkililik gibi konular endişe yaratmaktadır. Melatonin dozaj protokollerinin iyileştirilmesi, uzun vadeli etkilerin değerlendirilmesi ve bireysel yanıt farklılıklarının ele alınması için daha fazla araştırmaya ihtiyaç vardır. Melatonin implantları, özellikle cerrahi sterilizasyona alternatif arayan kedi yetiştiricileri ve sahipleri için umut vadeden bir üreme yönetimi aracı olarak öne çıkmaktadır.

Anahtar Kelimeler: *Melatonin, östrus baskılanması, kedi üremesi, kontraseptif implant, reprodüktif döngü, cerrahi olmayan kontrasepsiyon, ovaryum aktivitesi.*

Introduction

Cats are seasonal polyestrous animals, and ovulation in these species is induced mating. If mating does not happen and ovulation is not induced, cats will eventually return to estrus. During estrus, various behavioral changes such as vocalization, frequent urination, rolling, rubbing against their owner, and an increased desire to escape outdoors can be observed. These symptoms, along with the potential pregnancies resulting from escaping, pose significant challenges for cat owners (Goericke-Pesch, 2010; Johnston et al., 2001; Kutzler, 2007). Due to these issues, cat owners seek various methods to control reproduction (Goericke-Pesch, 2010; Kutzler &Wood, 2006). Estrus suppression can be achieved through surgical and non-surgical methods. Surgical methods permanently eliminate reproductive functions. However, many cat owners hesitate to opt for surgical sterilization due to the need for anesthesia, potential postoperative complications, the risk of obesity and diabetes mellitus, as well as the irreversible and costly nature of the procedure. Instead, they prefer medical treatments to suppress or delay the behavioral changes observed during estrus (Goericke-Pesch, 2010; Howe, 2006; Kutzler & Wood, 2006; Verstegen, 2000). Hormonal methods such as progestins (Romagnoli &Concannon, 2009; Tamada et al, 2003), androgens (Johnston et al., 2001; Tamada et al, 2003; Verstegen, 2000), gonadotropin-releasing hormone (GnRH) analogs (Munson et al., 2001), and melatonin are employed reproduction suppress reproduction temporarily.

Melatonin Synthesis, Secretion, and Metabolism: The pineal gland, also known as the epiphysis, is a neuroendocrine organ according located in the brain that regulates various bodily functions, primarily within the endocrine system, in response to environmental light-dark cycle. Working in conjunction with the suprachiasmatic nucleus, the pineal gland is considered a biological clock that synchronizes bodily activities with natural rhythms. The primary substance secreted by the pineal gland, melatonin, was identified in the late 1950s (Arendt &Aulinas, 2019).

Melatonin (N-acetyl-5-methoxytryptamine) is a neuromodulatory substance involved in numerous physiological processes in mammals. It is produced and secreted by the pineal gland of the central nervous system (Abecia et al., 2019). As a result of photoperiodic changes, melatonin synthesis and secretion follow both circadian (daily) and annual (yearly) rhythms, which are directly influenced by light-dark and day-length cycles (Cassone, 1990). Melatonin secretion duration is proportional to nighttime length (Schäfer-Somi, 2017).

In the pineal gland, melatonin production remains at low levels during the day and increases significantly at night. This synthesis involves the hydroxylation of tryptophan into 5-hydroxytryptophan, which is subsequently converted into serotonin. This process also plays a role in regulating ovarian activity in seasonally polyestrous animals like cats (Al-Hamedawi et al., 2020; AL-Shammary &Al-Yasiri, 2023). In cats, the highest melatonin concentrations are observed during anestrus and interest periods (Kassim et al., 2019). Exogenous melatonin, administered orally or via injection, has been shown to suppress estrous cycles in cats. Melatonin may serve as a signal for domestic female cats to detect exogenous melatonin levels, mimicking the effects of decreasing photoperiod-induced melatonin (Graham et al., 2004). A subcutaneous application of an 18 mg melatonin implant has been reported to suppress estrus for 2-4 months, without initial signs of estrus (Fontaine, 2021).

Estrous Cycle in Cats and the Role of Melatonin: The estrous cycle in female cats consists of four phases: proestrus, estrus, diestrus, and anestrus. The estrus phase, commonly referred to as vocalizations, increased affection, and restlessness, which can be challenging for cat owners exposure. Under long-daylight long daylight conditions (14–16 hours), estrous cycles occur more frequently (Hughes & Olson, 2000). Photoperiodic changes in reproductive capacity were first recognized in the 1930s (Baker & Ranson, 1932). Seasonal variations in photoperiod alter the duration of daily melatonin secretion, leading to behavioral changes associated with seasonal cycles (Wehr, 1997). Once it was discovered that seasonal differences influenced melatonin secretion, researchers established a connection between melatonin and the hypothalamic-pituitary-gonadal axis, demonstrating its critical role in both male and female reproductive systems (Shi et al., 2013) (Figures 1 and 2).

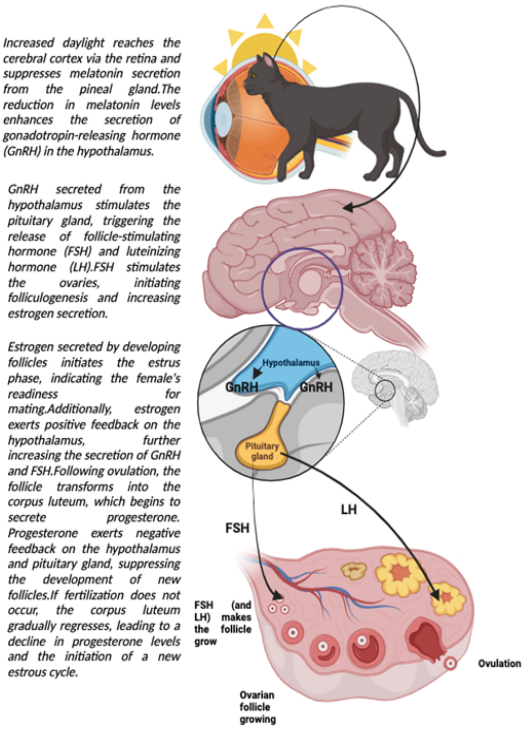

Figure 1. Effect of increasing day length on the hypothalamus–hypophysis–ovarian axis. The increasing light during early spring directly affects the cerebral cortex; consequently, melatonin secretion from the pineal gland is suppressed resulting in the suppressive effect on hypothalamic secretion of gonadotropin-releasing hormone (GnRH); the secretion of gonadotropins is increased, leading to an increase in serum sexual steroid hormone concentration. FSH = follicle-stimulating hormone; LH = luteinising hormone.

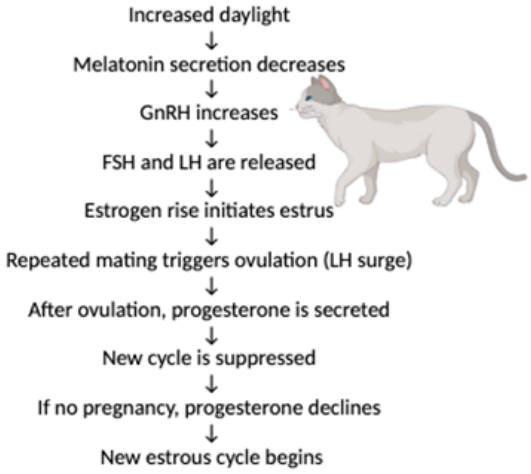

Figure 2. Estrous cycle in cats and the role of melatonin.

Melatonin, a hormone synthesized by the pineal gland, plays a crucial role in regulating reproductive cycles through its interaction with the hypothalamus. Melatonin production increases in darkness and decreases during long daylight periods. Elevated melatonin levels suppress gonadotropin-releasing hormone (GnRH) secretion, thereby inhibiting the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). This hormonal regulation is utilized in female cats to induce anestrus and suppress estrous behavior (Romagnoli & Concannon, 2003).

Increased daylight exerts a stimulatory effect on the hypothalamic-pituitary-ovarian axis. At the onset of spring, longer daylight exposure directly influences the cerebral cortex, leading to the suppression of melatonin secretion from the pineal gland. As a result, the inhibitory effect on GnRH release from the hypothalamus is diminished, causing an increase in gonadotropin secretion, which subsequently elevates serum sex steroid hormone concentrations (Leyva et al., 1984; Leyva et al., 1989). A reduced photoperiod is associated with elevated endogenous melatonin concentrations, followed by decreased sexual activity. Exogenous melatonin administration can mimic this effect. Oral or parenteral administration of exogenous melatonin or melatonin receptor agonists effectively suppresses reproductive function in cats (Kutzler, 2015).

Melatonin Implants and Their Mechanism of Action: Melatonin implants are subcutaneously placed devices that provide a continuous release of melatonin into the bloodstream. These implants mimic the natural effects of melatonin during short daylight periods, signaling the reproductive system to enter a state of quiescence. Consequently, ovarian activity is temporarily suppressed, leading to a reduction or complete elimination of estrus behavior. Sustained-release subcutaneous formulations of melatonin have been tested as a more practical alternative to oral administration in cats (Faya et al., 2011; Gimenez et al., 2009; Griffin et al., 2001). The interscapular space is typically used as the implantation site, and the implant can be administered to an unsedated patient with local anesthetic infiltration (Figure 3). Melatonin implants (18 mg, Melovine; Ceva Santé Animale) are commercially available for veterinary use in many countries. The application of an

The region of the interscapular region is shaved, and the area is then meticulously cleansed using an antiseptic solution.

Prior to the insertion of the melatonin implant 1 cm distal to the insertion site, the area is to be treated with lidocaine spray, after which the implant is to be placed subcutaneously.

Figure 3. The subcutaneous melatonin implant is administered via an applicator that is positioned in the interscapular region.

18 mg melatonin implant in female cats has been reported to extend the interest interval to 2–4 months (Gimenez et al., 2009).

Duration of Induced Anestrus: In female cats implanted during the interestrus phase, anestrus duration ranges from 1 to 3 months (Faya et al., 2011; Gimenez et al., 2009;

Schaefer-Somi, 2017), with the widest reported range in the literature being 21–277 days (Furthner et al., 2020). In female cats implanted during estrus, the suppression lasts approximately 2 months (Gimenez et al., 2009).

Estrus Symptoms Immediately Following Implantation; No estrus symptoms have been observed in female cats implanted during seasonal anestrus (Gulyuz et al., 2009) or very early interestrus (Faya et al., 2011). In female cats implanted during interestrus, estrus symptoms are rare, with an incidence of approximately 35% (Faya et al., 2011; Gimenez et al., 2009; Schaefer-Somi, 2017). In contrast, in female cats implanted during estrus, estrus symptoms are quite common, occurring in approximately 80% of cases (Gimenez et al., 2009).

Ovulation and Pseudopregnancy; Ovulation and pseudopregnancy have been observed in 43% of female cats implanted during interestrus (Faya et al., 2011).

Advantages of Melatonin Implants:

- Non-surgical nature: Provides a temporary alternative to sterilization, making it suitable for owners who wish to avoid permanent sterilization.
- Reversibility: Normal estrus cycles resume once the implant's effects diminish.
- Long-term efficacy: A single implant can be effective for several months, reducing the need for frequent interventions.
- Minimal side effects: Melatonin implants are generally well tolerated, with no significant adverse effects reported in clinical studies (Durán Frías, 2021).

Disadvantages of Melatonin Implants

- High individual variability in suppression duration: Estrus should be carefully monitored before administration.
- Application limitations: The subcutaneous insertion of an 18 mg melatonin implant can be performed using the applicator provided with the product; however, this applicator is designed for sheep, allowing multiple implants to be loaded and administered sequentially. If several queens are implanted on the same day, each requires a sterile needle, making needle availability a limiting factor. In some countries, implants are only available in packs of 2 × 25 with a single needle, necessitating additional purchases. The applicator, designed for sheep, must be bought separately, and obtaining extra needles can be challenging. Consequently, surgical placement via an incision after local anesthesia is often preferred, making repeated treatments less desirable (Romagnoli et al., 2022).
- Timing constraints: If the implant is not administered precisely during the interestrus phase, fertility cycles may resume shortly after implantation (Schaefer-Somi, 2017).
- Temporary nature: The effects of the implant are not permanent, requiring repeated applications for long-term estrus suppression.
- Cost: Melatonin implants may be more expensive than other hormonal methods.

Individual response variability: The efficacy of melatonin implants can vary among individual cats.

- Application procedure: Implantation requires veterinary expertise to ensure proper placement and dosage (Mason et al., 2015).

Conclusion

Melatonin implants provide a safe, effective, and reversible method for suppressing estrus in female cats. They are particularly beneficial for breeders needing reproductive control or cat owners seeking a non-surgical alternative to sterilization. Despite some limitations, such as temporary effects and cost, melatonin implants present a promising tool for feline fertility management. Further studies are needed to optimize dosage, understand individual variability, and evaluate long-term effects.

Conflict of Interest

The authors declare that there is no actual, potential or perceived conflict of interest for this article.

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HARRAN ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ YAYIN KURALLARI *

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8- Makale yayına kabul edildiği takdirde her türlü yayın hakkının devredildiğine dair beyanları kapsayan Telif Hakkı Devir Sözleşmesinin tüm yazarlar tarafından imzalanarak basımdan önce elektronik olarak dergi editörlüğüne gönderilmesi gerekmektedir. Telif Hakkı Devir Sözleşmesi gönderilmeyen makaleler yayımlamaya kabul edilmiş olsalar bile basılmazlar.

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YAZIM KURALLARI

Yazılar, MS Word formatında, Times New Roman yazı tipinde, 12 punto, çift satır aralıklı ve her kenardan 2.5 cm boşluk bırakılarak hazırlanmalıdır. Makaleye satır numaraları (makalenin 2. sayfasından başlamak üzere sürekli olacak şekilde) eklenmelidir. Bu şekildeki yazılar, şekil ve tablolar dâhil olmak üzere orijinal bilimsel araştırmalar ve derlemelerde 15, kısa bilimsel makale ve olgu sunumlarında 5 sayfayı geçmemelidir.

Birimler ve ölçüler için Uluslararası Standart birimleri (SI-sistem) kullanılmalıdır.

Araştırma Makaleleri: Orijinal araştırma makaleleri aşağıdaki ana konu sıralamasına göre dizilmelidir: Başlık, Yazar adları (Sorumlu yazar (*) ile işaretlenmeli), Yazar adresleri, Yazar ORCID numaraları, Özet ve Anahtar kelimeler (3 - 6 kelime), İngilizce başlık, Abstract ve Keywords ile Giriş, Materyal ve Metot, Bulgular, Tartışma ve Sonuç, Teşekkür veya Bilgilendirme ile Kaynaklar. Her bir Tablo ve Şekil ayrı sayfalarda yer almalıdır.

YAZIM DÜZENİ

Özet: Orijinal araştırma makalelerinde 250, diğer makale türlerinde 200 kelimeyi geçmeyecek şekilde hazırlanmalıdır.

Anahtar Kelimeler: En fazla 6 tane olmak üzere her iki dildeki özetin altında alfabetik sırayla verilmelidir. Anahtar kelimeler, Türkiye Bilim Terimleri arasından seçilmelidir. Anahtar kelimelerin seçiminde Türkiye Bilim Terimleri internet adresinden (http://www.bilimterimleri.com) yararlanılmalıdır.

Giriş: Sonuçların anlaşılabilmesi ve yorumlanabilmesi için o konu ile ilgili yapılmış olan çalışmalar hakkında bilgilere yer verilmelidir. Giriş'te çalışmanın hipotezi belirtilmelidir. Çalışmanın amacı bu bölümün en sonunda açık olarak yazılmalıdır.

Materyal ve Metot: Bu bölümde deneysel çalışmalar diğer araştırmacılar tarafından tekrarlanabilecek yeterlilikteki detayı ile verilmelidir. Uluslararası indeksli dergilerde yayınlanmış bir makalede açıklanan bir teknik kullanıldığında, metodun çok kısa açıklanması ve ilgili orijinal makaleye atıf yapılması gereklidir. Makalede etik kurul izini ve/veya yasal/özel izin alınmasının gerekip gerekmediği bu bölümde belirtilmelidir. Materyal olarak hayvan kullanılan orijinal araştırma makalelerinde (klinik, deneysel, saha çalışmaları vb.); etik kurul onayı alınmış olmalıdır. Etik kurul onay/izin belgesinin "alındığı etik kurulun ismini, sayısını ve tarihini" içeren açıklayıcı bilgiler materyal ve metot bölümüne yazılmalıdır. Yayın kurulu etik kurul onay belgesini isteme hakkına sahiptir.

Bulgular: Araştırma bulguları açık ve anlaşılabilir şekilde verilmelidir. Bulgular, gerektiğinde tablo ve şekillerle desteklenmeli ve kısa olarak sunulmalıdır.

Tartışma ve Sonuç: Bulgular gereksiz ayrıntıya girmeden literatürler ışığında tartışılmalı ve bulguların önemi vurgulanmalıdır. Sonuç ya da öneri cümlesi ile bitirilmelidir.

Teşekkür: Çalışma veya makaleye kişisel katkı ve parasal destek burada belirtilmelidir.

Derleme: Derginin yayın alanlarındaki konularda yenilikleri içeren, güncel kaynaklardan yararlanılarak hazırlanmış makaleler olup, yazarların konu ile doğrudan ilişkili en az 3 adet çalışmalarının olması ve bunların derleme içinde kullanılması durumunda yayınlanmak üzere kabul edilebilecektir. Sorumlu yazar, derlemesini gönderirken konu ile ilgili makalelerinin de künye bilgilerini dergi editörlüğüne göndermelidir (makale künyeleri, makale metninin en son sayfasında sunulmalıdır). Harran Üniversitesi Veteriner Fakültesi Dergisi'nde değerlendirmeye alınan ve yayınlanan derlemeler **çağrılı derleme**lerden oluşmaktadır. Derlemelerde; Özet, Giriş, Sonuç ve Kaynaklar bölümleri bulunmalıdır.

Olgu Sunumu: Yazarların, karşılaştıkları yeni veya ender gözlemlenen olguların ele alındığı, bilimsel değere sahip bilgileri içeren eserlerdir. En fazla 15 kaynak kullanılmalı ve bu kaynakların güncel olmasına özen gösterilmelidir. Olgu sunumları; Özet, Giriş, Olgu tanımı, Tartışma ve Sonuç ile Kaynaklar bölümlerinden oluşmalıdır.

Kısa Bilimsel Makale: Kısa bilimsel makalelerde dar kapsamlı olarak ele alınmış, yeni bilgi ve bulgular sunulmalıdır. Araştırma makalesi formatında hazırlanmalı ve en fazla 5 sayfa olmalıdır. En fazla 2 tablo veya şekil içermelidir.

Kaynaklar

Metin içinde atıf yapılırken;

1. Yazar veya yazarların soyadından sonra parantez içinde kaynağın yayın yılı belirtilmelidir; Adams (1998) tarafından; Wilkie ve Whittaker (1997) tarafından; Doyle ve ark. (2007) tarafından….
2. Cümlenin sonunda atıf yapıldığında ise yazar ismi ve yayın yılı parantez içinde belirtilmelidir; ... bildirilmiştir (Adams, 1998); .... bildirilmiştir (Wilkie ve Whittaker, 1997); ..... bildirilmiştir (Doyle ve ark., 2007).
3. Birden çok kaynağa atıf yapılması durumunda önce alfabetik sonra kronolojik sıralama yapılmalıdır;
   …. bildirilmiştir (Adams, 1998; Adams, 2008; Doyle ve ark., 2007; Wilkie ve Whittaker, 2006).
4. Aynı yazarın aynı yıl yayınları söz konusu ise her biri "a" harfinden başlayarak küçük harflerle işaretlenmelidir;
   …. (Adams, 2005a; Adams, 2005b;…).

Kaynak listesi aşağıdaki şekilde hazırlanmalıdır:

1. **Kaynak listesi yazar soyadına göre alfabetik olarak sıralanmalıdır.**
2. **Kaynaklarda yer alacak dergi adları ISI web of Science'a göre kısaltılmalı ve italik yazılmalıdır.**
3. **Kaynakların yazın şekli aşağıdaki şekilde olmalıdır.**

Makale; Sullivan JC, Sasser JM, Pollock JS, 2007: Sexual dimorphism in oxidant status in spontaneously hypertensive rats. *Am J Phsiol Integr Comp Physiol,* 292 (1), 64-68.

Kitap; Cadenas E, Packer L, 2001: Handbook of Antioxidants. 2nd ed., Marcel Dekker Inc., New York, USA.

Kitaptan bir bölüm: Bahk J, Marth EH (1990). Listeriosis and Listeria monocytogenes In: Foodborne Diseases, Cliver DO (Ed), 248-256, Academic Press, San Diego. **Web sayfası:** Anonim (1) http://www.emea.europa.eu, Erişim tarihi; 01.04.2010.

Tez: Er A, 2009: Makrolid grubu antibiyotiklerin endotoksemide sitokin düzeylerine etkisi. Doktora tezi, SÜ Sağlık Bilimleri Enstitüsü, Konya.

Bilimsel toplantıda sunulan bildiri: Allen WR, Wilsher S, Morris L, Crowhurst JS, Hillyer MH, Neal HN, 2006: Re-establishment of oviducal patency and fertility in infertile mares. In: Proceedings of the Ninth International Symposium on Equine Reproduction, Kerkrade, Holland, pp. 27-28.

Tablo ve Şekiller: Her bir tablo ve şekil ayrı sayfalara yerleştirilmelidir. Kullanım sırasına göre numaralandırılmalı, kısa başlıklarla ifade edilmeli ve metin içinde tablo numarası verilerek atıfta bulunulmalıdır. Tablo başlıkları makalenin yazım dilinde tablonun üst bölümüne yazılmalıdır. Tabloda kullanılan kısaltmalar ve gerekli açıklamalar tablo altında verilmelidir. Şekil başlıkları makalenin yazım dilinde şeklin alt bölümüne yazılmalıdır.

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International Standard Units (SI-system) should be used for units and dimensions.

Research Articles: Original research articles should be arranged in the order of the following main topics: Title, Author names (must be marked with the responsible author (*)), Author addresses, Author ORCID numbers, Abstract and Keywords (3 - 6 words), English title, Abstract and Introduction to Keywords, Material and Method, Results, Discussion and Conclusion, Thanks or Information and References. Each Table and Figure should be on separate pages.

STYLE AND FORMAT

Abstract: It should be prepared not to exceed 250 words in original research articles and 200 words in other types of articles.

Keywords: It should be given in alphabetical order below the summary in both languages, maximum 6. Keywords should be selected from Turkey Science Terms. Turkey Science Terms in the selection of keywords from the internet address (http://www.bilimterimleri.com) should be utilized.

Introduction: In order for the results to be understood and interpreted, information about the studies done on that subject should be included. In the introduction, the hypothesis of the study should be specified. The purpose of the study should be clearly written at the end of this section.

Material and Method: Experimental studies should be given in this section with sufficient detail that can be repeated by other researchers. When using a technique described in an article published in international indexed journals, it is necessary to describe the method very briefly and to cite the relevant original article. In the article, it should be stated in this section whether the ethical committee permission and / or legal / special permission should be obtained. In original research articles using animals as materials (clinical, experimental, field studies, etc.); ethics committee approval must have been obtained. Explanatory information including the name, number and date of the ethics committee's ethics committee approval / permit document should be written in the material and method section. The editorial board has the right to request the ethics committee approval document.

Results: Research findings should be given clearly and understandably. Findings should be supported with tables and figures when necessary and presented briefly.

Discussion and Conclusion: Findings should be discussed in the light of the literature before going into unnecessary detail and the importance of the findings should be emphasized. It should be finished with a conclusion or suggestion sentence.

Acknowledgment: Personal contribution and monetary support to the study or article should be stated here.

Compilation: These are articles that contain innovations on the subjects of the journal's publications and are prepared by using current references. If the authors have at least 3 works directly related to the subject and they can be accepted for publication. When submitting his review, the responsible author should send the imprint information of the articles related to the subject to the editor of the journal (article tags must be presented on the last page of the article text). Reviews compiled and published in Harran University Veterinary Faculty Journal are invited reviews. in the compilation; Summary, Introduction, Conclusion and References sections should be available.

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Short Scientific Article: In short scientific articles, it should be handled narrowly and new information and findings should be presented. It should be prepared in the form of a research paper and should not exceed 5 pages. Must contain no more than 2 tables or figures.

References:

While citing in the text;

1. The publication year of the reference should be specified in parentheses after the surname of the author or authors; By Adams (1998); By Wilkie and Whittaker (1997); Doyle et al. (2007) by….

2. When cited at the end of the sentence, the name of the author and the year of publication must be indicated in parentheses; ... have been reported (Adams, 1998); .... has been reported (Wilkie and Whittaker, 1997); ..... has been reported (Doyle et al., 2007).

3. In case of reference to more than one reference, first alphabetical and chronological order should be done;

.... reported (Adams, 1998; Adams, 2008; Doyle et al., 2007; Wilkie & Whittaker, 2006).

4. If the same author has publications in the same year, each should be marked in lowercase letters, starting with the letter "a";

.... (Adams, 2005a; Adams, 2005b;…).

The list of references should be prepared as follows:

1. Reference list should be listed alphabetically by author surname.

2. The names of the journals in the references should be shortened according to the ISI web of Science and should be written in italics.

3. Type of references should be as follows.

Journal article; Sullivan JC, Sasser JM, Pollock JS, 2007: Sexual dimorphism in oxidant status in spontaneously hypertensive rats. Am J Phsiol Integr Comp Physiol, 292 (1), 64-68.

Book; Cadenas E, Packer L, 2001: Handbook of Antioxidants. 2nd ed., Marcel Dekker Inc., New York, USA.

Chapter in a book: Bahk J, Marth EH (1990). Listeriosis and Listeria monocytogenes In: Foodborne Diseases, Cliver DO (Ed), 248-256, Academic Press, San Diego. Web page: Anonymous (1) http://www.emea.europa.eu, Access date; 01.04.2010.

Thesis: Er A, 2009: Effect of macrolide antibiotics on cytokine levels in endotoxemia. PhD thesis, SU Health Sciences Institute, Konya.

Paper presented at the scientific meeting: Allen WR, Wilsher S, Morris L, Crowhurst JS, Hillyer MH, Neal HN, 2006: Re-establishment of oviducal patency and fertility in infertile mares. In: Proceedings of the Ninth International Symposium on Equine Reproduction, Kerkrade, Holland, pp. 27-28.

Tables and Figures: Each table and figure should be placed on separate pages. It should be numbered according to the order of use, expressed in short titles, and should be cited by giving the table number in the text. Table titles should be written in the writing language of the article in the upper part of the table. Abbreviations and necessary explanations used in the table should be given under the table. Figure titles should be written at the bottom of the figure in the writing language of the article.

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