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ANIMAL HEALTH, PRODUCTION AND HYGIENE

ANIMAL HEALTH, PRODUCTION AND HYGIENE

CONTENTS

ANIMAL HEALTH, PRODUCTION AND HYGIENE

İÇİNDEKİLER

Animal Health Prod and Hyg (2023) 12 (2) : 1 - 6

Animal Health, Production and Hygiene

www.dergipark.or.tr/tr/pub/aduveterinary

Research Article

Morphological Study of the Intraorbital Muscles *(Musculi bulbi)* in Rat and Guinea Pig

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ABSTRACT

Intraorbital muscles in the human are similar to those in the mammals as regards morphology and function, except for the absence of the retractor bulbi muscle in the human. The aim of this study is to determine the morphological and morphometric features of the eye muscles in the rat and guinea pig used as a model for various studies. Twenty-four bulbus oculi of 12 rats and 32 bulbus oculi of 16 guinea pig were investigated by subgross and histological methods. Certain morphometric data of musculi bulbi were obtained by stereo-microscope. Samples were fixed in 10% formalin for subgross procedures and preserved in Bouin solution for histological methods. The muscular part of the intraorbital muscles in the rat was examined and each muscle was determined to be originated from near the optic foramen and to be attached to sclera close to corneal limbus by tendinous ends. The intraorbital muscles of the guinea pig were relatively weak compared to the body size. The functional part of the intraorbital muscles located around the optic nerve was considerably short and attached to the sclera by the long tendinous ends. Retractor bulbi muscle was small muscle batch located ventrolateral of the optic nerve in rat and substantially weak muscle bundles that envelop the optic nerve in the guinea pig.

***Keywords**: Ocular muscles, rat, Guinea pig.*

Rat ve Kobay Ekstraoküler Göz Kaslarının *(Musculi bulbi)* Morfolojik Çalışması

ÖZET

İnsanlarda intraorbital göz kasları, m. retractor bulbi kasının yokluğu dışında memelilere benzer. Bu çalışmanın amacı çeşitli araştırmalara model olarak kullanılan rat ve kobaya ait göz kaslarının morfolojik ve morfometrik özelliklerini ortaya koymaktır. Çalışmada 12 adet rata ait 24 ve 16 adet kobaya ait 32 bulbus oculi subgros ve histolojik metotlarla incelendi. Ekstraoküler göz kaslarına ait morfometrik veriler stereo mikroskop ile elde edildi. Örnekler subgros incelemeler için %10 formalin solüsyonunda tespit edildi ve histolojik metotlar için Bouin solüsyonu kullanıldı. Ratta intraorbital göz kaslarının iyi geliştiği, her kasın foramen (for.) opticum yakınından başlangıç aldığı ve limbus cornea'ya yakın olarak sclera'da tendinöz olarak sonlandığı belirlendi. Kobay göz kaslarının vücut büyüklüğüne oranla oldukça zayıf şekillendiği gözlendi. Nervus (n.) opticus etrafında yer alan intraorbital kasların fonksiyonel kısımları oldukça kısa şekillendiği ve uzun tendo ile sclera'ya tutunduğu belirlendi. *Musculus retractor bulbi* kasının ratta *n. opticus*'un ventrolateral'inde küçük bir kas ve kobay'da *n. opticus*'un etrafında zayıf kas iplikleri halinde belirlendi.

***Anahtar Kelimeler:** Göz kasları, kobay, sıçan.*

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Introduction

Eye is responsible for transmitting the image from the environment to the brain. Position of orbita is different among the species. Orbita is located at rostral in the carnivore and omnivore and located at lateral in the herbivore (König and Liebich, 2007). In guinea pig and rats, orbita is located at lateral as in the herbivore. Due to the position of orbita, angle of sight is 340º in guinea pig and 360º in the rat. In contrast to well-developed extraocular muscles (EOMs) in the rat, they do not use their EOMs (O'Malley, 2005).

Intraorbital muscles are derived from prechordal mesoderm of the early embryo. A number of craniofacial muscles are different from trunk muscle as phenotypic properties such as myosin isoforms (Hyttel et al., 2010). Andrade et al. (2005), manifest that EOMs are more contractible than an extremity muscle by having more mitochondria and different transcription programme organization during aerobic exercise.

Extraocular muscles are responsible for voluntary and reflexive movement of eye (McMullen et al., 2009) and these movements are significant for anchoring the image on the retina (Jampolsky, 1953). EOMs are the fastest of the skeletal muscles (Andrade et al., 2005; McMullen et al., 2009). The muscles providing movement for three spatial-axis around the eye are *musculus (m.) rectus dorsalis* and *m. rectus ventralis*; *m. rectus lateralis* and *m. rectus medialis; m. obliquus dorsalis, m. obliquus ventralis* and *m. retractor bulbi* in domestic mammals (King and McLelland, 1985; Von Noorden and Campos, 2002; Felder et al., 2005; Dursun, 2007; König and Liebich, 2007). Therefore, retractor bulbi muscle is absent in humans (Dursun, 2002). Rectus muscles envelop the optic nerve and insert into orbita (Von Noorden and Campos, 2002; König and Liebich, 2007). EOMs must be used concurrent for binocular vision. Because of this, the muscles work as synergetic and antagonist. Medial rectus muscle and lateral rectus muscle work synergic for left or right movement of eye (Von Noorden and Campos, 2002).

Distance to limbus of EOMs' insertion is significant for surgical treatment (Von Noorden and Campos, 2002). Intraocular muscles have studied morphologically in the human (Kocabıyık et al., 2004), rabbit (Gultiken et al., 2006), roe deer (Gültiken et al., 2010), common buzzard (Gültıken et al., 2011), horse, donkey, mule, buffalo, camel, sheep, goat, pig (Hifny and Misk, 1982), dog (Hifny and Misk, 1982; Klećkowska et al., 2003; 2006) and cat (Hifny and Misk, 1982; Klec´kowska and Pospieszny, 2005) so far. Rat and guinea pigs are frequently used for clinical and surgical practice. This study could shed light on clinical and surgical application by morphological data in the rat and the guinea pig.

Materials and Methods

Twelve rats' and sixteen guinea pigs' heads were used in this study. The material used in the study consisted of sacrificed animals which were Ondokuz Mayıs University Experimental Animal and Research Center for various reasons and preserved in the laboratory of the Department of Veterinary Anatomy. No rat and guinea pig were killed for this study. All the eyes were clinically normal and there were no evidence of ocular trauma or infection in any. Thirty-two bulbus oculi from 8 rats and 8 guinea pigs were fixed in 10% formalin solution for subgross examination. Extraocular muscles were dissected after fixation. The width, length, thickness, distance to limbus and optic nerve of each muscle were measured using a Mitutoyo Digimatic Vernier Scale (15 mm) (code no: 500-311, Model CD-15D, serial no: 7175731, Mitutoyo Corporation, Japan), except for retractor bulbi muscle. Insertion tendons of muscles were observed with Olympus SZ-61 TRC stereo-microscope. Other eyes of 4 rats and 8 guinea pigs were fixed in Bouin solution for histological examination. Subsequently, the specimens were subjected to standard histological tissue sequencing procedures and blocked in paraffin. After cutting 5-µm sections from paraffin blocks, Crossmon's triple staining method was

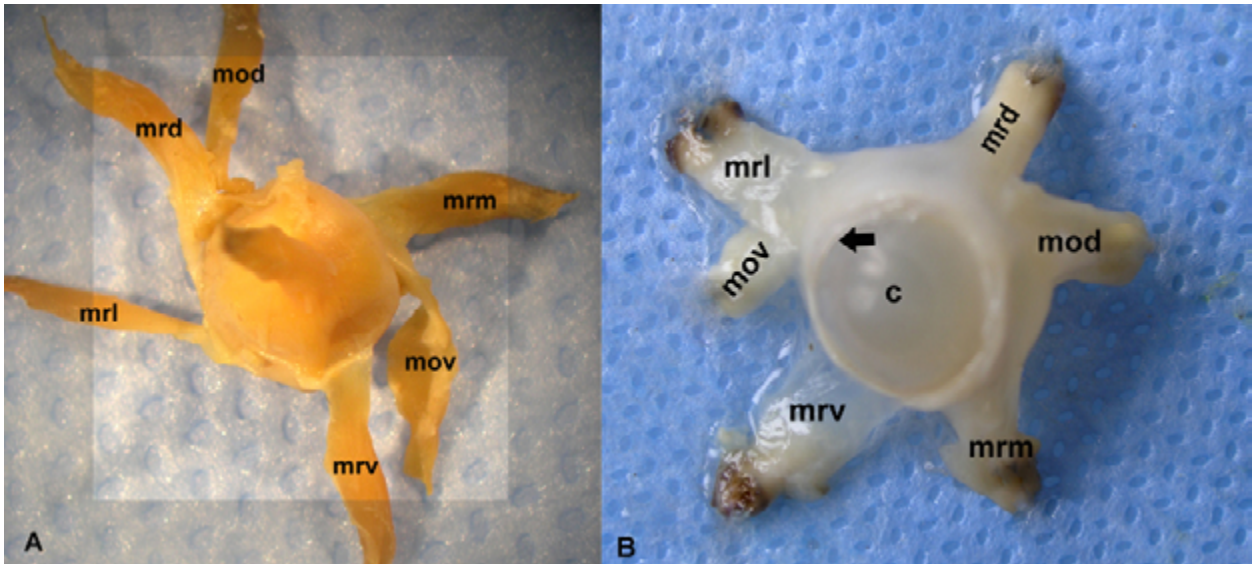

Figure 1. A: caudal aspect of extraocular muscle of the rat, B: rostral aspect of extraocular muscle of the guinea pig, mod: *m. obliquus dorsalis*, mov: *m. obliquus ventralis*, mrd: *m. rectus dorsalis*, mrl: *m. rectus lateralis*, mrv: *m. rectus ventralis*, mrm: *m. rectus medialis*, arrow: *limbus cornea*.

used to examine the histological structure.

Results

Morphologic Examinations

Intraorbital muscles were observed as *m. obliquus dorsalis* (dorsal oblique muscle), *m. obliquus ventralis* (ventral oblique muscle), *m. rectus dorsalis* (dorsal rectus muscle), *m. rectus ventralis* (ventral rectus muscle), *m. rectus lateralis* (lateral rectus muscle), *m. rectus medialis* (medial rectus muscle) and *m. retractor bulbi* (retractor bulbi muscle). Muscular part of rat EOMs were observed relatively well developed. Each muscle inserted near to limbus cornea on sclera. The functional part of the eye muscles that envelops the optic nerve of guinea pigs was relatively short. This part was observed to be originated from the surround of optic foramen. Nevertheless, muscular part terminated and inserted to the sclera with a long tendinous part (Figure 1). The morphometric data of the *musculi bulbi* of the rat and the guinea pig are given in "Table 1 and Table 2" respectively. Eye muscles of the rat determine to be thinner than in the guinea pig. It included numerous lymph follicles in guinea pigs (Figure 2). The Harderian gland of the guinea pig was relatively bigger than in rat. Localization of muscles resembled to each other, but dorsal oblique muscle and medial rectus muscle were thicker and distinct in guinea pig. The rectus muscles were virtually identical in the rat and the guinea pig. The rectus muscles run longitudinally, but the ventral and dorsal oblique muscles located transversely and then vertically.

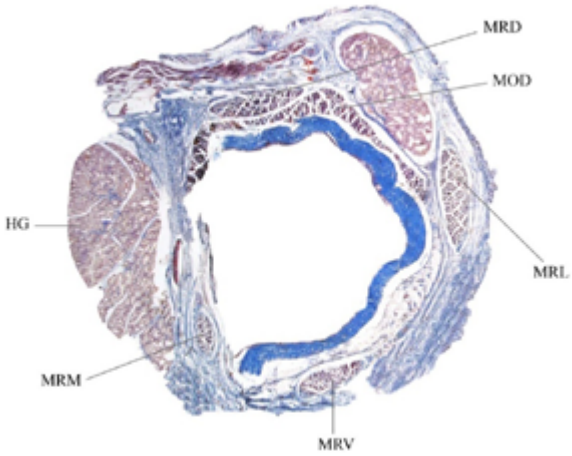

Figure 2. Histological aspect of extraocular muscles of the guinea pig. MOD: *m. obliquus dorsalis*, MRD: *m. rectus dorsalis*, MRL: *m. rectus lateralis*, MRV: *m. rectus ventralis*, MRM: *m. rectus medialis*, HG: *glandula palpebra tertia* (harderian gland).

Table 1. The morphometric data of the bulbus oculi of the rats except for the retractor bulbi muscle (mean ± standard deviation).

Rat (n*= 16)	Length (mm)	Width (mm)	Thickness (mm)	Distance to optic nerve (mm)
Dorsal oblique	9.32 ± 1.22	1.89 ± 0.33	0.54 ± 0.14	3.91 ± 0.38
Ventral oblique	7.33 ± 1.52	2.23 ± 0.39	0.48 ± 0.18	3.61 ± 0.51
Dorsal rectus	7.96 ± 1.01	2.31 ± 0.41	0.57 ± 0.13	3.59 ± 0.61
Ventral rectus	8.15 ± 1.81	2.23 ± 0.65	0.52 ± 0.09	3.63 ± 0.55
Lateral rectus	8.23 ± 1.22	2.58 ± 0.37	0.59 ± 0.10	3.73 ± 0.54
Medial rectus	8.18 ± 1.18	2.52 ± 0.41	0.57 ± 0.13	3.68 ± 0.59

* gives the total number of left and right bulbus oculi

Table 2. The morphometric data of the bulbus oculi of the guinea pigs except for the retractor bulbi muscle (mean ± standard deviation).

Guinea pig (n*= 16)	Length (mm)	Width (mm)	Thickness (mm)	Distance to optic nerve (mm)
Dorsal oblique	6.11 ± 1.23	3.04 ± 0.56	1.10 ± 0.16	7.10 ± 0.46
Ventral oblique	5.58 ± 1.00	2.86 ± 0.56	1.03 ± 0.12	6.46 ± 0.68
Dorsal rectus	6.45 ± 0.94	3.02 ± 0.50	1.15 ± 0.19	7.16 ± 0.44
Ventral rectus	6.60 ± 0.73	3.80 ± 0.67	1.18 ± 0.12	6.83 ± 0.62
Lateral rectus	6.44 ± 0.93	3.36 ± 0.55	1.04 ± 0.06	7.24 ± 0.63
Medial rectus	6.16 ± 0.80	3.18 ± 0.58	1.19 ± 0.08	6.21 ± 0.55

* gives the total number of left and right b

M. obliquus dorsalis

M. obliquus dorsalis twisted to under the dorsal rectus muscle over the trochlea in the rat. Additionally, it inserted obliquely to dorsomedial of the dorsal rectus muscle in the guinea pig (Figure 3).

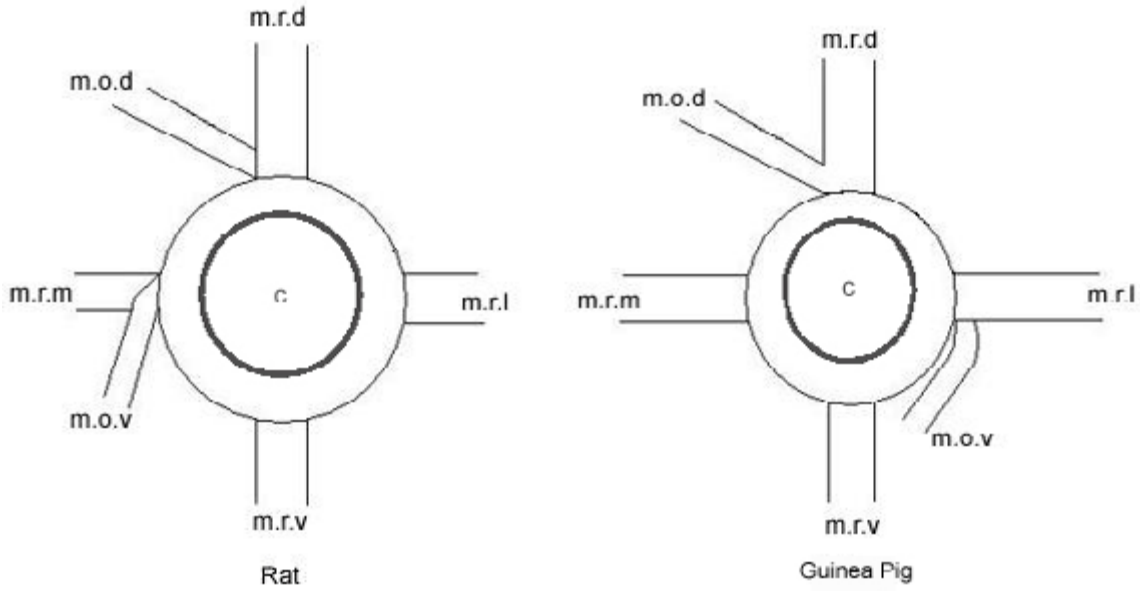

Figure 3. Insertion lines of eye muscles of the rat and guinea pig. c: cornea, m.r.d: *m.rectus dorsalis*, m.r.m: *m. rectus medialis*, m.r.v: *m. rectus ventralis*, m.r.l: *m.rectus lateralis*, m.o.d: *m. obliquus dorsalis*, m.o.v*: m.obliquus ventralis*.

M. obliquus ventralis

Ventral oblique muscle was embedded into fatty tissue in the rat and inserted to the sclera form dorsal of the medial rectus muscle. Insertion line of the ventral oblique muscle was different between the individuals. The muscle was inserted to the ventral of the lateral rectus muscle in the six guinea pigs, and inserted between the lateral rectus muscle and ventral rectus muscle in the one and inserted to ventral of the ventral rectus muscle in the another one. No differences have been detected between the right and left oculus.

M. rectus dorsalis

M. rectus dorsalis muscle inserted separately to sclera on the dorsal of dorsal oblique muscle in the rat and inserted to sclera joining the ventral of dorsal oblique muscle in guinea pig with tendinous part.

M. rectus ventralis

Ventral rectus muscle inserted to the ventral of the bulbus oculi in the rat. It inserted to the sclera on dorsal of ventral oblique muscle in only one guinea pig. In the other guinea pigs, it inserted as in the rat.

M. rectus lateralis

Tendon of the lateral rectus muscle inserted to sclera between the retractor bulbi muscle and ventral rectus muscle in the rat. It was attached to sclera on dorsal of the ventral oblique muscle in the six guinea pigs. The others were not involved in the ventral oblique muscle.

M. rectus medialis

Medial rectus muscle was connected to the sclera with tendinous insertion on the medial of bulbus oculi in both rats and guinea pigs.

M. retractor bulbi

Retractor bulbi muscle was seen as a small muscle on ventrolateral of the optic nerve in the rat. The retractor bulbi muscle has only a few muscle fibers around the optic nerve in the guinea pig.

Discussion

Extraocular muscles are different from the skeletal muscles and have multiple or focally innervated fiber (Pachter, 1983). These muscles are mentioned as having orbital and global layers before attaching to the sclera in rat (Khanna and Porter, 2001; Felder et al., 2005) Orbital and global layers have different nerve endings (Pachter, 1983). Orbital layer attaches to the orbita and global layer attaches to the sclera. They are of importance for visuomotor reflexes (Khanna and Porter, 2001). An external marginal zone was described for the first time in human eye muscles by Wasicky et al. (2000), except for orbital and global layers. Orbital and global layers were determined in dorsal rectus muscle in the present study but they were not seen in the ventral rectus muscle defined by Felder et al. (2005), and in the dorsal oblique muscle defined by Pachter and Colbjörnsen (1983).

There are manifold diseases regarding eye muscles such as Strabismus (Jampolsky, 1953; Lennerstrand, 2007; Schutte et al., 2009; Lai et al., 2012), Graves' disease (Maurits et al., 1990) and Sarcoidosis (Brooks et al., 1997). These muscles have substantial localization and morphometric properties for diagnosis (Maurits et al., 1990; Brooks et al., 1997; Wright, 2009; Lai et al., 2012). Lai et al. (2012) determined that the rectus muscles except for medial rectus muscle in Taiwan are shorter than in western Asian country. This situation might explain the diagnosis of strabismus types and surgical treatment

as well as ethnical differentiation. In the present study, intraorbital muscles insert into the sclera closed to the limbus in the rat and in the guinea pig, except for the retractor bulbi muscle. As rectus ventralis muscle attached with muscular part to the sclera and the others attached with tendinous insertion in New Zeland Rabbit (Gultiken et al., 2006), all rectus muscles attached with tendinous insertion in the dog (Klećkowska et al., 2003; 2006), the rat and the guinea pig. Thickness, width and length of the extraorbital muscles are major criteria for the diagnosis like sarcoidosis characterized with granulomas. Sarcoidosis occurs idiopathically and is diagnosed by the imaging techniques such as MR (Brooks et al., 1997). Autoimmune eye diseases, associated with thyroid function, are frequently formed by the mediation of the receptors in orbital fibroblasts and preadipocytes. Most of the patients with opthalmopathy are associated with Graves' disease (Khanna and Porter, 2001; Wall and Lahooti, 2010). The most successful treatment for Graves is surgical (Maurits et al., 1990). Because there has been aggregate lymph follicle, the guinea pig could be more suitable for experimental studies on Graves than the rat. However, concentration and presence of the related receptor must be investigated in the periorbital connective tissue. In our study, the harderian gland was observed in the medial part of the eye, but Demircioğlu et al. (2016), stated that the harderian gland is located on the caudal part of the guinea pig eye.

Retractor bulbi muscle envelops the optic nerve in mammals (Dursun, 2002; Klećkowska et al., 2003; 2006; Gultiken et al., 2006), except in humans (Kocabıyık et al., 2004; Woodburne and Burkel, 1994) and birds (Klec´-kowska-Nawrot et al., 2018). This muscle must be notice in experimental study in the rat and the guinea pig. Also, the insertion line of the ventral oblique muscle varies among animal species (Klec'kowska et al., 2003; Klec'kowska and Pospieszny, 2005; Gultiken et al., 2006; 2010; 2011; Zhou et al., 2011; Klec'kowska-Nawrot et al., 2018). Zhou et al. (2011) determined that the dorsal oblique muscle does not have a trochlea. In our study, dorsal oblique muscle was observed similar to the Zhou et Zhou et al. (2011). However, it was different from the findings of Zhou et al. (2011) that described for guinea pig with the single medial rectus muscle and lateral rectus muscle. The placement of the muscles in the guinea pig was similar to that of the New Zealand rabbit's intraocular muscles (Gultiken et al., 2006). It is mentioned that the lateral rectus muscle is absent in the guinea pig, and the ventral oblique muscle inserts below the ventral rectus muscle (Zhou et al., 2011). In this study, the ventral oblique muscle terminated below the ventral rectus muscle in only one guinea pig. In our study, the ventral oblique muscle, which has different termination points, generally inserts under the lateral rectus muscle. It is important to note that this study emphasized that the eye muscles, especially the lateral rectus muscle, medial rectus muscle, and ventral oblique muscle, may have different insertion points in studies to be performed on guinea pig eye muscles. That should be considered, especially when planning studies with guinea pig eye muscles.

Conclusion

Consequently, the morphologic features and connections to the sclera of the insertion tendons in the rat and guinea pig were clarified by morphometric evaluation of the width, length and thickness of intraorbital muscles and the distance to the optic nerve from the point where they attach to the sclera. The results of this study contribute to clinical research since the intraorbital muscle of these species may be used as a model in evaluating new approaches in the treatment of diseases that affect intraorbital muscles.

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Conflict of interest

There is no conflict of interest.

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Research Article

Effect of Vitamin C on Oxidant and Antioxidant Parameters in Cattle Immunized by Blackleg Vaccine

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ABSTRACT

The goal of this study is to determine the effect of vitamin C on certain oxidant and antioxidant parameters in cattle immunized against blackleg. In the study, a total of 40 cattle aged 12 to 18 months, of different breeds and genders, were used in the Ardahan region. The cattle were divided into four equal groups. The Control group received 2 mL of saline, the Vitamin group received 5 mg/kg dose of vitamin C, the Vaccine-Vitamin group received 2 mL of blackleg vaccine and 5mg/kg vitamin C, and the Vaccine group received 2 ml of blackleg vaccine. Blood samples were taken from all animals before vaccination (day 0) and on the 2nd, 14th, and 28th days of administration. On the 2nd, 14th, and 28th days, malondialdehyde (MDA) and nitric oxide (NO) levels were substantially increased (P<0.05) in the Vaccine group compared to the Control and Vitamin groups, while superoxide dismutase (SOD) and catalase (CAT) levels were considerably decreased (P<0.05). It was noted that the amount of MDA and NO measured in the 2nd, 14th, and 28th days in the Vaccine-Vitamin group reduced compared to the Vaccine group, while the amount of SOD and CAT enhanced. The study concluded that the blackleg vaccine administered to cattle causes oxidative stress and lipid peroxidation, and that vitamin C acts as an antioxidant. Vitamin C administration with blackleg vaccine in cattle is believed to be beneficial for lowering oxidative stress.

Keywords: *Blackleg vaccine, cattle, lipid peroxidation, oxidative stress, vitamin C.*

Yanıkara Aşısı ile Aşılanan Sığırlarda C Vitamininin Oksidan ve Antioksidan Parametreler Üzerine Etkisi

ÖZET

Bu araştırmanın amacı Yanıkara aşısı ile aşılanan sığırlarda C vitamininin bazı oksidan ve antioksidan parametreler üzerine etkisini belirlemektir. Araştırmada, Ardahan yöresinde ırk ve cinsiyetleri farklı, 12-18 aylık toplam 40 sığır kullanıldı. Sığırlar dört eşit gruba ayrıldı. Kontrol grubuna 2 mL serum fizyolojik, Vitamin grubuna 5 mg/kg dozda c vitamini, Aşı-Vitamin grubuna 2 ml yanıkara aşısı ve 5mg/kg C vitamini, Aşı grubuna ise 2 mL yanıkara aşısı enjekte edildi. Aşı ve ilaç uygulaması öncesinde (0.gün) ve uygulamanın 2., 14. ve 28. gününde tüm hayvanlardan kan örnekleri alındı. Uygulamanın 2., 14. ve 28. gününde Aşı grubunda malondialdehit (MDA) ve nitrik oksit (NO) düzeyinin Kontrol ve Vitamin grubuna göre anlamlı oranda (P<0,05) arttığı, süperoksit dismutaz (SOD) ve katalaz (CAT) düzeylerinin ise anlamlı oranda (P<0,05) azaldığı görüldü. Aşı-Vitamin grubunda 2., 14. ve 28. gün ölçülen MDA ve NO miktarının Aşı grubuna göre düştüğü, SOD ve CAT miktarının ise yükseldiği görüldü. Bu araştırmada, sığırlara uygulanan yanıkara aşısının oksidatif strese ve lipid peroksidasyonuna neden olduğu ve C vitamininin antioksidan etki gösterdiği sonucuna varıldı. Sığırlarda yanıkara aşısı ile birlikte C vitamini uygulamasının oksidatif stresi azaltma açısından faydalı olacağı düşünülmektedir.

Anahtar Kelimeler: *C vitamini, lipid peroksidasyonu, oksidatif stres, sığır, yanıkara aşısı.*

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Introduction

Blackleg disease is an infectious disease caused by *Clostridium chauvoei* (*C. chauvoei*). The pathogen of the disease is a gram-positive, spore-forming, anaerobic bacterium. Bacteria are very resistant to adverse environmental conditions due to their spore forms. The spores taken in the organism go into a vegetative form when favourable conditions present themselves. Certain toxins are synthesized by bacteria which pass into the vegetative form. These toxins cause injury to regional tissues and death (Nicholson et al., 2019). High mortality rates lead to significant economic losses. In animal species, cattle are the most vulnerable to illness. Cattle between 6 and 30 months old are generally affected. Cases of disease have also been reported in pregnant bovine animals and foetuses (Abreu et al., 2017). It may also be observed in sheep and other domestic animals in addition to cattle (Frey and Falquet, 2015).

The disease begins in the muscle tissue. Sick animals may experience high fever, oedema and cremation in the damaged area (cracking due to gas accumulation). The disease progresses quickly and the affected area turns dark red-black. The muscular tissue is haemorrhagic and necrotic. Additionally, areas of diffuse fibrin can be seen in the epicardium and dark red multifocal areas in the myocardium. The prognosis for the disease is bad and usually causes death (Araujo et al., 2010; Abreu et al., 2017).

Cells require oxygen to carry out their metabolic activities. In oxygen utilization, certain free radicals (reactive groups) are released. The most important of these are superoxide (O_2), singlet oxygen (O^-), and hydrogen peroxide (H_2O_2) (Heidarpour et al., 2013). These oxygen groups interact with nucleic acids, enzymes, proteins, and lipids in cells and break down their structures (Deveci and Güven, 2008). The reaction of these reactive groups with fatty acids in the cellular membrane is known as lipid peroxidation. Malondialdehyde (MDA) is released as the final product in this reaction chain. MDA is known to be an indicator of cellular membrane degradation (Atakisi et al., 2010; Esmaeilnejad et al., 2018).

Nitric oxide (NO) is a molecule produced from L-arginine. It has important physiological functions such as the immune system, blood pressure regulation and nerve conduction in living organisms. The NO produced by macrophages in inflammatory cases plays a significant role in defence against pathogens (like viruses, bacteria, and parasites) (Bozukluhan et al., 2016). When nitric oxide is produced in excess, it changes to peroxinitrite. That metabolite suppresses the immune system (Bozukluhan et al., 2013). It also causes cellular damage and lipid peroxidation (Mavangira and Sordillo, 2018).

The quantity of reactive oxygen groups increases in mastitis, metritis, tissue damage, heavy exercise, pregnancy, and infections (Jaguezeski et al., 2018). As a result of overproduction of oxidant substances or inadequate antioxidant substances, these reactive substances cause cellular damage (Kırbaş et al., 2021). Such a condition is known as oxidative stress. It is claimed that living beings are more sensitive to some diseases (like sepsis and lung diseases) in the case of oxidative stress (Jaguezeski et al., 2018). The antioxidant defence mechanism is divided into non-enzymatic substances and enzymatic. Catalase (CAT) and superoxide dismutase (SOD) are enzymatic, vitamin C (ascorbic acid) is non-enzymatic antioxidants (Mavangira and Sordillo, 2018).

Vaccination is the main way to prevent blackleg disease, which progresses quickly and leads to the death of cattle (Mamak et al., 2018). The blackleg vaccine given to cattle is thought to cause oxidative stress and vitamin C can reduce oxidative stress. The study was carried out to define the effect of vitamin C on some oxidant and anti-oxidant parameters in cattle immunized with blackleg vaccine.

Materials and Methods

This study was approved by the Kafkas University Animal Experiments Local Ethics Committee (27.08.2020, 2020/120 Number Ethics Committee Decision) and the Ministry of Agriculture and Forestry (21.08.2020-E.29486769-622.01-2331582).

The research was conducted on 40 cattle of different races and genders, aged between 12 and 18 months, in the Ardahan region. The animals used in the study were maintained in the same environment and equal feeding conditions (tap water from the same resource and grassland and *ad libitum*) were applied. The cattle were divided into four equal groups, each consisting of 10 animals. The groups were the Control, Vitamin, Vaccine-Vitamin and Vaccine groups, respectively. 2 ml saline physiological solution (Polifleks-Polifarma®) was administered to the Control group and 5 mg/kg vitamin C (Maxivit-C®-baVET) was administered subcutaneously to the Vitamin group. Vitamin C was administered within the recommended dose range (4-6 mg/kg) in the drug (Maxivit-C-baVET®) prospectus. In the Vaccine-Vitamin group, 2 ml of blackleg vaccine (VBR CHAUVOEI®-Ata-Fen) and 5 mg/kg of vitamin C were injected subcutaneously different areas using separate injectors. In the Vaccine group, only 2 mL. of blackleg vaccine was given subcutaneously. 10 mL. blood samples were taken from the vena jugularis in the serum tube (BD Vacutainer® CAT) before the administration (day 0) and on the 2nd, 14th, and 28th days of the administration from all animals in the groups. The blood samples were centrifuged at 3000 rpm for 15 minutes. Then, the obtained serum stored at -20°C until the test.

Enzymatic activities of CAT and SOD in the serum were identified by ELISA (Epoch, Biotek, USA) with commercial kits (Cayman Chemical Company, USA). MDA analysis was determined as colorimetric (Epoch, Biotek, USA) using the methodology reported by Yoshioka et al. (1979) and NO was determined by Miranda et al. (2001).

In this study, the data was statistically evaluated using the statistical package program (IBM SPSS Statistics®, Chicago, IL, USA). The normal distribution of the data was verified using the Shapiro-Wilk assay. The Group means were compared with one-way analysis of variance (ANOVA). Tamhane's T2 and Tukey HSD tests were used to perform multiple group comparisons. The results obtained are presented in the form of a mean ($\bar{X}$) and standard deviation (SD). P<0.05 was found to be statistically important in this study.

Results

The results of the analysis of some oxidant and antioxidants obtained from this study are shown in Table 1 below.

Table 1. The analysis results of the cattle groups

Parameters	Days	Control (n=10) $\bar{X}$ ± SD	Vitamin (n=10) $\bar{X}$ ± SD	Vaccine-Vitamin (n=10) $\bar{X}$ ± SD	Vaccine (n=10) $\bar{X}$ ± SD	P-value
MDA (µmol/L)	0	1.89 ± 0.27	1.84 ± 0.39	1.95 ± 0.43	1.81 ± 0.27	0.815
	2	1.81 ± 0.23 [a]	1.77 ± 0.23 [a]	3.90 ± 0.37 [b]	4.03 ± 0.45 [b]	0.001
	14	1.92 ± 0.23 [a]	1.75 ± 0.35 [a]	4.32 ± 0.30 [b]	4.66 ± 0.82 [b]	0.001
	28	1.82 ± 0.38 [a]	1.86 ± 0.40 [a]	4.14 ± 0.37 [b]	4.49 ± 0.64 [b]	0.001
NO (µmol/L)	0	27.32 ± 4.59	31.66 ± 5.72	28.50 ± 6.28	31.28±5.03	0.228
	2	41.02 ± 7.70 [a]	34.86 ±5.82 [a]	49.45 ± 10.00 [b]	53.41 ± 9.62 [b]	0.001
	14	44.36 ± 9.80 [a]	36.71±5.46 [a]	53.64 ± 6.14 [b]	57.62 ± 10.54 [b]	0.001
	28	46.78 ± 11.14 [a]	35.98 ± 8.51 [a]	55.62 ± 9.58 [b]	62.70 ± 12.45 [b]	0.001
SOD (U/mL)	0	233.00 ± 15.37	230.82 ± 15.30	224.62 ± 14.40	227.59 ± 13.96	0.608
	2	236.42 ± 12.23 [a]	233.61 ± 16.70 [a]	201.33 ±12.55 [b]	174.82 ± 36.57 [b]	0.001
	14	241.97 ± 16.30 [a]	236.42 ± 12.16 [a]	186.54 ± 18.69 [b]	182.02 ± 33.92 [b]	0.001
	28	239.23 ± 16.70 [a]	230.60 ± 17.12 [a]	200.63 ± 29.33 [b]	194.76 ± 27.04 [b]	0.001
CAT (nmol/min/mL)	0	42.62 ± 9.68	41.34 ± 4.41	39.85 ± 7.84	43.92 ± 5.83	0.632
	2	46.82 ± 10.14 [a]	36.90 ± 3.95 [a]	29.35 ± 6.70 [b]	19.57 ± 3.76 [c]	0.001
	14	47.00 ± 9.12 [a]	40.07 ± 5.53 [a]	31.20 ± 7.35 [b]	15.44 ± 4.21 [c]	0.001
	28	43.21 ± 4.89 [a]	43.58 ± 4.33 [a]	35.55 ± 5.21 [b]	20.83 ± 5.26 [c]	0.001

[a, b, c] Those with different letters in the same row were statistically significant in the range of P<0.05

As shown in Table 1 above, for the zero day, there is no difference among the MDA, NO, SOD, and CAT amounts of the groups. However, on the 2nd, 14th, and 28th days, in the Vaccine group, the mean amount of NO and MDA increased substantially (P<0.05) compared to the Control and Vitamin groups, and insignificantly (P>0.05) compared to the Vaccine-Vitamin group. It is seen that the amount of SOD measured in the 2nd, 14th, and 28th days decreased significantly (P<0.05) in the Vaccine group compared to the Control and Vitamin groups, and insignificantly (P>0.05) compared to the Vaccine-Vitamin group. It is observed that the amount of CAT in the Vaccine group decreased significantly (P<0.05) on the 2nd, 14th, and 28th days compared to the Vitamin, Control and Vaccine-Vitamin groups. There was an important boost (P<0.05) in the CAT enzyme level in the Vaccine-Vitamin group compared to the Vaccine group during the 2nd, 14th, and 28th days.

Discussion

NO is a molecule synthesized by the nitric oxide synthetase (NOS) enzyme and has important biological functions. This molecule, whose half-life is very short, has significant physiological and metabolic functions in many species, from bacteria to mammals (Atakişi and Merhan, 2017). It is reported that the level of NO increases in foot-and-mouth disease (FMD) in cattle (Bozukluhan et al., 2013; Uzlu et al., 2016), mastitis (Atakisi et al., 2010), omphalitis (Bozukluhan et al., 2016) and vaccination with anthrax vaccine (Doğan et al., 2021). In this study, on 2nd, 14th, and 28th days, NO levels in the Vaccine-Vitamin group boosted considerably (P<0.05) relative to the Vitamin and Control groups (Table 1). The findings in this study are similar to those in the studies reported by Bozukluhan et al. (2013), Atakişi et al. (2010), Uzlu et al. (2016), Doğan et al. (2021), and Bozukluhan et al. (2016). This increase in nitric oxide can be explained by the antigenic characteristic of the blackleg vaccine. The bacterial blackleg vaccine (antigenic stimulus) is thought to activate macrophages and induce the synthesis and secretion of NO. The reactions between the antigen and the macrophages explain the increase in the quantity of NO. It was found that the amount of NO of days 2, 14, and 28 in the Vaccine-Vitamin group was reduced compared to the Vaccine group. This reduction in NO is due to the reaction of vitamin C with the oxidant groups (antioxidant effect).

MDA is the final product formed when free oxygen groups react with fatty acids in the cell membrane. Detection of MDA is accepted as an indicator of lipid peroxidation. In studies conducted on cattle, MDA levels were reported to be affected in some bacterial, viral, and parasitic diseases. It is reported that the level of MDA increases in skin papilloma (Arslan et al., 2018), *Fasciola gigantica* (Bahrami et al., 2014), Neosporiosis (Glombowsky et al., 2017), Coccidiosis (Yilmaz and Issi, 2014), Babesiosis (Saleh, 2009), Anaplazmosis (Esmaeilnejad et al., 2018), *Dictyocaulus viviparus* (Değer et al., 2008), *Hypoderma* spp (Merhan et al., 2017), Theileriosis (Kızıl et al., 2011), *Toxocara vitulorum* (Bozukluhan et al., 2017), Hidatidosis (Heidarpour et al., 2013), Tuberculosis (Kızıl and Keltek, 2017), listeriosis (Jaguezeski et al., 2018), FMD (Khoshvaghti et al., 2014; Uzlu et al., 2016), Omphalitis (Bozukluhan et al., 2016), Brucellosis (Perin et al., 2017), Mastitis (Deveci and Güven, 2008), anthrax vaccine administration (Doğan et al., 2021), Dystocia (Bayyit and Merhan, 2020), and transplanted cattle (Chirase et al., 2004).

In this study, it was noted that the MDA quantity for the 2nd, 14th, and 28th days of the Vaccine group boosted significantly ($P<0.05$). The findings in this study are similar to those reported by Bahrami et al. (2014), Glombowsky et al. (2017), Perin et al. (2017), Yılmaz and Issı (2014), Saleh (2009), Arslan et al. (2018), Esmaeilnejad et al. (2018), Değer et al. (2008), Merhan et al. (2017) Kızıl et al. (2011), Heidarpour et al. (2013), Bozukluhan et al. (2017), Kızıl and Keltek (2017), Jaguezeski et al. (2018), Khoshvaghti et al. (2014), Uzlu et al. (2016), Bozukluhan et al. (2016), Deveci and Güven (2008), Doğan et al. (2021), Bayyit and Merhan (2020), and Chirase et al. (2004). This increase in the amount of MDA can be explained by the fact that oxidant substances produced during the recognition and processing of antigens by macrophages cause lipid peroxidation. Because these oxidants, which are produced in excess, react with unsaturated fatty acids in the cellular membrane. MDA, which is produced in excess through a series of reactions, is released into the bloodstream. Based on the increase in the amount of MDA in the blood, it can be suggested that the blackleg vaccine causes lipid peroxidation.

CAT is a significant enzyme whose antioxidant properties are present in the body. It is reported that the amount of CAT in cattle decreases in Tuberculosis (Kızıl and Keltek, 2017), Coccidiosis (Tufan and Çam, 2008), *Theileria annulata* (Kızıl et al., 2011), *Dictyocaulus viviparus* (Değer et al., 2008), *Listeria monocytogenes* (Jaguezeski et al., 2018), Brucellosis (Perin et al., 2017), *Anaplasma marginale* (Esmaeilnejad et al., 2018), Etiyonin (Abd Ellah et al., 2009), and administration anthrax vaccine (Doğan et al., 2021). In this study, on the 2nd, 14th, and 28th days, the amount of CAT in the Vaccine group declined substantially compared to the Control, Vitamin, and Vaccine-Vitamin groups. The study results are similar to those studies results reported by Kızıl and Keltek (2017), Tufan and Çam (2008), Esmaeilnejad et al. (2018), Değer et al. (2008), Jaguezeski et al. (2018), Perin et al. (2017), Kızıl et al. (2011), Abd Ellah et al. (2009), and Doğan et al. (2021). The reduction in the amount of CAT may be due to the depletion of the CAT enzyme reacting with the increasing oxidant groups after vaccination.

In the present study, on the 2nd, 14th, and 28th days, the amount of CAT in the Vaccine-Vitamin group boosted substantially compared to the Vaccine group (Table 1). The reason for this boost in the CAT quantity is thought to be due to the antioxidant property of vitamin c. As ascorbic acid reacts with oxidant groups and reduces their effect. This means that the CAT enzyme is less used in the Vaccine-Vitamin group than in the Vaccine group. It is reported that the amount of CAT in cattle increases when FMD vaccine is administered (Kızıl and Gül, 2004) while does not change significantly in Papillomatosis (Arslan et al., 2018) and FMD (Khoshvaghti et al., 2014). These differences in the CAT level are thought to be because of the content of the vaccines used (such as viral, bacterial, live, attenuated, inactive, and adjuvant), care and nutritional conditions.

One of the antioxidant enzymes is SOD. It transforms superoxide into hydrogen peroxide (H_2O_2). The resulting hydrogen peroxide loses its harmful effects by transforming into water and oxygen by means of the CAT enzyme (Aslankoç et al., 2019). It is reported that the amount of SOD in cattle decreases in Coccidiosis (Tufan and Çam, 2008), FMD (Khoshvaghti et al., 2014), *Fasciola gigantica* (Bahrami et al., 2014), *Anaplasma marginale* (Esmaeilnejad et al., 2018), Hidatidosis (Heidarpour et al., 2013), *Dictyocaulus viviparus* (Değer et al., 2008), administration anthrax vaccine (Doğan et al., 2021), and Bovine Leukemia Virus (Ali et al., 2019).

In this study, on the 2nd, 14th, and 28th days, the SOD quantity in the Vaccine group decreased substantially ($P<0.05$). Results from this study are similar to those reported by Bahrami et al. (2014), Tufan and Çam (2008), Doğan et al. (2021), Khoshvaghti et al. (2014), Esmaeilnejad et al. (2018), Heidarpour et al. (2013), Değer et al. (2008), and Ali et al. (2019). Based on the decrease in the amount of SOD in the Vaccine group, it can be claimed that the blackleg vaccine enhanced the amount of superoxide. The amount of SOD that reacts with the excess superoxide radical is reduced. Naturally, serum level also decreases. This reaction explains the decrease in the amount of SOD in the Vaccine group.

It is reported that the amount of SOD increases in Brucellosis (Perin et al., 2017), *Tenia saginata* (Łuszczak et al., 2011), septicemia (Meral et al., 2017), and cattle administered Ethionine (Abd Ellah et al., 2009). In another study, it is reported that vitamin c does not affect the amount SOD in cattle vaccinated with FMD vaccine (Kızıl and Gül, 2004). Variable amount of SOD in studies conducted on cattle is thought to be caused by the formulation of the vaccine, the kind of infection (like bacterial, viral, and parasitic), course of infection (acute, subacute or chronic), and care and food conditions.

Conclusion

In conclusion, in cattle vaccinated with blackleg vaccine, on the 2nd, 14th, and 28th days following the vaccination, it is observed that the amounts of NO and MDA boosted, the amount of CAT and SOD reduced. Based on the findings, it could be argued that blackleg vaccines in cattle lead to oxidative stress and peroxidation of lipids. It was noted that the amounts of MDA and NO in the Vaccine-Vitamin group reduced and the amounts of SOD and CAT boosted. It is recommended to use vitamin c with the blackleg vaccine in cattle.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Research Article

Determination of Chemical and Microbiological Quality of Strained Yoghurt Samples Marketed in Aydın Province

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A B S T R A C T

In this study, 60 strained yoghurt samples, including 30 public bazaar and 30 dairy-markets were examined in terms of chemical and microbiological properties. According to chemical analysis of bazaar samples, average levels of pH, acidity, fat, protein, salt, total solid and ash are determined; 3.94, 2.19% (LA), 8.09%, 11.21%, 1.83%, 21.74%, 0.71%, respectively. The average levels of given elements above in samples gathered from dairy stores are determined; 3.94, 1.86% (LA), 9.47%, 11.95%, 1.81%, 23.26%, 0.71%, respectively. No presence of starch was found in any of the samples. The chemical analysis results of the strained yoghurt samples were determined in accordance with the values specified in Turkish Food Codex except for the amount of fat analysis gave a result over the specified value. Coliform bacteria level was determined <3 MPN/g in 17 samples, 4-1100 MPN/g in 10 samples and 1100 MPN/G in 3 samples, also *Escherichia coli* Type 1 presence was determined in 12 samples. Lactic acid bacteria were found at 7.32 log cfu/g (Lactobacillus number) and 8.09 log cfu/g (*Streptococcus* number), respectively in bazaar samples. Yeast and mold levels were determined as 5.89 log cfu/g on average. In dairy-market samples, coliform bacteria level was <3 MPN/g in 19 samples, 4-1100 MPN/g in 9 samples and >1100 MPN/g in 2 samples. In addition, in 6 samples the presence of *E. coli* Type 1 has been determined. Lactic acid bacteria in the samples were determined on average at 7.67 log cfu/g (Lactobacillus number) and 8.09 log cfu/g (Streptococcus number), respectively. The yeast and mold levels of dairy-market samples were determined 5.94 log cfu/g on average.In this study, it was determined that strained yoghurt samples were subjected to contamination from raw material to consumption. This situation is very important because it can negatively affect public health as well as product quality.

***Keywords**: Microbial quality, public health, strained yoghurt.*

Aydın'da Tüketime Sunulan Süzme Yoğurtların Kimyasal ve Mikrobiyolojik Kalitesinin Belirlenmesi

Ö Z E T

Bu çalışmada, 30 adet halk pazarlarından ve 30 adet de mandıra-marketlerden olmak üzere toplamda 60 adet süzme yoğurt örneği, kimyasal ve mikrobiyolojik özellikleri yönünden incelenmiştir. Kimyasal analizler sonucunda pazar örneklerinin pH, asitlik, yağ, protein, tuz, kuru madde ve ham kül analiz sonuçlarının ortalama değerleri sırasıyla, 3,94, %2,19 (LA), %8,09, %11,21, %1,83, %21,74 ile ham kül %0,71 olarak tespit edilmiştir. Mandıra-market örneklerinin kimyasal analiz sonuçlarının ortalama değerleri ise sırasıyla, 3,94, %1,86 (LA), %9,47, %11,95, %1,81 %23,26 ve ham kül %0,71 olarak belirlenmiştir. Yapılan nişasta analizi sonucunda hem pazar örneklerinde hem de mandıra-market örneklerinde nişasta varlığına rastlanmamıştır. Süzme yoğurt örneklerinin yağ miktarı hariç kimyasal analiz sonuçları, Türk Gıda Kodeksi'nde belirtilen değerlere uygun olarak tespit edilmiştir. Yağ analizi belirtilen değerin üzerinde sonuç vermiştir. Koliform bakteri düzeyi pazar örneklerinden, 17'sinde <3 EMS/g, 10'unda 4-1100 EMS/g arasında ve 3'ünde ise >1100 EMS/g olarak tespit edilmiş, ayrıca 12 örnekte *Escherichia coli* Tip 1 varlığı belirlenmiştir. Pazar örneklerinde laktik asit bakterileri ortalama olarak sırasıyla 7,32 log kob/g (Lactobacillus sayısı) ve 8,09 log kob/g (Streptococus sayısı) düzeyinde tespit edilmiştir. Maya ve küf düzeyi ise ortalama 5,89 log kob/g olarak saptanmıştır. Mandıra-market örneklerinde, koliform bakteri düzeyinin 19 örnekte <3 EMS/g, 9 örnekte 4-1100 EMS/g arasında ve 2 örnekte de >1100 EMS/g olduğu tespit edilmiştir. Bununla birlikte 6 örnekte, *E. coli* Tip1 varlığı belirlenmiştir. İncelenen örneklerdeki laktik asit bakterileri ortalama olarak sırasıyla 7,67 log kob/g (Lactobacillus sayısı) ve 8,09 log kob/g (Streptococus sayısı) düzeyinde saptanmıştır. Mandıra-market örneklerinin maya ve küf düzeyi ise ortalama 5,94 log kob/g olarak belirlenmiştir. Bu çalışma ile süzme yoğurt örneklerinin ham maddenin elde edilmesinden tüketimine kadar kontaminasyona maruz kaldığı tespit edilmiştir. Bu durum ürün kalitesinin yanı sıra halk sağlığını da olumsuz etkileyebileceği için oldukça önemlidir.

***Anahtar Kelimeler**: Mikrobiyal kalite, halk sağlığı, süzme yoğurt.*

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Introduction

Yoghurt is a coagulated dairy product obtained through lactic acid fermentation of milk by symbiotic bacteria such as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarus* subsp. *thermophilus.* Yoghurt is an important food source in terms of the amount of protein, carbohydrate, fat, vitamin and mineral substances in its chemical structure, and the dry matter content it contains is higher than milk. Since most of the lactose in milk is broken down during yoghurt production, consumption of yoghurt is quite comfortable for lactose intolerant individuals. The bacteria used in yoghurt production have a suppressive effect on pathogenic bacteria in the gastrointestinal tract and prevent their growth; therefore, it makes yoghurt an important food for human health (Sömer, 2013).

Due to the high-water content of yoghurt, which is widely consumed around the world, its durability and shelf life are short (Kırdar and Gün, 2007). From past till now, many methods have been applied to increase the shelf life and durability of yoghurt (Sömer, 2013). Removing most of the water in yoghurt is among the known and commonly used methods (Kırdar and Gün, 2007).

The cultural richness of our country has helped to produce various types of yoghurt and thus to consume yoghurt during periods of reduced milk production. Some of these are resistant yoghurt varieties such as Silivri, tulum, strained, winter yoghurt and kurut (Ünsal, 2007; Sömer, 2013). Strained yoghurt, also known as bag or pouch yoghurt among this durable yoghurt, is a type of yoghurt that is more popular and consumed more than the others (Tekinşen and Bayar, 2008). Strained yoghurt is a type of yoghurt with a higher dry matter content than regular yoghurt, with its spreadable feature, and which has a color that can vary between white and yellow depending on the type and chemical structure of the milk used in production (Baladura, 2011).

The production of strained yoghurt is done more commonly within the family in the middle regions of Anatolia, and in small-scale enterprises (Atamer et al., 1990; Uysal, 1993). With the development of technology, the production of strained yoghurt in modern dairy enterprises has also increased and has become a more hygienic product (Baladura, 2011). However, strained yoghurt produced in family and small-scale enterprises are still sold for sale in the market places. For this reason, there is no standard production technique in the production of strained yoghurt. Therefore, there are significant differences in chemical and microbiological quality between strained yoghurt samples offered for sale in bazaar and strained yoghurt samples sold in dairy stores and markets (Tekinşen and Bayar, 2008).

In the production of strained yoghurt; traditionally, natural yoghurt is filtered in cloth bags and clay pots, while reverse osmosis, centrifugation and ultrafiltration processes are applied industrially (Nsabimana et al., 2005).

With this study, it has been aimed to determine the microbiological and chemical properties of strained yoghurt offered for consumption in markets and dairy-markets in Aydın.

Materials and Methods

In this study, 60 samples of strained yoghurt that were obtained from dairy/markets in Aydın, with 30 samples from each source, were used. The samples taken were brought to the laboratory under the cold chain and subjected to microbiological and chemical analyses within the same day.

Chemical Analysis of Strained Yoghurt Samples

Dry matter determination: Dry matter values of strained yoghurt samples were determined using gravimetric method (Sömer, 2013).

Determination of raw ash: In order to determine the amount of ash in strained yoghurt samples, the crucibles were kept in a drying oven at 105 °C for 1 hour, then cooled in a desiccator and their tare was determined. Then, 3-3.5 g of strained yoghurt samples were added to the crucibles and dried in a drying oven at 105±2 °C for one day. At the end of the period, the crucibles were burned in the muffle furnace at 550 °C for 5-6 hours. After the burning process, the crucibles were removed from the muffle furnace, taken into the desiccator, and after cooling, they were weighed again and transferred to the muffle furnace. This process was continued until the crucible weight was stabilized. The ash amount with the fixed value determined in the weighing was calculated as % (Kırdar, 2001).

pH determination: 10 g of strained yoghurt sample was taken into a beaker and homogenized with the help of a glass baguette. Afterwards, pH meter (AD 1030, Hungary) probe was immersed in the sample and waited until a constant value was obtained in the pH meter, and the fixed value was accepted as the pH value (Biberoğlu and Ceylan, 2013).

Titratable acidity determination: In order to determine the titratable acidity of the strained yoghurt samples, 10 g of the homogenized strained yoghurt sample was weighed and 90 ml of distilled water was added into an erlenmeyer flask. The sample in the erlenmeyer flask was mixed thoroughly with the help of a glass baguette. Afterwards, a few drops of 1% phenolphthalein solution (Merck 1.07233.0100) were dropped into the erlenmeyer flask and titrated with 0.1 N NaOH solution (Merck 1.06498.500) until a permanent pink colour was formed. The titratable acidity of strained yoghurt samples was calculated with formula and determined as % lactic acid (TSE, 2006).

The formula used in the analysis: % lactic acid= [(V×N×0.009) / m] × 100 (V: Amount of NaOH spent in the titration (ml), m: Amount of strained yoghurt sample used in titration (g), N: Normality of NaOH solution).

Fat determination: The fat analysis of strained yoghurt was performed by the Gerber method, as specified in TSE 1330 Yoghurt Standard (TSE, 2006).

Crude protein determination: Crude protein analysis of strained yoghurt samples was made by Kjeldahl method (Kurt et al., 2003; Sömer, 2013).

Salt determination: 5 g of strained yoghurt sample was taken homogeneously into an erlenmeyer flask, and hot distilled water was added and shaken vigorously for 10 minutes. The resulting solution was filtered through a filter paper into a 500 ml volumetric flask. In order for the salt remaining in both the erlenmeyer flask and the filter paper to pass into the water, the filtration process was repeated by adding hot distilled water 4-5 times to the solution in the erlenmeyer flask. When the filtrate in the volumetric flask came to room temperature, distilled water was added to the volumetric flask up to the volume line. 25 ml of this filtrate was taken, transferred to an erlenmeyer flask and 2-3 drops of 5% potassium chromate solution (K_2CrO_4) (MERCK 104952.0250) was added. Then, the filtrate was titrated with 0.1 N silver nitrate solution ($AgNO_3$) (MERCK 109990.0001) until a permanent brick red colour was observed in the erlenmeyer flask (Kırdar, 2001).

Starch determination: In order to determine the presence of starch in strained yoghurt samples, 2-3 g strained yoghurt samples were taken into a test tube and a few drops of Lugol's solution (MERCK 109261.1000) were dripped onto it. It was examined whether the blue colour, which is a positive result, was present in the samples or not (Şahan, 2012).

Microbiological Analysis of Strained Yoghurt Samples

Preparation of strained yoghurt for microbiological analysis: From the collected strained yoghurt samples, 10 grams of samples were weighed under sterile conditions, placed in stomacher bags and 90 ml of sterile physiological peptone water (Oxoid CM0009) was added to them. Stomacher bags were homogenized in a stomacher (Bagmixer, Interscience, France) for 2 minutes. Serial dilutions were prepared from the homogenate to determine the number of LAB, yeast and mold and coliform bacteria and to detect the presence of *E. coli.*

Enumeration of lactic acid bacteria

Enumeration of Lactobacilli: Lactobacillus counts were made by the spread plate method using Man Rogosa and Sharpe (MRS) Agar (OXOID CM0361B) medium. From the previously prepared serial dilutions, they were inoculated into 0.1 ml medium, and the sown mediums were left to incubate at 35 °C for 48-72 hours (Özçelik, 1998; Sömer, 2013).

Enumeration of Streptococci: M17 Agar (OXOID CM0785B) was used to determine Streptococcus measurements in strained yoghurt samples. Serial dilutions of 0.1 ml were left in their medium, seeded using the spread plate method and incubated at 42 °C for 48-72 hours (Özçelik, 1998; Halkman, 2005; Sömer, 2013).

Enumeration of yeast and molds: In order to determine the yeast and mold levels in the examined samples, seeding was carried out on Potato Dextrose Agar (PDA) (OXOID CM0139B) medium with 10% tartaric acid addition using the pour plate method. The seeded media were incubated at 22 °C for 5 days (Karahan et al., 2002; TSE, 2006; Sömer, 2013).

Enumeration of coliform bacteria: Coliform group bacteria analysis was performed using the Lauryl Sulphate Tryptose (LST) broth (OXOID CM0451B) medium using the Most Probable Number (MPN) method (Halkman, 2005).

Determining the presence of E. coli Type 1: In order to determine the presence of *E. coli* Type 1, confirmation and completion tests were respectively applied to positive tubes with turbidity and gas formation in coliform bacteria analysis.

A total of 60 strained yoghurt samples of microbiological and chemical data analysis were performed using Microsoft Excel version 2016, mathematical functions and Statistical Package for Social Sciences (SPSS) version 22. Maximum, minimum, mean and standard deviation values were specified for descriptive statistics.

Results

In this study, some chemical (dry matter, fat, acidity, salt, pH, starch, ash and protein presence) and microbiological (coliform bacteria, *E. coli*, lactic acid bacteria and yeast-mold) properties of 60 strained yoghurt samples sold in Aydın province, 30 from the bazaar and 30 from the dairy-markets, were examined.

Chemical Analysis Results of Strained Yoghurt

The chemical analysis results of the strained yoghurt samples obtained from the market are given in Table 1. No starch was found in these samples.

Chemical analysis results of dairy-market samples are given in Table 2. No starch was found in these samples

Microbiological Analysis Results of Strained Yoghurt

Lactic acid bacteria and yeast-mold analysis results in the analysed market samples are shown in Table 3.

The lactic acid bacteria and yeast-mold counts of the samples taken from dairy-markets in our study are given in Table 4.

As a result of the analysis, coliform bacteria were detected in 13 of 30 strained yoghurt samples from bazaars and 11 of 30 samples from dairy-markets.

When the samples were examined in terms of *E. coli* Type 1 presence, *E. coli* Type 1 was detected in 12 (40%) of 30 bazaar samples and 6 (20%) of 30 dairy-market samples.

When evaluated in general, it was determined that 18 (30%) of 60 strained yoghurt samples were contaminated with *E. coli* Type 1.

Table 1. Chemical analysis results of strained yoghurt samples obtained from the bazaar

Chemical analysis		Minimum	Maximum	Mean±SD
Dry Matter	g	0.55	0.87	0.72±0.08
	%	17.43	26.47	21.74±2.41
Raw Ash	g	0.02	0.02	0.02±0.002
	%	0.56	0.81	0.71±0.06
pH		3.79	4.10	3.94±0.1
Acidity	%	1.86	2.88	2.19±0.002
Fat	%	6.30	9.70	8.09±0.01
Protein	%	8.93	13.98	11.21±1.09
Salt	%	0.94	3.39	1.83±0.005

Table 2. Chemical analysis results of strained yoghurt samples obtained from dairy-markets

Chemical analysis		Minimum	Maximum	Mean±SD
Dry Matter	g	0.61	0.90	0.78±0.07
	%	19.21	27.22	23.26±1.84
Raw Ash	g	0.02	0.03	0.02±0.002
	%	0.56	0.90	0.71±0.08
pH		3.70	4.09	3.91±0.08
Acidity	%	1.35	2.30	1.86±0.001
Fat	%	6.60	13.80	9.47±0.02
Protein	%	9.65	13.17	11.95±0.77
Salt	%	0.99	2.36	1.81±0.002

Table 3. Bazaar samples LAB and Yeast-Mold analysis results (log cfu/g)

Microbiological Analysis	Minimum	Maximum	Mean±SD
Lactobacillus number	5.97	8.45	7.32±0.61
Streptococci number	6.66	9.19	8.09±0.43
Yeast and Mold number	4.84	7.08	5.89±0.63

Table 4. LAB and Yeast-Mold analysis results of dairy-market samples (log cfu/g)

Microbiological Analysis	Minimum	Maximum	Mean±SD
Lactobacillus number	6.04	8.47	7.67±0.61
Streptococci number	7.43	8.54	8.09±0.28
Yeast and Mold number	4.67	7.13	5.94±0.73

Discussion

Akın (1999) reported that the dry matter value of concentrated yoghurt made from cow's milk is 23.35% in yoghurt produced by the traditional method in which straining is done with cloth bags, and 22.30% in strained yoghurt produced using ultrafiltration technique. Töral et al. (1985) stated that the average dry matter ratio of 90 bags of yoghurt samples obtained from Denizli, Burdur, Isparta, Antalya and Aydın provinces was 22.74%. According to Şimşek et al. (2010) found the dry matter ratios of 22 strained yoghurt samples collected from the provinces of Isparta and Burdur to be between 17.84% and 27.72%. In our study, dry matter analysis results were found to be 21.740% on average. The ratios obtained in our study show similar results to the studies mentioned above.

Akın (1999), observed that the ash value in concentrated yoghurt made using cow's milk was 0.79% in traditionally produced yoghurt and 0.87% in yoghurt obtained by ultrafiltration technique. In their studies, Kayıkçılar (1971), Seçkin and Nergiz (1997), Çağlar et al. (1997), Kırdar and Gün (2002), Şimşek et al. (2010) determined the ash values as, 1.61%, 0.82%, 0.98%, 0.7%, 0.92%. Sömer (2013) determined the values as 0.79%, 0.65% and 0.67%, respectively, in the determination of ash in 3 samples taken from Aydın province in this study. The values obtained in our study are approximately the same as those of the researchers mentioned above. In our study, the average value found for bazaar and dairy market samples was 0.71%. The differences may be due to differences in the type of milk used, milk composition, and straining time.

Sömer (2013) reported that the pH values of strained yoghurt samples obtained from Afyon province ranged from 3.62 to 4.17, while the values of strained yoghurt samples obtained from Muğla province were 3.53 on average. And also, he reported that the pH values of strained yoghurt samples obtained from Isparta, Burdur, and Aydın provinces were determined to be 3.95, 3.80, and 3.78 on average, respectively. Biberoğlu and Ceylan (2013) determined the average pH value to be 3.81 in their studies on yoghurt obtained from Erzurum and Kars provinces. Akarca and Tomar (2019) found out that the average pH value of 50 yoghurt samples collected at different times from the yoghurt offered for sale in Afyonkarahisar province markets as 3.51. The pH value of yoghurt was approximately determined in the studies of the researchers mentioned in our study. The value for marketing and dairy samples was determined as 3.94 and 3.91, respectively. It is thought that the differences that may occur in the pH values of strained yoghurt may be related to the storage temperatures. As the storage temperatures increase, the pH value decreases and the amount of lactic acid increases.

Kalender (2014), in this study examining the effect of inulin addition in the production of reduced-fat strained yoghurt, stated that the titration acidity of strained yoghurt varied between 1.58% and 1.73% during the storage period. Demirci and Gündüz (1983), as a result of their research on yoghurt with different proportions of milk powder added, stated that the titration acidity increased proportionally as the milk powder ratio increased. In our study, while the average acidity value was 2.19 in market samples, it was determined as 1.86 in dairy-market samples. It is thought that this difference, which emerged as a result of our study, may be related to the storage times, since the increase in the storage time causes the acidity value to decrease.

The percentage fat values obtained in our study were determined as the lowest 6.30%, the highest 9.70% and the average 8.09% in the market samples and in dairy-market samples, the highest 13.80%, the lowest 6.60% and average 9.47%. Sömer (2013), in his study on resistant yoghurt, examined the percentage change in fat values of strained yoghurt, and obtained the lowest 3.00% and the highest 19.60% results. He stated that the fat values obtained as a result of the analyzes in 3 samples taken from Aydın province were 3.80%, 7.30% and 8.40%, respectively. Obtaining different results in terms of fat analysis is thought to be due to the difference in fat ratios of the raw milk used.

In our study, while the average protein value was 11.21% in bazaar samples, it was determined as 11.95% in dairy-market samples. Sömer (2013) stated that he determined protein values between 3.45% and 11.68% in his study on strained yoghurt. He stated that he found the protein values of the strained yoghurt samples taken from Aydın province as 10.99%, 7.74% and 7.00%. Kalender (2014), in this research on strained yoghurt, examined the protein values during storage and stated that the highest protein value was 11.78% on the 1st day, and the lowest protein value was 9.51% on the 21st day. Protein results obtained from other studies are similar to the results of our study.

As a result of the salt analysis made with the samples taken, the salt values of the bazaar samples were the lowest 0.94%, the highest 3.39% and an average of 1.83%, while the salt values of the dairy-market samples were the lowest 0.99% and the highest %. 2.36 and an average of 1.81%. The fact that the salt content of the strained yoghurt produced by the traditional method (home type) and sold in the bazaar reaches up to 3.39%, suggests that the producer may use too much salt in the production of strained yoghurt in order to prevent the deterioration that may occur in yoghurt. Sömer (2013) examined the salt values of strained yoghurt samples in his study and reported that the salt values in 32 samples collected from different provinces were in the range of 0.47-4.10%. He expressed the salt values of 3 samples taken from Aydın as 0.82-0.53-0.76%.

Şahan (2012), in his study on yoghurt samples offered for sale in Erzurum, stated that he detected starch in 3 of the 40 samples he examined, but in our study, starch was not detected in a total of 60 samples taken from the bazaar and dairy-markets.

In our study, the average value of Lactobacilli in bazaar samples was determined as 7.32 log cfu/g and 7.67 log cfu/g in market-dairy samples. The average value of Streptococci in bazaar and market-dairy samples was determined as 8.09 log cfu/g. Sömer (2013), in his study on the microbiological and physicochemical properties of resistant yoghurt, stated that he observed the lowest and highest Lactobacillus numbers in the samples taken from the province of Burdur and were respectively 5.25 and 8.92 log cfu/g. He stated that the lowest value of Streptococci numbers was obtained from the province of Burdur, and the highest value was obtained from the province of Afyon, as 4.40 and 8.74 log cfu/g, respectively. Lactobacillus numbers in 3 samples taken from Aydın province were 6.61, 6.37 and 6.94 log cfu/g, respectively, and Streptococci numbers are 6.20,

7.18 and 7.09 log cfu/g. Şahan (2012), in his study on the physical, chemical and microbiological properties of yoghurt offered for sale in Erzurum, examined the lowest, highest and average Lactobacillus numbers of 7.48, 9.10 and 8.50 log cfu/g, respectively, in 40 samples. indicated its values. He stated that the Streptococci numbers were the lowest 6.30 log cfu/g, the highest 9.01 log cfu/g and the average 8.16 log cfu/g. Considering the average values in our study similar results were obtained in terms of both Lactobacillus numbers and Streptococci numbers in bazaar and dairy-market samples.

Atasoy et al. (2003) stated that they found the yeast and mold numbers of the samples in the range of $1.5x10^4$ cfu/g and $3.6x10^6$ cfu/g in their study on the yoghurt offered for consumption in Urfa. Keleş (2003) determined the average number of yeast and molds as $3x10^5$ cfu/g in his study on homemade yoghurt produced in Konya. Demirkaya and Ceylan (2013), in their study on the chemical and microbiological quality of yoghurt offered for consumption in Bilecik, stated that the number of yeast and molds was between <1.00-5.87 log cfu/g and on average 1.36 log cfu/g.

In this study, no significant difference was observed between bazaar and dairy-market samples in terms of yeast and mold numbers. It is thought that the contamination may be caused by factors such as the tools-equipment used in the process from the supply of raw milk to the final product, working personnel, and storage conditions.

Tekinşen and Bayar (2008), in their study examining the chemical and microbiological properties of strained yoghurt samples produced in Konya, stated that they detected an average of 14.22 EMS/g coliform bacteria between 19-150 MPN/g in 20% of 45 samples from 9 different brands. Çağlar et al. (1997) stated that they detected an average of 16 MPN/g coliform bacteria in 5 of 13 strained yoghurt samples in their study. Kırdar and Gün (2002) detected 1.0-1.25 x 10^3 cfu/g coliform bacteria in 17.5% of the strained yoghurt samples they examined.

In our study, coliform bacteria were detected in 13 of the bazaar samples and in 11 of the dairy-market samples, with values between 4 and >1100 MPN/g. However, the presence of *E. coli* Type1 was determined in 12 of the examined bazaar samples and in 6 of the dairy-market samples. As some researchers stated (Kırdar and Gün, 2002; Karabıyık, 2006), both between bazaar and dairy-market samples, and this study and also the sample studies mentioned above, it is thought that the number of coliform bacteria and the presence of *E. coli* Type1 vary depending on the equipment used in the production of strained yoghurt and the hygiene of the employees and/or the methods preferred in the sanitation processes used in the enterprise.

Conclusion

Strained yoghurt; it is one of the dairy products that is widely consumed in our country and especially in the province of Aydın, also has a very important place in human nutrition. When the results obtained are evaluated, it is concluded that adequate hygiene and sanitation conditions should be provided and precautions should be taken in the production of strained yoghurt. For the production of quality milk and dairy products, both the producer and the consumer should be informed. In addition, controls and inspections should be carried out by experts at frequent intervals without interruption, products offered for sale under inappropriate conditions should not be allowed, and sanctions with a deterrent effect should be applied.

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

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Research Article

The Effects of Hot Pepper Seeds Added to the Diet in Quails on the Morphology of the Oviduct and Ovary

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ABSTRACT

The aim of this study is to reveal how red-hot pepper seeds added to the diet in quails change ovarian follicle dynamics and the morphological structure of the oviduct. For this reason, a total of 48 female Japanese quails (*Coturnix coturnix Japonica*) aged 14 weeks were used as a material. While red hot pepper (*Capsicum annuum L.*) seeds were added to the diet of the animals in the experimental group at the rate of 2%, 4%, and 6%, it was not added to the control group. At the end of the study, while the number of white follicles showed a statistically significant increase in all groups compared to the control group (P<0.05), a significant increase was recorded in the number of yellowish follicles in the groups in which 2% and 4% red pepper seeds were added to the diet compared to the control group. Also, the correlation analysis revealed that there was a positive correlation between the yellowish follicle and the white follicle. In the present study, it was observed that there was a negative correlation between magnum length and isthmus length (r=-0.369, P=0.019), while there was a positive correlation between the total length of the oviduct and yellow follicle diameter (r=0.335, P=0.034). Consequently, it was concluded that the addition of red-hot pepper (2% and 4%) seeds to the diet may increase egg yield in quails.

***Keywords**: Capsicum annuum L., Coturnix coturnix Japonica, folliculogenesis, oviduct.*

Bıldırcınlarda Rasyona Eklenen Acı Biber Tohumunun Yumurta Kanalı ve Yumurtalık Morfolojisi Üzerine Etkileri

ÖZET

Bu çalışmanın amacı, bıldırcınlarda rasyona eklenen kırmızı-acı biber tohumlarının ovaryum folikül dinamikleri ve oviduk morfolojik yapısı üzerine etkilerini ortaya koymaktır. Bu amaçla, materyal olarak 14 haftalık toplam 48 adet dişi Japon bıldırcını (*Coturnix coturnix Japonica*) kullanıldı. Kırmızı acı biber (*Capsicum annuum L.*) tohumları deney grubundaki hayvanların rasyonlarına %2, %4 ve %6 oranlarında katılırken, kontrol grubundaki hayvan rasyonlarına katılmadı. Çalışma sonunda kontrol grubu dışındaki tüm gruplarda beyaz folikül sayısında istatistiksel olarak anlamlı bir artış gözlendi (P<0,05). Rasyonuna %2 ve %4 oranında kırmızıbiber tohumları eklenen gruplar kontrol grubuna göre sarımsı folikül sayısında anlamlı bir artış kaydedildi. Ayrıca yapılan korelasyon analizi, sarımsı folikül ile beyaz folikül arasında pozitif bir korelasyon olduğunu ortaya koydu. Bu çalışmada magnum uzunluğu ile isthmus uzunluğu arasında negatif korelasyon olduğu görülürken (r=-0,369, P=0,019), oviduk kanalı toplam uzunluğu ile sarı folikül çapı arasında pozitif korelasyon olduğu görüldü (r=0,335, P=0,034). Sonuç olarak, kırmızı acı biber (%2 ve %4) tohumlarının diyete eklenmesinin bıldırcınlarda yumurta verimini artırabileceği kanısına varıldı.

***Anahtar Kelimeler**: Capsicum annuum L., Coturnix coturnix Japonica, folikülogenezis, ovidukt.*

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Introduction

Industrial poultry farming is one of the most effective ways to supply the demand for animal protein increasing in parallel with the growth in world population (Wallinga et al., 2022). The ovary and the oviduct are the two primary organs in avians that are responsible for reproductive performance. The avian oviduct is divided into four regions: the infundibulum where fertilization takes place; the magnum where egg white (albumin) and chalazae form; the isthmus where the inner and outer shell membranes form; and the uterus where the shell forms (Assersohn et al., 2021).

Scientists have been studying many herbal and nature-identical compounds as feed additives in recent years due to some objectives such as consumer demands in the poultry sector, avoiding antibiotics and anti-coccidiosis, and preserving the ecological balance by reducing the use of maize and soy (Rossi et al., 2020). One of the most extensively studied products in this field is red hot pepper (*Capsicum annuum L*). Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the active ingredient in hot pepper (Srinivasan, 2016). In addition to the different pharmacological properties of hot pepper or capsaicin such as analgesia (Kim et al., 2003), antineoplastic (Clark and Lee, 2016), antioxidant (Chaudhary et al., 2019; Li et al., 2019), immune modulator (Antonious, 2018), anti-obesity (Zsiboras et al., 2018), antibacterial (Careaga et al., 2003), and DNA protective (Oğuzkan et al., 2018), and hormone profile changes reported in avian (Erdost et al., 2006), anti-helmentic (Gentiles et al., 2019), anti-coccidial (Lozada-Ortiz et al., 2022) and antibiotic (Soliman and AlAfifi, 2020; El-Hack et al., 2022) properties, it also has properties that improve ovarian antioxidant capacity, egg production (Liu et al., 2021a) and meat quality (Liu et al., 2021b), and regulate lipid metabolism (Puvača et al., 2019).

When the studies in which red hot pepper or capsaicin was used as a feed additive in avian diets were examined (Ozfiliz, 2002; Ozer et al., 2005; Daş et al., 2022), none of the studies on the effects of red hot pepper or capsaicin on ovary and oviduct, which are the organs mainly responsible for egg yield, were found. When the seeds of red-hot pepper, which is widely produced in Turkey, are used as a feed additive in poultry, no information is available about the morphological changes it causes in the genital system organs. This study aims to reveal the changes caused by seeds of red hot pepper added to the diet as a feed additive on quail ovarian follicle dynamics and the morphological structure of the oviduct. Besides, the first findings to be obtained are intended to guide other studies to be carried out in this field and contribute to the literature.

Materials and Methods

Animal Material

In the animal material of the study, 48 female Japanese quails (*Coturnix coturnix Japonica*) at 14 weeks of age were used. The present study was conducted after ethical approval (date 22/03/2022, session 2022/003, decision 01-06) obtained from Harran University Animal Experiments Local Ethics Committee.

All stages of the study were carried out at the Avian unit, the Faculty of Veterinary Medicine at Harran University. During the experiment (14 weeks), the subjects were given feed and drinking water *ad libitum*. The animals were fed with isocaloric and isonitrogenous diets. The diet was prepared based on maize and soya. The subjects in the groups were kept in daylight and artificial lighting with 16-hour light and 8-hour darkness cycles in their henhouses. The conditions such as feeding and lighting were maintained uninterruptedly for 14 weeks. The ambient temperature was kept in the range of 18-22 °C, which is in the thermal neutral zone suitable for avian.

Formation of the Study Groups

The subjects were randomly divided into four groups according to the hypothesis of the study. No additive was included in the diet of the first group (Group Control, n=12). Red hot pepper (*Capsicum annuum L*.) seeds were added to the diets of the experimental groups at the rate of 2% in the second group (Group 2%, n=12), 4% in the third group (Group 4%, n=12) and 6% in the fourth group (Group 6%, n=12).

Provision of red hot pepper seeds

Red hot pepper (*Capsicum annuum L*.), which holds the Certificate of Geographical Indication Registration as Şanlıurfa Isot Pepper since 29/01/2001 with registration number 33 by the Turkish Patent Institute, was employed in the study. The seeds that stuck to the placenta of this pepper were sorted out, dried and stored in closed boxes to be added to the diet.

Morphological Assessment of Oviduct and Ovary

At the end of the experimental period of 14 weeks, ovaries and oviducts were resected from the slaughtered without euthanasia quails. The follicles in the ovaries of quail were counted and recorded as follows: those with white color and 1-4 mm diameter were called "white follicles", those with yellowish color and a 5-8 mm diameter were called "yellowish follicles" and those with yellow color and 9-40 mm diameter were called "yellow follicles" as reported by Anna (2021). The widths of the yellow follicles in the resected ovaries and the morphological length and width of the oviduct sections were digitally measured in mm through ImageJ software by photographing the genital system of each subject from a fixed distance (Apple iPhone 11, 12 MP. 3024x4032, Wide Camera-26 mm ƒ1.8) and saving the photograph as a HEIF file. The length unit on the ruler placed next to the specimen in the photograph was used for calibration of the software in mm.

The data obtained in the present study were statistically analyzed using the Statistical Package for the Social Sciences 24.0 package program (IBM SPSS Statistics®,

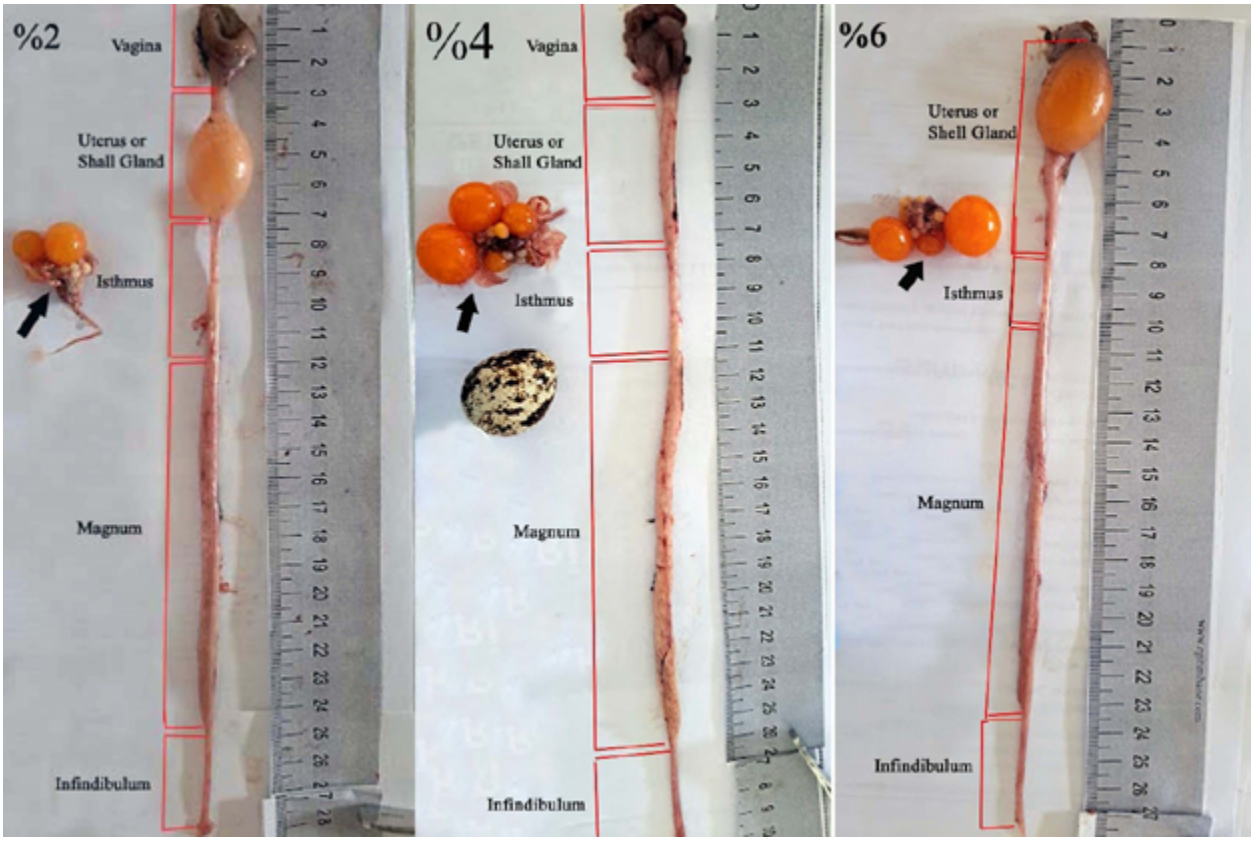

Figure 1. Group %2, Group %4, Group %6 arrow: ovarian follicles.

Chicago, IL, USA). Shapiro-Wilk test indicated that the values were normally distributed. One-way ANOVA test was used to determine whether or not there was a difference between the groups in terms of number, width and length, and the statistical significance of the difference between the groups was measured by the Duncan's test. Also, the Pearson's correlation analysis was used to determine the correlation between the measured values in all groups. The difference between the groups was considered as statistically significant when the P value was ≤0.05 (Heiberger and Neuwirth, 2009).

Results

In the study, the number of follicles (white, yellowish, yellow) of the quails in the control group, to which nothing was added to the diet, and of those in the groups to which red hot pepper (*Capsicum annuum L.*) seeds were added to the diet at the rates of 2% (Group 2%), 4% (Group 4%) and 6% (Group 6%) (Figure 1) as well as the mean diameter of yellow follicles, infundibulum, magnum and isthmus lengths and widths (Figure 2) and the statistical significance of the difference of these values between the groups are given in detail in Table 1.

In the present study, the numbers of white, yellowish and yellow follicles of the ovarian follicular activity for Groups Control, 2%, 4% and 6% were measured as 14.50±0.58, 17.10±1.02, 18.20±0.53 and 16.90±0.97 for white follicles, 1.20±0.13, 4.50±0.56, 3.60±0.69, and 2.60±0.43 for yellowish follicles, and 2.60±0.16, 2.50±0.17, 2.80±0.20, and 2.70±0.15 for yellow follicles, respectively. While the number of white follicles showed a statistically significant increase in all groups compared to the control group ($P<0.05$) the number of yellowish follicles had a significant increase in the groups 2% and 4% compared to the control group.

The correlation analysis indicated a positive correlation between the yellowish follicle and the white follicle. In the present study, there was a negative correlation between magnum length and isthmus length ($r=-0.369$, $P=0.019$), and a positive correlation was determined between the total length of the oviduct and diameter of yellow follicle ($r=0.335$, $P=0.034$) (Table 2).

Discussion

In the present study, the highest number of white follicles in relation to ovarian follicular activity was measured in Group 4%; whereas, the highest number of yellowish follicles was determined in Group 2%. Although the highest number of hierarchical yellow follicles, which are potential eggs, was obtained in Group 4%, the difference was not statistically significant. These findings are compatible with the studies (Ozer et al., 2005; Liu et al., 2021b) reporting that follicle growth and development of reproductive organs in avians is accelerated by red hot pepper or capsaicin. In the present study, it was thought that the reason for the increase in the number of quail follicles induced by hot pepper seeds was related to the changes in the number and size of the epithelial layer and theca cells surrounding the follicle in the avian ovary as reported by Ozer et al. (2005). The histological reason why this difference was observed was considered to be

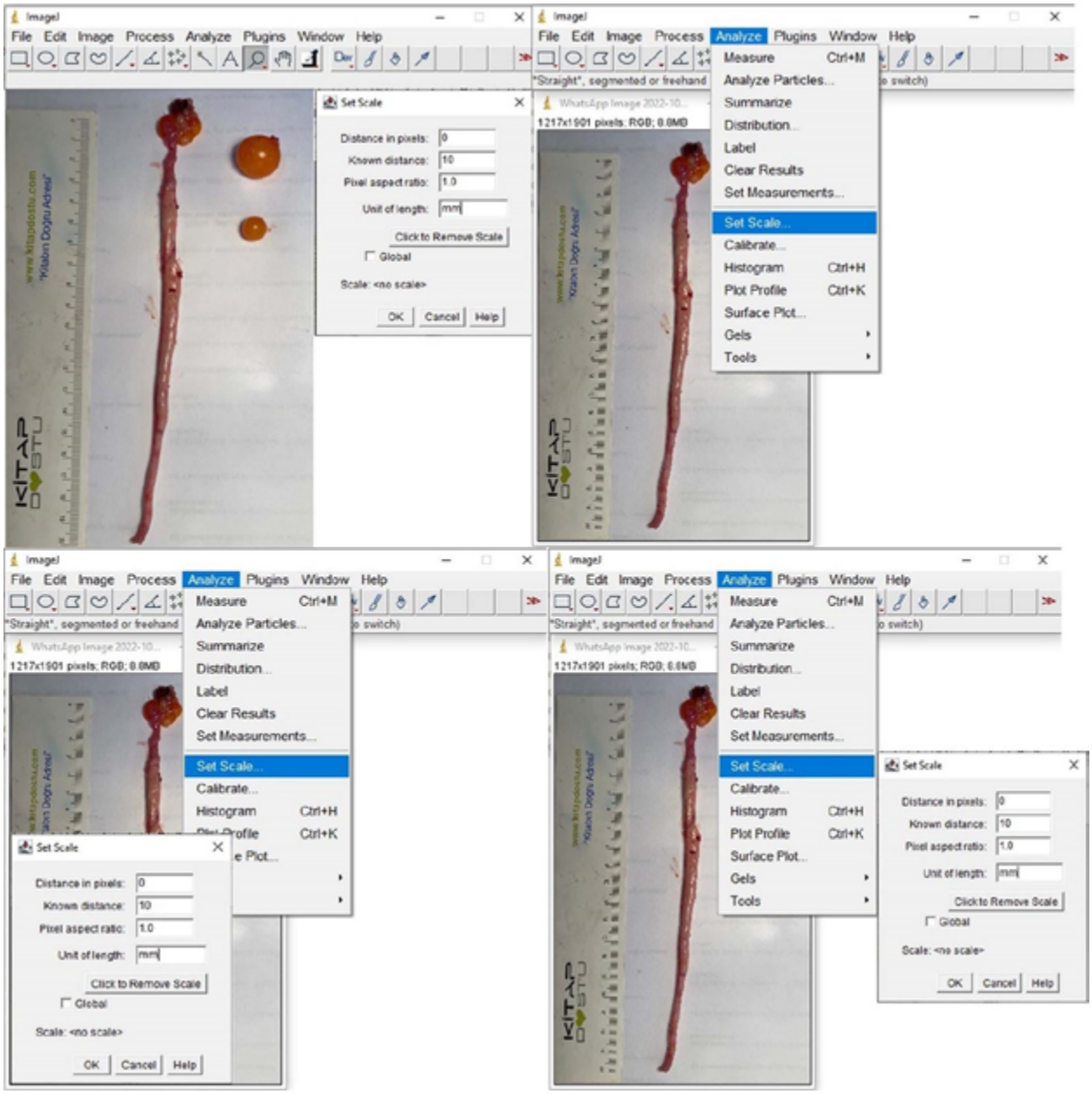

Figure 2. Group control. Digitally measurement of the genital system with ImageJ software.

due to the protective effect of capsaicin on follicles from apoptosis and atresia (Zik et al., 2010; Güler and Zik, 2018). Liu et al. (2021b) argued that capsaicin increased follicular growth and maturation in avians by activation of TRPV4 (Transient Receptor Potential Vanilloid) and calcium (Ca^{++}) signaling pathway in the ovary along with improvement in ovarian antioxidant capacity. It was considered that this condition stated by Liu et al. (2021b), may be another reason for the increased follicular activity in the present study.

Among the study groups in the present study, a significant increase was measured in the number of yellowish follicles in the group 2% compared to the group 6%. Such a high result in follicular development is compatible with the finding reported by Zik et al. (2010) that low-dose capsaicin protects ovarian follicles from apoptosis and stimulates follicular development. Likewise, the study by Güler and Zik (2018), which examined the proliferative and apoptotic effects of different doses of capsaicin on granulosa cells in the avian ovary, also reported that low-dose capsaicin treatment had positive effects on avian folliculogenesis. Alatriste et al. (2013) reported that high-dose capsaicin may affect steroidogenesis by damaging the hypothalamus-pituitary-ovarian pathway with its neurotoxic properties. In the present study, the reason why the number of yellowish follicles decreased as the dose of capsaicin increased was considered to be this effect reported by Alatriste et al. (2013).

Also, the lengths of the infundibulum, magnum and isthmus and the width of the anatomical midpoint were individually measured to assess how hot pepper seeds added to the diet affect the morphology of the oviduct in quails. The magnum length measured under this scope was significantly longer in Group 6% compared

Table 1. Comparison of the difference of measured values between groups (as Mean±SE).

	Group Control (n=12)	Group 2% (n=12)	Group 4% (n=12)	Group 6% (n=12)	P
BW (g)	232.93±6.74	230.05±8.17	230.33±3.75	221.22±5.90	0.589
In. L (mm)	35.86±3.48	37.79±4.19	36.67±3.50	41.64±4.40	0.735
In. W (mm)	3.47±0.21	3.37±0.25	3.64±0.29	3.96±0.49	0.608
Mg. L (mm)	147.76±5.57^{bc}	137.16±3.69^{c}	151.14±3.61^{b}	167.11±5.24^{a}	0.001
Mg. W (mm)	6.23±0.19	6.20±0.30	6.82±0.26	6.14±0.38	0.317
Is. L (mm)	59.30±1.86	70.53±4.91	65.46±2.57	60.30±3.56	0.095
Is. W (mm)	4.28±0.15	4.03±0.18	4.70±0.21	4.37±0.19	0.101
TL (mm)	242.92±7.26	245.48±7.30	253.28±7.06	269.05±7.01	0.060
AW (mm)	4.66±0.10	4.54±0.11	5.05±0.11	4.82±0.27	0.171
WF	14.50±0.58^{b}	17.10±1.02^{a}	18.20±0.53^{a}	16.90±0.97^{a}	0.019
YWF	1.20±0.13^{c}	4.50±0.56^{a}	3.60±0.69^{ab}	2.60±0.43^{bc}	<0.0001
YF	2.60±0.16	2.50±0.17	2.80±0.20	2.70±0.15	0.641
AYFD (mm)	14.89±0.20	14.93±0.57	15.61±0.54	16.25±0.48	0.151

$^{a, b, c}$Between groups with different letters in the same column mean difference is significant. BW: Body Weight, In.: Infundibulum, Mg.: Magnum, Is.: Isthmus, L: Length, W: Width, TL: Total Length, AE: Average Width, WF: White Follicle, YWF: Yellowish Follicle, SF: Yellow Follicle, AYFD: Average of Yellow Follicle Diameters.

to the other groups. This finding, which has no meaning at first consideration, turns out to be significant in the correlation analysis in the present study. The results of the present study indicated a negative correlation between magnum length and isthmus length (r=-0.369, P=0.019). When the significant correlations between the number of ovarian follicle types and the parts of the oviduct were examined, a positive correlation was measured between the number of yellowish follicles and the length of the isthmus (r=0.317, P=0.046). When combined with these findings in the present study, which is compatible with the results reported by Yıldırım et al. (2022), it can be asserted that as the number of yellowish follicles decreases, so will the length of the isthmus, and the length of the magnum will increase in parallel with the decrease in the length of the isthmus. This might be due to the anatomical reflection of follicular activities on the oviduct, a hollow and dynamic

Table 2. Correlation of measured values.

	In. L	In. W	Mg. L	Mg. W	Is. L	Is. W	TL	AW	WF	YWF	YF	AYFD
BW	.133	-.077	-.372*	-.094	.001	-.051	-.207	-.120	-.022	.070	.049	.014
In. L	1	-.248	.330*	.317*	-.014	-.043	.739**	.009	.033	.010	.150	.212
In. W		1	.374*	.063	-.117	.144	.096	.710**	-.289	-.226	-.122	.006
Mg. L			1	.190	-.369*	.152	.729**	.396*	-.176	-.298	.172	.147
Mg. W				1	.102	.108	.348*	.645**	-.097	-.086	.095	.067
Is. L					1	.120	.193	.030	.136	.317*	.075	.255
Is. W						1	.147	.523**	.276	.038	.039	.198
TL							1	.311	-.050	-.065	.238	.335*
AW								1	-.131	-.175	-.007	.115
WF									1	.564**	.128	.014
YWF										1	-.181	.133
YF											1	.293

BW: Body Weight. In.: Infundibulum. Mg.: Magnum. Is.: Isthmus. L: Length. W: Width. TL: Total Length. AE: Average Width. WF: White Follicle. YWF: Yellowish Follicle. SF: Yellow Follicle. AYFD: Average of Yellow Follicle Diameters. * (P<0.05). ** (P<0.01).

organ, in quails and it was considered that the magnum section of the oviduct was more intensely affected by follicular activities morphologically. When the results are analyzed, our opinion is supported by the fact that Group 2%, which had the highest mean number of yellowish follicles (4.50±0.56), also had the shortest magnum length (137.16±3.69). The present study also revealed a positive correlation between total oviduct length and yellow follicle diameter (r=0.335, P=0.034), which supports our opinion that ovarian activities would affect oviduct morphology. Hot pepper seeds in the diet were found to increase ovarian activities at certain doses and to a certain extent. Therefore, it was considered that the oviduct parts also changed morphologically in relation to the numerical changes indicating ovarian follicular activity such as yellowish follicles depending on the ratio of the additive to the diet. While a correlation was found between the length and width of the infundibulum and the total length (r=0.739, P=0.000) and mean width (r=0.710, P=0.000) of the oviduct, no significant correlation was measured between the infundibulum and ovarian follicular activities. This remarkable finding is considered to be associated with the fact that ovarian follicular activity is more related to the morphological changes of some oviduct segments, such as the isthmus, whereas the infundibulum is relatively less correlated with ovarian follicular activity. Besides our conclusion, the correlation of the infundibulum with the total length and width of the oviduct suggests that the infundibulum may be more dynamic than the other oviduct parts, which may affect the total length and average width of the oviduct. Consequently, a negative correlation was observed between the magnum and isthmus of oviduct segments in quail and it was determined that the length of the oviduct increased as the ovarian yellow follicle diameter increased. A very strong correlation was measured between the length and width of the infundibulum and the total length and average width of the oviduct. When the ovarian follicle dynamics of quails were examined, it was observed that the best results were obtained in terms of white follicle number when 4% of red hot pepper seeds were added to the diet and in terms of yellowish follicle number when 2% of red hot pepper seeds were added to the diet.

Conclusion

These results suggested that the addition of 2% and 4% of red hot pepper seeds to the diet may increase egg yield in quails. In the present study was observed, red hot pepper or capsaicin increase egg yield and was supported to the follicular activity in the ovary. Based on the results of the present study, it was concluded that further comprehensive and molecular studies to be carried out in this field may shed light on how red hot pepper seed affects the mechanism of egg yield increase.

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Conflict of interest

The authors have any conflict of interest.

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Research Article

Conformational Characteristics in Arabian and Thoroughbred Horses

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ABSTRACT

Horses are expected to maintain their racing lives with healthy and high performances. The body structure of an ideal racehorse should have ideal athletic characteristics. By selecting horses with good body structure, it is possible to increase both race achievements and breeding values. Studies are ongoing to evaluate the ideal body structure of horses in an objective and measurable way. This helps identify the strengths and weaknesses of horses. The study was aimed at determining the overall body conformations of Arabian and Thoroughbred racing horses by morphometric measurements. Photographs of Arabian and Thoroughbred horses in standard positions were scaled on a computer to measure angle and length values. Using the data obtained, body structures were tried to be revealed for both races. In addition, structural differences that increased the risk of disability in both races were assessed. There were significant differences between the head, leg, and body structures of Arabian and Thoroughbred horses. Lover length measurements were found for Arabians than for Thoroughbreds. It was noted that differences between the two races were significant, especially in distal extremity measures. It was found that in Thoroughbreds, the rump is generally higher than the withers, and this may result in a greater loading on the forelegs. In addition to identifying the body structures and differences of both races, the results of the study are thought to be useful for selection practises and to contribute to the understanding of the aetiology of disabilities.

***Keywords**: Conformation, morphometry, Thoroughbred, Arabian horse.*

Arap ve İngiliz Atlarında Konformasyon Farklılıkları

ÖZET

Atların yarış hayatlarını sağlıklı ve üst düzey performans ile sürdürmesi istenir. İdeal bir yarış atının vücut yapısı ideal atletik özelliklerde olmalıdır. İyi vücut yapısına sahip olan atların belirlenerek seçilmesi ile hem yarış başarılarının hem de damızlık değerlerinin artması mümkündür. Atlarda ideal vücut yapısının objektif ve ölçülebilir şekilde değerlendirilmesi için çalışmalar devam etmektedir. Bu sayede atların güçlü ve zayıf yönlerinin ortaya koyulması mümkün olmaktadır. Bu çalışmada Arap ve İngiliz yarış atlarında genel vücut konformasyonlarının morfometrik ölçümler ile tespit edilmesi amaçlandı. Arap ve İngiliz atlarının standart duruş halinde yandan çekilmiş fotoğraf görüntüleri bilgisayarda ölçeklendirilerek açı ve uzunluk değerleri ölçüldü. Elde edilen veriler kullanılarak her iki ırk için vücut yapıları ortaya koyulmaya çalışıldı. Bunun yanında her iki ırkta sakatlık riskini arttıran yapısal farklılıklar değerlendirildi. Arap ve İngiliz atlarının baş, bacak ve gövde yapıları arasında önemli farklar olduğu görüldü. Arap atlarının uzunluk ölçümlerinde İngiliz atlarına oranla daha düşük değerlere sahip oldukları görüldü. Açısal ölçümlerde ise iki ırk arasında özellikle distal ekstremite ölçümlerinde farklılıkların önemli olduğu dikkati çekti. İngiliz atlarında genellikle sağrının cidagodan daha yüksek olduğu ve bu durumun ön bacaklara daha fazla yüklenmeye neden olabileceği düşünüldü. Çalışma sonuçlarının her iki ırkın vücut yapılarının ve farklılıklarının tanımlanması yanında seleksiyon uygulamalarına faydalı olacağına ve sakatlıkların etiyolojisinin anlaşılmasına katkı sağlayacağı düşünülmektedir.

***Anahtar Kelimeler**: Konformasyon, morfometri, İngiliz atı, Arap atı.*

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Introduction

The horse racing industry is experiencing significant expansion (Belloy and Bathe, 1996). In the year 2022, the Jockey Club of Turkish hippodromes saw a total of 3789 Thoroughbred and 3130 Arabian horses in competition (Türkiye Jokey Kulübü, 2022). The number of racehorses increases every year (Figure 1). The significance of the industry becomes more evident with the incorporation of domains such as horse owners, breeders, attendants, and veterinary provisions.

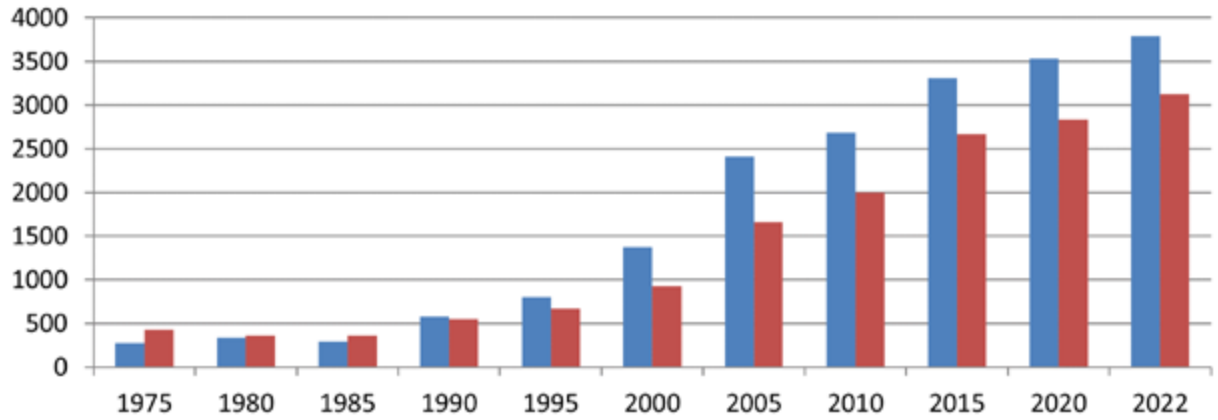

Figure 1. Arabian (red bars) and Thoroughbred (blue bars) racing horse population in Jockey Club of Türkiye hippodromes.

In the horse racing industry, it is necessary for horses to maintain healthy racing lives and show the highest level of performance. It is requested that horses can last many years without dividing their racing performance by disabilities (Belloy and Bathe, 1996). Horse injuries are harming the racing industry (Stover, 2003).

Conformation in horses is used to define the body structure suitable for work. The effects on balance and the structural compatibility of conformation in horses have been known for a long time (Green, 1975; Harris, 1993). Working on the relationship between conformation and performance is of great importance (Moore, 2010). Because insufficient knowledge about the effect of conformation on performance and health can lead to the wrong horse selection (Sanchez et al., 2013). The goal of raising racing horses should focus on conformation characteristics (Jakubec et al., 2009). The ultimate goal of the selection programmes is to obtain horses with the ideal conformation characteristics that stand out in sporting performance (Belloy and Bathe, 1996).

Clinical experience has shown that some diseases of the movement system in sports horses are associated with defects in the extremities. It is therefore suggested that conformity information can be used as a pre-selection criterion for racing horses (Dolvik and Klemetsdal, 1999).

Quantitative conformation studies may help us get new data about different breeds (Belloy and Bathe, 1996). Quantitative methods to understand conformation can be used for an objective evaluation, and some studies have been carried out in this area (Fedorski and Pikula, 1988; Delahunty et al., 1991; Mawdsley et al., 1996; Kavazis and Ott, 2003; Stover, 2003; Anderson and McIlwraith, 2004). In Sweden horse, a method is used that was originally developed by Magnuson and Thafvelin (1990) for a study on standardbred trotters.

Thoroughbred horse is the fastest of the world's horse breeds and conformation has an important role in performance of this breed (Bakhtiari and Heshmat, 2009). Among horse breeds such as Thoroughbred, Arabian, and Trotter, it has been found that they have an average higher cidago height and a longer body length (Saastamoinen et al., 1998; Sadek et al. 2006; Molina et al., 1999; Gharahveysi et al., 2008). The cidago height of adult horses is positively associated with racing performance (Dolvik and Klemetsdal, 1999) and step length (Galisteo et al., 1998). Munahi (2016) studied the head and neck structure of Arabian horses and shared some data. Cervantes et al. (2009) have worked on body structure in Arabian horses and have some values.

The increased incidence of injuries in the front legs, which carry more than 60% of the horse's weight when standing, and more than 40% when carrying it, is supported by the findings of epidemiological studies (Williams et al., 2001; Ely et al., 2004; Perkins et al., 2005; Cogger et al., 2008). The high tensions experienced by the superficial digital tendency (SDFT) undoubtedly contribute to the high incidence of injury (Ely et al., 2015). However, the increasing incidence with age suggests that injury is not caused by a simple mechanical overload (Kasashima et al., 2004). Also, some horses compete at the highest level and never suffer tendon damage (Thorpe et al., 2010). Conformity and incoordination defects are among the causes of SDFT disabilities (Jorgensen et al. 2003; Weller et al. 2006). In a study on carpal joint disabilities, it was found that conformity had a close relationship with the frequency of disability (Steel et al., 2006).

A short, streep croup with a short pelvis is a weak hindquarter. A very flat croup may place the hind legs far back, making it difficult for the horse to engage his hind legs. A long line from hip to hock permits a long stride with better engagement of the hind leg. This goes with short hind cannons and hocks well let down, which is a more powerful conformation (Harris, 1993). Meanwhile, a long and forwardly sloping femur places the hindlimb more under the horse, which allows the horse to keep

its balance more easily and carry more weight on the hindlimbs. In standardbred trotters, a positive correlation has been found between stifle angle and performance (Magnusson and Thafvelin, 1990).

The angle of the pastern is important for the equine athlete and crucial to length of stride, ease of gait, and durability. The actual "ideal" angle varies depending on a horse's leg and body conformation. A 45-55 degree angle in the front pasterns is usually appropriate, with a corresponding 49-59 degree angle in the hind pasterns. If pasterns have steeper angles and are too upright, the front legs will suffer excessive concussion, and the horse will ride rough. Pasterns that slope too much with an angle less than 45 degrees tend to be weak and more prone to breakdown (Thomas, 2005).

Conformation data is used to understand the causes of the injuries in addition to evaluating horses breeds (Bakhtiari and Heshmat, 2009; Mostafa and Elemmawy, 2020). Horses with good conformity get better market prices.

The aim of this study is to demonstrate the conformation characteristics of Arabian and Thoroughbred horses in Turkey using objective values. The information obtained makes it possible to reveal the current state of conformation and the differences among these breeds. The results of the study provide information that will help the breeding programmes.

Materials and Methods

Study Population

In this study, 400 horses were controlled, but only 50 Arabian and 50 Thoroughbred racing horses were used. The horses have not had any orthopaedic problems or injuries, and they were training during the study (Table 1). All horses were healthy and active racehorses. In the selection of horses, the conditions were that they were healthy, had no orthopedic defects, registered in the stud book, and actively participating in races during the study period.

Photographs Characteristic

The staff maintains horses' proper body posture on level ground. After a proper body posture was achieved, a photo was taken with a digital camera (Canon EOS 350D) with a resolution of 3456x2304 dpi, from the left side of the horse (Anderson and McIlwraith, 2004; Sadek et al., 2006), from a distance of three metres, using a fixed tripod, in the horizontal plane from the middle point of the body.

Image Analysis

All image analysis was performed by the first author (IGY). Photographs were transferred to a computer and calibrated in the Vet Eickemeyer® Medizintechnik für Tierärzte (EIVIS) programme. Each photograph was calibrated using the length of the withers. The following measurements and angles were recorded from the left lateral view of each horse.

Measuring

All reference points are shown in Figure 2 and described in Table 2.

Data Analysis

The same investigator (IGY) completed all measurements in an attempt to eliminate any possibility of interobserver variability. The statistical analysis of the data was performed using the statistical package program (SPSS 13, IBM SPSS Statistics®, Chicago, IL, USA). The mean, standard deviation, range and minimum-maximum were calculated for each parameter. Student's t-test was used to compare horses. Statistical significance was described at $P<0.05$ level.

Results

The study's goal was to find out objectively what Arabian and Thoroughbred horses in Turkey look like in terms of their conformation. The information gathered showed the current conformation values and how these breeds are various (Table 3).

Thoroughbreds have a larger head area than Arabian horses. The nose and neck angles used to assess the structure of the head were found to be larger in Thoroughbred horses, while the angle of the mandibula was smaller. The head was found to have structural differences in Thoroughbreds and Arabian horses.

When measurements were made with regard to the front leg, it was found that all the length values were greater in Thoroughbred horses. While there is no general difference between the field values, it was noted that the front wrist angle and axle values are lower in Thoroughbred horses. When compared to Arabians, it was found that the front wrist structures of Thoroughbreds had a more flexible structure.

In the back legs, length measurements such as the femur, tibia, metatarsus, and rear pastern height were found to have higher values than in Thoroughbreds. *Articulatio coxae, articulatio genu, articulatio tarsi*, and rear hoof angle measurements were statistically higher in Arabian horses.

When measurements of the overall body structure between the two breeds were assessed, it was found that the measurements of the height of the withers, the head height, the length of the body, the back length, the thorax height, the abdomen depth, the front thorax angle, and the croup angle had higher statistical values in Thoroughbreds. The rear thorax angle and abdomen angle measurements were found to have greater values in Arabian horses.

The difference between the conformation structures in the ways in which the values obtained from body measurements of Arabian and Thoroughbred horses are drawn using averages for both breeds is more visible (Figure 3).

In these models drawn from the mean values of the two races, the differences between the length values across the body between the two breeds are usually statistically

Table 1. The descriptive properties about the horses in the study.

Races	Sex	n	Age (year) mean (min-max)	Withers Length (cm) mean (min-max)
Arabian	♂	25	3.96 (3-4)	152 (147-155)
	♀	25	3.68 (3-4)	151 (145-156)
Thoroughbred	♂	25	3.40 (3-4)	165 (160-170)
	♀	25	3.36 (3-5)	164 (160-168)

valuable, and when the angular values for the race are examined, the statistical differences in angular value for the back legs appear to be greater.

It has been found that the height of the withers in Arabian horses is greater than the height of the withers in Thoroughbred horses.

Discussion

Some values for the conformational characteristics of healthy and injured thoroughbred horses have been reported (Mostafa and Elemmawy, 2020). Between the two studies, the length of the radius, Mc3, femur, tibia, and Mt3 measurements have very similar values. In addition, the angular angles of the carpi, front and rear pastern, hock, and hip also yielded extremely compatible results. When the data are consistent between the two studies, the effect is great because the measurement points are taken from the same anatomical points. This should be taken into account when determining measurement points and taking measurements in conformation studies. Sadek et al. (2006) in their study on Arabians, measured the height of withers as 142 cm (in study 151.56 cm), height of croup 141 cm (in study 148.64 cm), body length as 135 cm (in study 151.62 cm), thorax depth as 51 cm (in study 69.60 cm). Based on the fact that the measured values were higher, it is believed that the Arabian horses used in the study were selected for very long years for racing purposes.

Figure 2. Specific anatomical points on the photos. 1: nose, 2: apex of upper lip, 3: forehead, 4: mandibular ramus, 5: withers, 6: shoulder joint, 7: shoulder, 8: elbow joint (midpoint), 9: olecranon, 10: deep point of thorax, 11: mid-carpus, 12: dorsal surface of carpal joint, 13: mid-meta-carpophalangeal joint, 14: mid-hoof the coronary band, 15: front hoof apex, 16: deepest point of back, 17: croup, 18: origin of tail, 19: hip, 20: buttock, 21: articulatio coxae, 22: patella, 23: plica lateris, 24: calcaneal tubercle, 25: mid-hock, 26: mid-metatarsophalangeal joint, 27: mid-hoof the coronary band, 28: rear hoof apex

Table 2. List of 37 traits of the variables studied.

Traits	Units	Description
Head Area	cm^2	Nose to forehead to ramus mandible
Nasal Angle	degree	Forehead to nose to ramus mandible
Forehead Angle	degree	Nose to forehead to ramus mandible
Mandibular Angle	degree	Nose to ramus mandible to forehead
Length of Scapula	mm	Prominence of withers to shoulder joint
Length of Humerus	mm	Shoulder joint to elbow joint
Length of Radius	mm	Elbow joint to mid-carpal joint
Length of Mc3	mm	Mid-carpal joint to mid-metacarpophalangeal joint
Height of Front Pastern	mm	Mid-metacarpophalangeal joint to ground
Angle of Scapula	degree	Prominence of withers to shoulder joint to a line parallel with the ground (horizontal axis)
Shoulder Angle	degree	Scapulohumeral angle: Withers to shoulder joint to lateral epicondyle of humerus
Elbow Angle	degree	Shoulder joint to lateral epicondyle of humerus to mid-carpus
Carpal Angle	degree	Radius to mid-carpus to mid-metacarpophalangeal joint for carpi
Front Pastern Angle	degree	Mid-carpus, along the third metacarpal bone to mid-metacarpophalangeal joint to hoof axis
Front Hoof Pastern Axis	degree	Mid-metacarpophalangeal joint to mid-hoof the coronary band to hoof axis
Front Hoof Angle	degree	Dorsal surface of the front hoof to a line parallel with the ground
Length of Femur	mm	Greater trochanter of femur to lateral condyle of the tibia
Length of Tibia	mm	Lateral condyle of the tibia to mid-hock at the talus
Length of Mt3	mm	Mid-hock to mid-metatarsophalangeal joint
Height of Rear Pastern	mm	Mid-metatarsophalangeal joint to ground
Coxae Angle	degree	Tuber coxae to articulatio coxae to patella
Genu Angle	degree	Articulatio coxae to patella to mid-hock
Hock Angle	degree	Patella to mid-hock to mid-metatarsophalangeal joint
Rear Pastern Angle	degree	Mid-hock to, along the third metatarsal bone to mid-metatarsophalangeal joint to hoof axis
Rear Hoof Pastern Axis	degree	Mid-metatarsophalangeal joint to mid-hoof the coronary band to hoof axis
Rear Hoof Angle	degree	Dorsal surface of the rear hoof to a line parallel with the ground
Wither Height	mm	Highest point of withers to ground.
Croup Height	mm	Highest point of croup to ground.
Body Length	mm	Shoulder to buttock
Back Length	mm	Withers to croup
Thoracic Depth	mm	Withers to deep point of thorax
Abdomen Depth	mm	Croup to plica lateralis
Front Thorax Angle	degree	Withers to shoulder joint to deep point of thorax
Rear Thorax Angle	degree	Withers to articulatio coxae to deep point of thorax
Back Angle	degree	Withers to the deepest point of back to croup
Abdomen Angle	degree	Shoulder to deep point of thorax to plica lateralis
Croup Angle	degree	Croup to origin of tail to a line parallel with the ground

Pura Raza Espanola horses they used in their study were obtained in the past by melting Arabian and Thoroughbred horses. Pura Raza Espanola horses have been as height of withers 157.89 cm, heights of croup 158.02 cm (Sanchez et al., 2013). In study, Thoroughbred horses had a height of withers of 164.98 cm and a height

Table 3. The conformational measurements on the horses (mean and standard deviation)

Measurement	n	Arabian	n	Thoroughbred	P
Head Area	46	76.47 (11.02)	44	83.20 (12.71)	0.005
Nasal Angle	46	43.60 (2.08)	44	44.44 (1.74)	0.042
Forehead Angle	46	56.00 (4.59)	44	58.58 (3.46)	0.003
Mandibular Angle	46	81.31 (4.79)	44	77.43 (4.26)	0.000
Length of Scapula	50	57.50 (4.03)	50	60.88 (2.92)	0.000
Length of Humerus	50	26.65 (2.74)	50	29.76 (2.55)	0.000
Length of Radius	50	42.12 (2.82)	50	45.94 (29.59)	0.000
Length of Mc3	50	27.33 (1.88)	50	29.95 (1.77)	0.000
Height of Front Pastern	50	16.67 (1.10)	50	17.98 (1.10)	0.000
Angle of Scapula	50	56.38 (4.43)	50	56.71 (4.47)	0.717
Shoulder Angle	50	102.51 (4.76)	50	101.01 (5.05)	0.130
Elbow Angle	50	116.36 (10.45)	50	114.06 (4.21)	0.152
Carpal Angle	50	180.59 (1.47)	50	181.06 (1.82)	0.163
Front Pastern Angle	50	150.25 (4.91)	50	144.38 (4.94)	0.000
Front Hoof Pastern Axis	49	168.30 (3.77)	50	171.16 (6.14)	0.006
Front Hoof Angle	49	54.67 (2.11)	50	53.88 (3.27)	0.157
Length of Femur	50	44.83 (3.51)	50	49.24 (3.52)	0.000
Length of Tibia	50	48.88 (4.92)	50	55.75 (4.29)	0.000
Length of Mt3	50	33.91 (2.86)	50	36.22 (2.56)	0.000
Height of Rear Pastern	50	16.79 (1.24)	50	18.83 (1.22)	0.000
Coxae Angle	48	98.72 (7.66)	50	89.70 (8.05)	0.000
Genu Angle	49	126.78 (6.38)	50	123.58 (6.11)	0.013
Hock Angle	48	146.19 (3.71)	50	144.19 (2.98)	0.004
Rear Pastern Angle	49	155.78 (4.50)	50	154.52 (4.89)	0.186
Rear Hoof Pastern Axis	47	170.67 (4.93)	50	171.97 (6.07)	0.251
Rear Hoof Angle	47	58.50 (1.73)	50	56.56 (3.32)	0.001
Wither Height	50	151.56 (2.96)	50	164.98 (2.81)	0.000
Croup Height	50	148.64 (6.09)	50	166.12 (6.73)	0.000
Body Length	50	151.62 (7.40)	50	163.69 (4.79)	0.000
Back Length	50	74.99 (4.44)	50	81.96 (4.68)	0.000
Thoracic Depth	50	69.60 (2.92)	50	75.38 (2.14)	0.000
Abdomen Depth	50	54.24 (3.56)	50	56.66 (2.81)	0.000
Front Thorax Angle	50	78.01 (3.00)	50	80.50 (2.80)	0.000
Rear Thorax Angle	50	39.75 (2.47)	50	38.56 (1.91)	0.008
Back Angle	50	152.65 (3.72)	50	153.25 (2.81)	0.372
Abdomen Angle	50	145.08 (4.73)	50	140.60 (3.70)	0.000
Croup Angle	50	20.29 (1.60)	50	22.73 (1.75)	0.000

Measurements were made using units “cm and degree”

of croup of 166.12 cm. Height of withers 151.56 cm, height of croup148.64 cm were found in Arabian horses. These values for Pura Raza Espanola horses were found to be close to the averages of the two breeds.

According to the results obtained in a study on Arabian horses (Cervantes et al., 2009); height of withers 150.02cm (in study 151.56 cm) , length of scapula 60.64 cm (in study 57.50 cm), body length 147.64 cm (in study 151.62 cm) , length of back 104.74 (in study 74.99 cm), height of thorax 62.56 cm (in study 69.60 cm), length of radius 41.25 cm (in study 42.12), length of metacarpus 25.63 cm (in study 27.33 cm), length of metatarsus 34.74 cm (in study 33.91 cm) was reported. These measurements appear to be compatible with our work. Cervantes et al. (2009) found in angular measurements; art. cubiti angle 30.97 degree (in study 116.36 degree), front pastern angle 56.94 degree (in

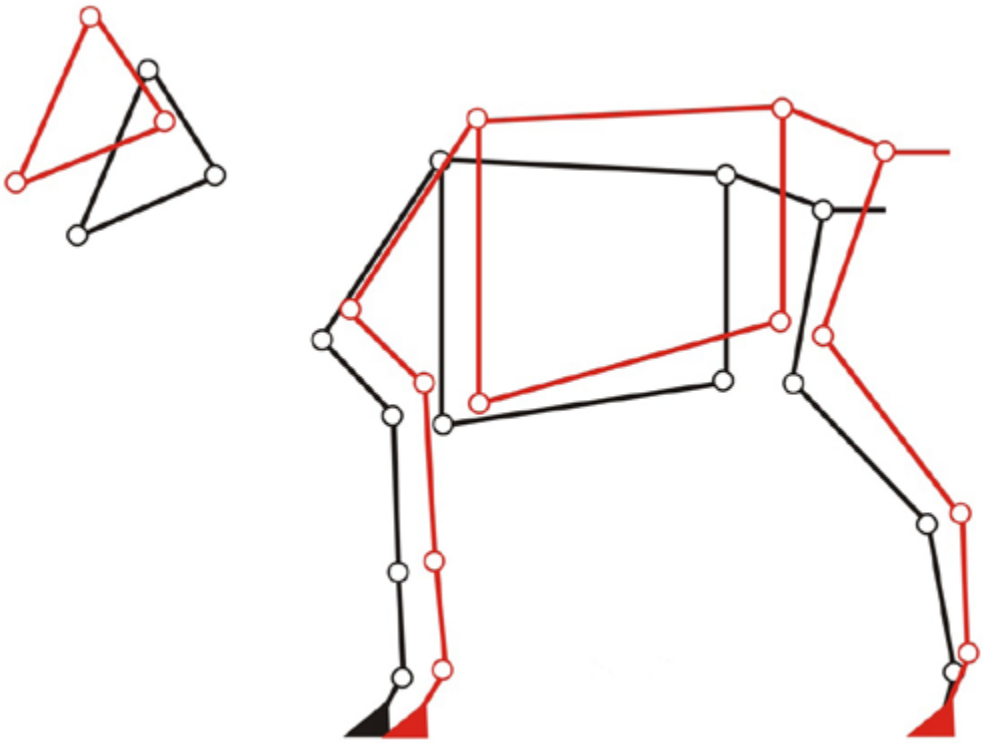

Figure 3. Schematic representation of Arabian and Thoroughbred horses in accordance with the average results obtained in the study.

study 150.25 degree), rear pastern angle 59.43 degree (in study 155.78 degree). The difference between the two measurements is thought to be due to the use of the angle between the joint and the horizontal axis in studies by Cervantes et al. It is thought and suggested that measuring the angle between the bones that form the joint in the measurement of joint angles can reduce the difference between studies.

A study on the body structure of Thoroughbred horses found that the length of humerus 29.97 cm (in study 29.76 cm), length of radius 43.38 cm (in study 45.94 cm), length of Mc3 30.25 cm (in study 29.95 cm), scapula angle 55.54 degree (in study 56.71 degree), shoulder angle 104.90 degree (in study 101.01 degree), carpal angle 181.38 degree (in study 181.06 degree) results are consistent with our study (Andersson and McIlwraith, 2004). The difference in measurements between the two studies is believed to be due to the measurement difference in the front pastern angle 54.24 degree (in study 144.38), and rear pastern angle 52.85 degree (in study 154.52 degree).

The horse may move forward with a direct driving force from its hind legs. A horse's speed and agility are significantly influenced by its hindquarter angles. More range of movement and typically greater agility are characteristics of a horse with acute angles and a longer swing of the leg. Wide angles and a shorter stride enable him to normally outrun the horse while exerting less effort (Thomas, 2005). According to our research, Arabian horses' back legs are shorter on average, and higher angular values could shorten their steps and put them in risk.

The front pastern and nail angles should ideally be between 45 and 55 degrees; greater angles could result in more trauma to the front legs, whilst lower values would make the legs weaker since they are too inclined (Thomas, 2005). Andersson and McIlwraith (2004) on Thoroughbred horses as front hoof angle 48.01degree (in study 53.88 degree), rear hoof angle 48.28 degree (in study 56.56 degree). In study, we found that hoof angles have higher values. Higher angular and axial values in Arabian horses were hypothesised to affect the prevalence of impairments in this location in our investigation. Researchers believe that studies on the incidence of disabilities in the front wrist region between the two breeds will be more obvious.

In our research, we discovered that both Arabian and Thoroughbred horses had high hoof angles. This circumstance has been interpreted in such a way that the frequency of impairments may increase in tandem with the observation of values below the required axis angles of 180 degrees. Hoof maintenance and trimming should be performed more frequently and at the proper angles on racehorses.

For optimal agility, balance, and locomotion, the croup and withers should be the same height. If the croup is higher than the withers, the rear legs are disproportionately longer than the front legs, which can cause stride and forging issues. A croup that is too elevated causes the saddle to move forward over the withers. The long hind legs provide the rider with more propulsion with each stride, resulting in a more uncomfortable voyage (Thomas, 2005). Changes in conformation shift the centre of gravity forward and cause variations in gait and leaping techniques, placing asymmetrical loads on the musculoskeletal system and predisposing the individual to jump (Ross and Dyson, 2011). Higher values for the height of the cidago in Thoroughbred horses would cause the body weight to place a greater burden on the front

legs, according to the results of our study. This should not be overlooked because it can result in injuries to the front legs, particularly in high-performance horses. This should be considered during the selection programs.

The sacral slope has allowed horses to extend their hind legs and gallop faster (Thomas, 2005). This information led to the conclusion that Thoroughbred horses had larger sacrum angles than Arabians.

It has been hypothesised that the differences in certain conformational values are the result of different measurement sites (Belloy and Bathe, 1996). In order to eliminate these disparities, it will be possible to establish more standardised measurement points and decrease measuring disparities between studies. Although much progress has been made in the determination of body structure in the cattle industry, it is thought that more studies should be done on this issue in the horse industry.

The study examined differences in cranium structure between Thoroughbred and Arabian horses. The larger angle of the mandible in Arabian horses was interpreted as indicating that the upper respiratory system has a larger area to settle. Due to their lengthy heads and faces, it was believed that Thoroughbreds had a large nose and forehead angle. It is believed that in-depth research is required to disclose the potential consequences of these structural differences in the head region between breeds.

Limitation of this study: The purpose of the study was to compare and contrast the conformation structures of Arabian and Thoroughbred horses. Therefore, an equal number of females and males measurements were obtained, and the effect of gender was not considered in statistical comparisons.

Conclusion

The horse's body structure must be proportional to the labour it performs. This is particularly significant in horse competition. Otherwise, the horse's racing career will be brief. Only horses with optimum conformation should be selected for breeding purposes. Using morphometric measurements such as length and angle, the research aimed to establish the body structure and reference values of Arabian and Thoroughbred horses. It is believed that the results could be useful for Arabian and Thoroughbred horse industry. It is expected that this study will make a scientific contribution to the horse industry and form the basis for future research on breeder selection and racing performance.

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

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Animal Health Prod and Hyg (2023) 12 (2) : 36 - 41

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Research Article

The Effect of Human Chorionic Gonadotropin and Ketoprofen Applications on Pregnancy Rates in Dairy Cows After Artificial Insemination

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A B S T R A C T

The presented study aims to investigate the effect of creating a luteotropic effect on the establishment and maintenance of pregnancy and suppressing the luteolysis mechanism during the maternal recognition process on pregnancy rates in dairy cows. For this purpose, the ovulations of 96 Holstein cows aged between 2-8 years were synchronised with the Ovsynch protocol. After artificial insemination, the cows were divided into four groups. The group receiving Human Chorionic Gonadotropin (hCG) on the 6th day after artificial insemination (H, n=20), the group receiving Ketoprofen on the evening of the 15th day, and the morning of the 16th day after artificial insemination (K, n=24), the group receiving both hCG on the 6th day and Ketoprofen on the 15th day evening and 16th day morning after artificial insemination (HK, n=25), and the control group (C, n=27) that did not receive any post-insemination treatment were established. Pregnancy checks were conducted on the 30th, 45th, and 60th days through rectal and ultrasonographic (USG) examinations. According to the results of the pregnancy examinations, it was determined that 9 out of 20 cows (45.00%) in group H, 10 out of 24 cows (41.67%) in group K, 16 out of 25 cows (64.00%) in group HK, and 12 out of 27 cows (44.44%) in group C were pregnant. No statistically significant difference (P>0.05) was observed among the groups. The study found that separately providing luteotropic support with hCG or suppressing the luteolysis mechanism with Ketoprofen after artificial insemination did not improve pregnancy rates in cows. However, when both interventions were combined, there was a 20% increase in the pregnancy rate, indicating that different treatment approaches may be required. Further research with larger sample sizes and detailed hormone profiles is needed to optimise and utilise these combination therapies to enhance pregnancy rates.

Keywords: *Human chorionic gonadotropin, ketoprofen, pregnancy rate, cow.*

Sütçü İneklerde Suni Tohumlama Sonrası İnsan Koryonik Gonadotropin ve Ketoprofen Uygulamalarının Gebelik Oranına Etkisi

Ö Z E T

Sunulan çalışmanın amacı, sütçü ineklerde gebeliğin oluşum ve devamlılığında luteotropik etki oluşturmanın ve maternal kabul sürecinde luteolizis mekanizmasını baskılamanın gebelik oranı üzerine etkisini araştırmaktır. Bu amaçla yaşları 2-8 arasında değişen 96 adet Holstayn ırkı ineğin Ovsynch protokolü ile ovulasyonları senkronize edildi. Suni tohumlama sonrası inekler dört gruba ayrıldı. Suni tohumlama sonrası 6. günde İnsan Koryonik Gonadotropin (hCG) uygulanan grup (H, n=20), suni tohumlama sonrası 15. gün akşamı ve 16. gün sabahı Ketoprofen uygulanan grup (K, n=24), suni tohumlama sonrası 6. günde hCG ve 15. gün akşamı ile 16. gün sabahı Ketoprofen uygulanan grup (HK, n=25) ve suni tohumlama sonrası herhangi bir uygulama yapılmayan kontrol grubu (C, n=27) oluşturuldu. Gebelik kontrolleri 30., 45. ve 60. günlerde rektal ve ultrasonografik (USG) muayenelerle yapıldı. Gebelik muayenelerinin sonuçlarına göre, H grubundaki 20 inekten 9'u (%45,00), K grubundaki 24 inekten 10'u (%4,67), HK grubundaki 25 inekten 16'sı (%64,00) ve C grubundaki 27 inekten 12'si (%44,44) gebeydi. Gruplar arasında istatistiksel olarak anlamlı bir fark (P>0,05) bulunmadı. Çalışmada, suni tohumlamayı takiben hCG ile luteotropik destek sağlamanın veya Ketoprofen ile luteolizis mekanizmasını baskılamanın gebelik oranı üzerinde olumlu bir etkisinin bulunmadığı ancak birlikte uygulandığında gebelik oranında %20 artış olduğu belirlendi. Belirlenen bu artış ineklerde gebelik oranını artırmak için farklı tedavi protokollerinin gerekebileceğini işaret etmektedir. Ancak, gebelik oranlarını artırmak amacıyla bu ve benzeri kombinasyonlu tedavilerin optimize edilmesi ve kullanılması için materyal sayısı artırılarak hormon profilleri ile birlikte daha ayrıntılı yeni çalışmalara ihtiyaç olduğu düşünülmektedir.

Anahtar kelimeler: *İnsan koryonik gonadotropin, ketoprofen, gebelik oranı, inek.*

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Introduction

In order to increase the conception rate in insemination or embryo transfer practices in dairy cows, new approaches to reduce pregnancy losses based on coordinated and timely physiological interactions that occur among the embryo, uterine and corpus luteum (CL) during early pregnancy are still being tried to be developed (Kirbas et al., 2014; Wiltbank et al., 2014)primarily by the corpus luteum (CL). One of these strategies is to improve the function of the CL and/or to improve the concentration of peripheral progesterone (P_4) and, consequently, embryonic development through the creation of a new CL or direct application of exogenous P_4. For this purpose, one of the hormones that have been widely tested in cows is the Human Chorionic Gonadotropin (hCG) (rensis et al., 2010) induces ovulation throughout the estrous cycle, promotes the formation of accessory corpora lutea when applied in the early luteal phase, and modifies follicular wave dynamics increasing the frequency of three-wave dominant follicular cycles. As hCG acts on ovarian cells independently of the pituitary gland and its effect is longer lasting than that produced by endogenous LH release, use of hCG rather than gonadotropin-releasing hormone (GnRH). Many studies have reported increased pregnancy rates and P_4 levels after hCG administration in cows in the days following insemination (Santos et al., 2001; Alnimer and Shamoun, 2015). However, some studies report that P_4 levels increase after hCG administration, but there is no positive effect on the pregnancy rate (Hanlon et al., 2005; Rossetti et al., 2011; Stevenson and Pulley, 2012; Kucukaslan et al., 2022). A detailed analysis determined that the preference for hCG administration after insemination to improve fertility should be focused on cows that are expected to have low or moderate fertility, as well as on the day and dose of administration (Besbaci et al., 2020).

One of the strategies to improve pregnancy rates in dairy cows is to prolong the life of the CL by using non-steroidal anti-inflammatory drugs just before the time of possible onset of luteolysis, giving time for embryos with developmental delay to produce sufficient amounts of interferon tau (IFNT) (Binelli et al., 2001). One of the most basic conditions for the formation and continuity of pregnancy in cows during early pregnancy is the initiation of endometrial anti-luteolytic functioning, which causes lysis of the CL in order to maintain a high concentration of P_4 in the peripheral blood and endometrial function in order to prevent the formation of new estrus and improve implantation (Bazer et al., 2017; Tinning et al., 2023). This process is initiated by IFNT, which is secreted by the conceptus and blocks the synthesis of Prostaglandin F_2 alpha ($PGF_{2\alpha}$) in the endometrium during maternal recognition of pregnancy in cows, at about the 16th day in cows (Sánchez et al., 2018; Wiltbank et al., 2023) the oocyte is fertilized. Therefore, pharmacological strategies aimed at inhibiting the synthesis of $PGF_{2\alpha}$ are used to prevent the onset of endometrial $PGF_{2\alpha}$ release between the 15th and 17th days, which are defined as the critical period for the recognition of pregnancy (Binelli et al., 2001; Paksoy and Das, 2013; Alkan and Erdem, 2018). Non-steroidal anti-inflammatory drugs prevent the formation of luteolytic functioning by inhibiting cyclooxygenase enzymes (COX-1, COX-2) involved in the synthesis of $PGF_{2\alpha}$. Non-steroidal anti-inflammatory drugs, such as flunixin meglumine, carprofen, ketoprofen, and meloxicam, are mainly used for their pain-relieving properties (Smith et al., 2008). However, non-steroidal anti-inflammatory drugs have also been tried to improve fertility by reducing $PGF_{2\alpha}$ release due to manipulations performed during artificial insemination and embryo transfer in cows (Bülbül et al., 2010; Gaievski et al., 2022), or by preventing luteolysis by inhibiting endometrial $PGF_{2\alpha}$ synthesis on the 15-16th days after insemination (Guzeloglu et al., 2007; Erdem and Guzeloglu, 2010; Dursun, 2011). Using non-steroidal anti-inflammatory drugs after insemination it was reported to have a positive effect on the pregnancy rate in some studies (Guzeloglu et al., 2007; Bülbül et al., 2010), but there was no positive effect on the pregnancy rate in some studies (Erdem and Guzeloglu, 2010; Von Krueger and Heuwieser, 2010). It has been stated that various factors such as the type, dose, number of applications, time, and frequency of non-steroidal anti-inflammatory agents, were effective in the formation of these results. In addition, the use of these drugs to improve conception in cows that produce milk should be carefully evaluated (Daeseleire et al., 2003). Ketoprofen is a non-steroidal anti-inflammatory agent that inhibits both COX enzymes. Compared to flunixin meglumine, which tested the effect on pregnancy rate in more studies, ketoprofen stays in circulation for a shorter time than flunixin meglumine, has a narrower volume of distribution, is eliminated from the kidneys in a short time. and It has fewer side effects but is more expensive (Smith et al., 2008). The fact that there are few studies examining the advantages of ketoprofen over other non-steroidal anti-inflammatory drugs and their effects on fertility compared to other non-steroidal drugs suggests that its use should be investigated to improve fertility in lactating cows.

The aim of this study is to investigate the effect on the pregnancy rate in dairy cows of providing luteotropic support with hCG or suppressing the luteolysis mechanism with Ketoprofen separately or in combination after artificial insemination.

Materials and Methods

The study was conducted with the approval of the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (ADU-HADYEK) under the protocol number 64583101/2023/32 and date 09/03/2023.

The study was performed on a private dairy farm located in Aydin province, Türkiye. The cattle in the farm were fed with a diet consisting of self-prepared concentrated ration, hay, and silage. Water was provided through automatic water troughs ad libitum. A total of 96 Holstein breed cows, aged between 2 to 8 years, without any puerperal problems (such as retained placenta, uterine

infections, ovarian cysts) as determined during examinations, were included in the study. The study was conducted between the months of March to May.

Experimental Design

All cows were synchronised using the classical Ovsynch method for ovulationsynchronisation. In the Ovsynch method, cows were initially administered 10 mcg of Buserelin acetate (Receptal®, 0.004 mg/mL Buserelin, MSD), which is a GnRH analogue, IM. Seven days later, a PG-$F_{2\alpha}$, 500 mcg of cloprostenol (Eustramate®, 500 mcg/2mL cloprostenol, MSD), was administered IM. The second GnRH analogue injection was administered 48 hours later. Artificial insemination was performed on all cows 16-20 hours after the second GnRH injection, and this day was recorded as Day 0 of the study.

To determine the effects of hCG and ketoprofen applications on pregnancy rates at different time intervals, all synchronised cows were randomly divided into four groups: hCG (H), ketoprofen (K), hCG and Ketoprofen (HK), and Control (C). In the H (n=20) group, cows were given 1500 IU of hCG (Chorulon®, Intervet) IM on the 6th day after artificial insemination, as specified by Singh et al. (2020). In the K (n=24) group, cows were administered 3 mg/kg of Ketoprofen (Ketojezik®, 100 mg/mL ketoprofen, Teknovet) IM in the evening of the 15th day and the morning of the 16th day after artificial insemination, as specified by Richards et al. (2009). In the HK (n=25) group, cows received hCG on the 6th day and ketoprofen injections on the evening of the 15th day and the morning of the 16th day after artificial insemination. The C (n=27) group did not receive any additional treatments. Pregnancy examinations were performed in all cows using USG examination (Kaixin 5100v, Hasvet) between days 35 and 45 after artificial insemination. Pregnancy outcomes were confirmed on day 60 through rectal and USG examinations.

Statistical Analysis

Statistical analyses were conducted using the SPSS 15.0 software package (SPSS Inc., Chicago, Illinois, USA). Pregnancy rates obtained in the groups were evaluated using the chi-square test.

Results

In the pregnancy examination conducted, it was determined that 9 out of 20 cows (45.00%) in the H group, 10 out of 24 cows (41.67%) in the K group, 16 out of 25 cows (64.00%) in the HK group, and 12 out of 27 cows (44.44%) in the C group were pregnant. No statistically significant difference (P>0.05) was observed among the groups. In the presented study, the pregnancy rates obtained in the groups were presented in Table 1 and Figure 1.

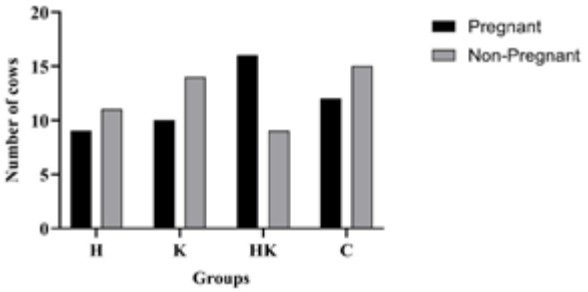

Figure 1. Number of pregnant and non-pregnant cows in the study groups (P>0.05). H; Human Chorionic Gonadotropin (hCG) group, K; Ketoprofen group, HK; hCG and Ketoprofen group, C; Control group

Discussion

In this study, it was evaluated whether the single or combined use of hCG and ketoprofen affected the pregnancy rate at the first insemination in lactating dairy cows. So far, various studies have been conducted to increase plasma P_4 levels or prevent luteolysis aim to reduce pregnancy losses during early pregnancy in dairy cows. This study that was anti-luteolytic administration at the time of possible onset of luteolysis following luteotropic hormone administration to increase of plasma P_4 level was slightly different from other studies aimed only at increasing P_4 levels or preventing luteolysis from affecting the pregnancy rate. To achieve this, hCG was injected on the 6th day after insemination, and ketoprofen was injected on the 15th and 16th days in lactating cows. One of the original aspects of this study is the twice-daily application of ketoprofen, at a dose used in very few studies, to increase the pregnancy rate by stopping luteolysis during the probable onset period of luteolysis 12 hours apart. The study's main finding was that the administration of hCG on the 6th day after insemination and two ketoprofen injections at 12-hour intervals on the 15th and 16th days had a positive effect on the pregnancy rate, although not at a statistically significant level. Another important finding is that hCG administration on only the

Table 1. Percentages of pregnant and non-pregnant cows in the study groups [n (%)]

Groups	Age*	n	Pregnant	Non-pregnant	P value
H	4.64±0.36	20	9 (45.00%)	11 (55.00%)	0.37
K	4.91±0.25	24	10 (41.67%)	14 (58.33%)	
HK	4.96±0.24	25	16 (64.00%)	9 (36.00%)	
C	5.04±0.21	27	12 (44.44%)	15 (55.56%)	

H; Human Chorionic Gonadotropin (hCG) group, K; Ketoprofen group, HK; hCG and Ketoprofen group, C; Control group, *; Ages of the groups were presented as Mean±SEM (Standard Error of Mean)

6th day resulted in a very small increase in pregnancy rate compared to the pregnancy rates of the control group and the ketoprofen-treated groups, whose pregnancy rates were very close to each other.

It has been shown in some previous studies that the formation of a new CL by ovulation of the dominant follicle of the first wave with hCG administration on the 4th-7th days after insemination in cows or the peripheral P_4 concentration is increased by improving the functions of the luteal cells of the existing CL (Santos et al., 2001; Fantini Filho et al., 2004; Walker et al., 2005; De Rensis et al., 2008). It is well known that P_4, also known as the pregnancy hormone, plays an essential regulatory role in the formation and maintenance of pregnancy, both before and after insemination. However, it is also stated that the course and timing of the increase in P_4 concentration after ovulation is more important than the maximum P_4 level in the luteal phase for the formation and maintenance of pregnancy in cows (Mann and Lamming, 1999). Other studies have also shown that the level and profile of P_4 in the early period after fertilisation have a more important and positive relationship with embryonic viability than in the late period (Diskin et al., 2006; McNeill et al., 2006; Starbuck et al., 2006). Pandey et al. (2016) stated hCG on the 5th day after insemination improved both the conception rate and the plasma P_4 concentration. In this study, the effects of hCG administration on the formation of new CL or P_4 levels could not be investigated. However, compared to the pregnancy rates in the ketoprofen group and the control group, higher pregnancy rates were obtained in the hCG and hCG with ketoprofen groups. This result corresponds to the statements that, as previously reported in some studies, elevated P_4 levels in early pregnancy benefit the embryo from the effects of P_4 on embryo development for a more extended period until the critical period of maternal acceptance, allowing it to grow enough to trigger the anti-luteolytic mechanism. In this study, the pregnancy rate obtained in the ketoprofen group was very similar to that in the control group. It was also stated that hCG administration can delay luteolysis if the dominant follicle of the first wave ovulates and can provide an additional effect that increases the chance of survival by growing more if a live embryo is found. Moreover, it is thought that stopping endometrial $PGF_{2\alpha}$ synthesis with ketoprofen application, which has been exposed to the effect that makes the uterus microenvironment more suitable for conceptus development and the ability to develop larger conceptuses to produce more IFNT that can block $PGF_{2\alpha}$ synthesis, thus increases the likelihood of pregnancy continuation. A similar result as in our study was obtained in a study in which GnRH was injected on the 5th day after insemination, and ketoprofen was injected on the 11th day (Kraevskiy et al., 2020). However, in the other study, it has been reported that the pregnancy rate in Nelore cows administered hCG on the 7th day after insemination and flunixin meglumine on the 16th day did not differ from other groups. In addition, in the same study, it was reported that the pregnancy rate of the group in which hCG was applied on the 7th day and flunixin meglumine on the 16th day was remarkably higher than the pregnancy rate of the group in which only flunixin meglumine was administered and that hCG application in the early pregnancy period was more effective in preventing pregnancy losses (Rossetti et al., 2011).

In the past, various kinds of non-steroidal anti-inflammatory drugs have been studied extensively to prevent endometrial $PGF_{2\alpha}$ synthesis and reduce pregnancy losses by applying them just before the time of possible luteolysis in inseminated cows. However, some studies have reported that these drugs positively affect the pregnancy rate while they do not affect or even negatively affect others. The different results obtained in studies showed it put up various factors, including the variable inhibitory effects on COX-1 and COX-2 of these drugs, the dose, number and time of application of the drug, and animal-dependent factors, etc.

Ketoprofen is a short-acting, non-selective COX-1 and COX-2 inhibitor. In this study, administration of ketoprofen at twice the prospectus dose on days 15 and 16 after insemination did not positively affect the pregnancy rate compared to the control group. In a conducted study (Dursun, 2011), it was reported that a single dose of ketoprofen administered on the 15th day after artificial insemination did not have a positive impact on the pregnancy rate in non-lactating cows, and only a slight increase in pregnancy rate was observed, consistent with the findings of our own study. Ketoprofen's short half-life and the 12-hour interval between two drug applications may have led to the no complete inhibition of $PGF_{2\alpha}$ synthesis and, as a result, to the regression of the CL. A similar view has also been put forward in a study in which flunixin meglumine was applied twice every 24 hours (Von Krueger and Heuwieser, 2010).

Conclusion

In this study, it was determined that after artificial insemination providing luteotropic support with hCG on the 5th day or suppressing the luteolysis mechanism with ketoprofen applications on the 15th and 16th days at 12-hour intervals did not have a positive effect on the pregnancy rate. However, in the group where both applications were performed after artificial insemination (hCG on the 5th day and ketoprofen on the evening of the 15th day and morning of the 16th day), there was a 20% increase in the pregnancy rate. These results are believed to be attributed to the combination of hCG supporting luteal function in the early stages of pregnancy, allowing the embryo to develop sufficiently until maternal acceptance, and the inhibitory effect of ketoprofen on $PGF_{2\alpha}$ synthesis. It has been concluded that more comprehensive studies should be conducted by determining hormone profiles during the early embryonic period with a larger number of animals.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Research Article

Doppler Ultrasonography Assessment of Uterine Artery Blood Flow throughout Late Pregnancy in Awassi Ewes

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ABSTRACT

Monitoring uterine blood flow during pregnancy allows us to gain information about fetal development. The present study was carried out to determine the changes in uterine artery blood flow after the second half of pregnancy in Awassi ewes. The study included a total of 20 ewes with a single pregnancy, separated into 4 groups according to the period of the pregnancy, as 90-105-120-135 days. By monitoring the uterine artery blood flow with Doppler ultrasound, measurements were taken of the pulsatilite index (PI), resistance index (RI), end diastolic velocity (EDV), peak systolic velocity (PSV), systolic/diastolic ratio (S/D), and time-averaged peak (TAP). The data obtained were analyzed with repeated measures ANOVA (variance analysis). Throughout the pregnancy periods, a time-related significant difference was seen between the groups in respect of the PSV, and RI values ($P<0.001$). In addition, a time-related significant difference was seen between the groups in respect of the EDV, TAP, PI, and S/D values ($P<0.01$). The PSV value was observed to be significantly higher on the 135th day of the pregnancy compared to the 90th, 105th, and 120th days ($P<0.001$). The EDV, and TAP values were observed to be significantly higher on the 135th day of the pregnancy compared to the 90th, 105th, and 120th days ($P<0.01$). The RI value was seen to be significantly lower on the 135th day compared to the 90th, 105th, and 120th days ($P<0.001$). The PI, and S/D values were seen to be significantly lower on the 135th day compared to the 90th, 105th, and 120th days ($P<0.01$). As a result, significantly differences were observed in the changes in the uterine artery blood flow after the second half of the pregnancy of Awassi ewes. It was concluded that as the vast majority of fetal development occurs in the second half of the pregnancy, determination of changes in Doppler ultrasound parameters will contribute to increasing hemodynamic information.

Keywords: *Doppler ultrasound, ewe, pulsatilite index, resistance index, uterine artery.*

İvesi Irkı Koyunlarda İleri Gebelik Boyunca Uterin Arter Kan Akımının Doppler Ultrasonografi ile Değerlendirilmesi

ÖZET

Gebelik sırasında uterus kan akışının izlenmesi, fetal gelişim hakkında bilgi edinmemizi sağlar. Sunulan çalışma İvesi ırkı koyunlarda gebeliğin ikinci yarımından sonra uterin arter kan akımı değişikliklerinin belirlenmesi amacıyla yapıldı. Çalışmada toplam 20 adet tekiz gebe koyun kullanıldı. Koyunlar gebeliğin dönemine göre 90-105-120 ve 135. gün olmak üzere 4 gruba ayrıldı. Doppler ultrason ile uterin arter kan akımı izlenerek pulsatilite indeks (PI), rezistans indeks (RI), diyastol sonu hız (EDV), maksimum sistolik hız (PSV), sistol/diyastol oranı (S/D) ve ortalama azami hız (TAP) ölçüldü. Elde edilen veriler tekrarlı ölçümler ANOVA (varyans analizi) ile analiz edildi. Gebelik dönemleri boyunca gruplar arasında PSV ve RI değerleri açısından zamana bağlı olarak anlamlı farklılık görüldü ($P<0.001$). Ayrıca EDV, TAP, PI ve S/D değerleri açısından da gruplar arasında zamana bağlı olarak anlamlı farklılık belirlendi ($P<0.01$). Gebeliğin 135. gününde PSV değerinin 90, 105 ve 120. günlere göre anlamlı derecede yüksek olduğu görüldü ($P<0.001$). Gebeliğin 135. gününde EDV ve TAP değerlerinin 90., 105. ve 120. günlere göre anlamlı derecede yüksek olduğu belirlendi ($P<0.01$). Gebeliğin 135. gününde RI değerinin gebeliğin 90, 105 ve 120. günlerine kıyasla daha düşük olduğu görüldü ($P<0.001$). Gebeliğin 135. gününde PI ve S/D değerlerinin gebeliğin 90, 105 ve 120. günlerine kıyasla daha düşük olduğu belirlendi ($P<0.01$). Sonuç olarak İvesi ırkı koyunlarda gebeliğin ikinci yarımından sonra uterin arter kan akımı değişikliklerinde önemli oranda farklılık gözlendi. Fetal gelişimin büyük kısmının gerçekleştiği gebeliğin ikinci yarımından sonra Doppler ultrason parametre değişimlerinin belirlenmesi ile hemodinamik bilgilerin arttırılmasına katkıda bulunacağı kanısına varıldı.

Anahtar Kelimeler: *Doppler ultrason, pulsatilite indeksi, rezistans indeksi, koyun, uterin arter.*

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Introduction

Physicians routinely use Doppler ultrasonography (USG) to monitor human pregnancies, and there has been a recent increase in studies of this by veterinarians (Herzog and Bollwein, 2007). The monitoring blood flow during pregnancy allows us to learn about fetal development. Doppler ultrasound is used to evaluate anatomic and functional vascular information such as blood flow velocity, direction, and type (Nicolaides et al., 2000). Optimal establishment of feto-maternal blood flow is necessary for the normal development of the fetus during pregnancy. Doppler ultrasound provides valuable information about physiological and pathological differences in the blood flow between the mother and fetus (Fleischer et al., 1994). Doppler USG is generally used gynaecologically to examine changes in the uterine artery (Herzog and Bollwein, 2007). Uterine artery Doppler examination performed in pregnancy makes it possible to diagnose high-risk pregnancies that threatens the health or life of the mother or fetus (Ferrell, 1991).

The increase in fetus and uterus volume in the final trimester are associated with the increased demand for nutrients and oxygen provided by the increase in uterus and fetal perfusion (Kim-Egloff et al., 2016). The uterine and placental blood flow increases during pregnancy to meet the metabolic requirements of the growing fetus (Reynolds et al., 2005). The uterine arteries primarily feed the maternal part of the placenta (Kim-Egloff et al., 2016). Uterine vascularisation is evaluated using the resistance index (RI) and pulsatile index (PI) of the uterine arteries, which are good markers of the vascular perfusion of the reproductive organs (Bollwein et al., 1998; Lemos et al., 2017). As an increase in RI is a sign of a decrease in vascular perfusion, there is a negative correlation with vascular perfusion (Dickey, 1997; Sharma et al., 2019). Abnormal vascular findings in the fetal and/or maternal structures are associated with restricted intrauterine growth, fetal stress, or signs of early embryonic death (Özkaya et al., 2007; Abdelhalim et al., 2014). The measurement of uterine blood flow in pregnant ewes from the middle of pregnancy until the last phase of the pregnancy is an important diagnostic tool to explain the results of intrauterine fetal growth restriction and evaluate fetal health during the pregnancy (Gomez et al., 2006; Papageorghiou and Leslie, 2007; Wallace et al., 2008).

The aim of this study was to determine uterine artery blood flow changes after the second half of the pregnancy of Awassi ewes. This study was conducted to contribute to increasing hemodynamic information about the physiology of ewes in the last period of pregnancy.

Materials and Methods

This study was conducted with the permission of Harran University Animal Experiments Local Ethics Committee (HRU-HADYEK) (dated 28/08/2021 and numbered 2021/007).

Animal Selection and Experimental Protocol

The study material comprised 20 Awassi ewes at the Veterinary Faculty Practice Farm of Harran University, in Eyyübiye, Şanlıurfa province. The farm records were examined to select ewes, aged 2-3 (2.25±0.05) years, each weighing mean 56.86±2.29 kg, with a body condition score ranging from 2-3 (1=Extremely weak, 5=Obese) (2.55±0.04), which had previously given birth only once, as a normal single birth with no problems following the birth. The study was conducted between August and February. The sheep were fed a mixture of hay (5.2%), clover (32.9%) and milk feed (61.9%). To ensure that all the ewes were in the same period of pregnancy, progesterone-based oestrus synchronisation was performed in the breeding season. A vaginal sponge (60 mg medroxyprogesterone acetate, Esponjavet®, Hipra Animal Health, Türkiye) containing progesterone was placed in the vagina of each ewe (day 0) to remain there for 12 days. On the 11th day, 2 ml PGF2a (Dinoprost tromethamine, Dinolytic®, Zoetis, Türkiye) was administered intramuscularly. On the 12th day, the vaginal sponge was removed and 500 IU PMSG (PMSG, Oviser®, Hipra Animal Health, Türkiye) was injected intramuscularly. For 3 days following the PMSG injection, oestrus follow-up was made with a search ram for 30 mins at 8-hour intervals, and the ewes showing oestrus were naturaly insaminated by rams that had previously been determined as fertile. The ewes were kept all together throughout the study and their nutritional and water needs were provided ad libitum. On the 35th day after the insemination, the ewes were performed transrectally examined for pregnancy as single or twin, and those with a single pregnancy were included in the study for analysis. The same ewes were used in each group at different stages of pregnancy. In the second half of the pregnancy, all the ewes underwent Doppler ultrasound examinations were performed at 15-day intervals; on the 90th day of the pregnancy (Group 1, n=20), on the 105th day (Group 2, n=20), on the 120th day (Group 3, n=20), and on the 135th day (Group 4, n=20) (Figure 1).

Doppler Ultrasound Examination

The uterine artery Doppler examination was performed transabdominally. Before the transabdominal scan, the ewes were restrained in standing position and both inguinal regions were shaved extensively towards the cranial direction. No sedative drugs were administered before and during the examinations. All the ultrasound examinations were performed by the same experienced operator using a 5-10 MHz linear transducer (Sonosite Edge II, USA). Observing the placenta-uterus border of the fetus, the artery imaged at the highest quality within the uteroplacental area was used. For the anatomic evaluation of the uterine arteries, first with selected B-mode ultrasound the bladder was identified then differentiation of the uterus tissue was made and using colour Doppler, the vessels to be measured were determined. Blood flow velocity wave forms were obtained from the proximal section (before the bifurcation) of the uterine artery

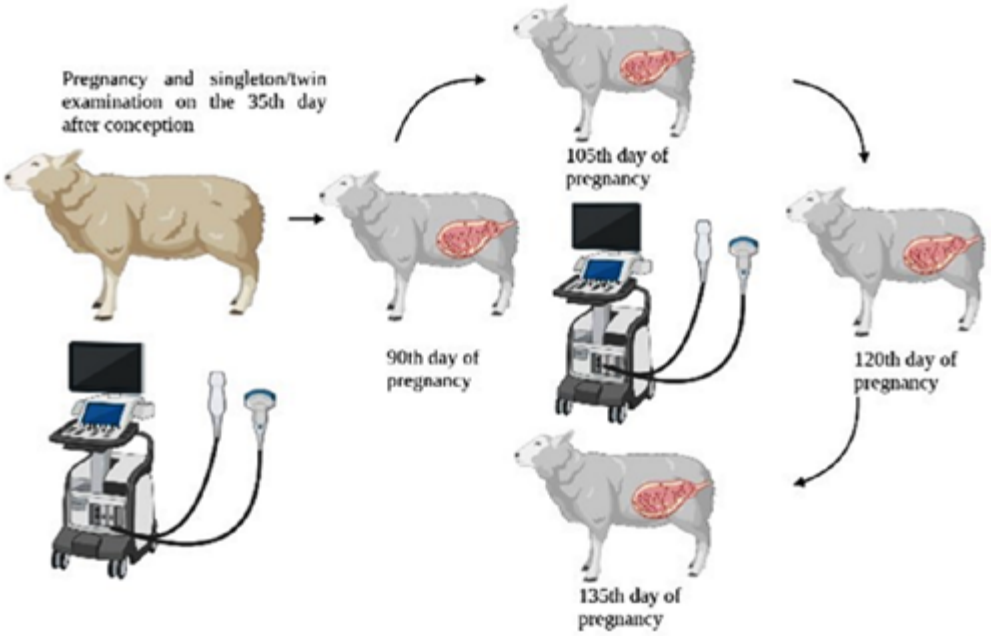

Figure 1. Study design.

after mapping the iliac vessel orientation (Elmetwally, 2016). Measurements were created using manual mode after obtaining a minimum of 3 consecutive healthy artery wave (without artefacts) images (Figure 2). In all of the examinations, the measurements were disregarded when the angle of insonation was above 20°. In the traces obtained taking Pulse Doppler USG measurements from the arteries in question, the PI, RI, EDV, PSV, S/D, and TAP values were measured. These parameters were calculated automatically by the Doppler device software using mathematical formulas or the Pourcelot index [RI: (PSV-EDV)/PSV, PI: (PSV-EDV)/mean velocity, S/D: PSV/

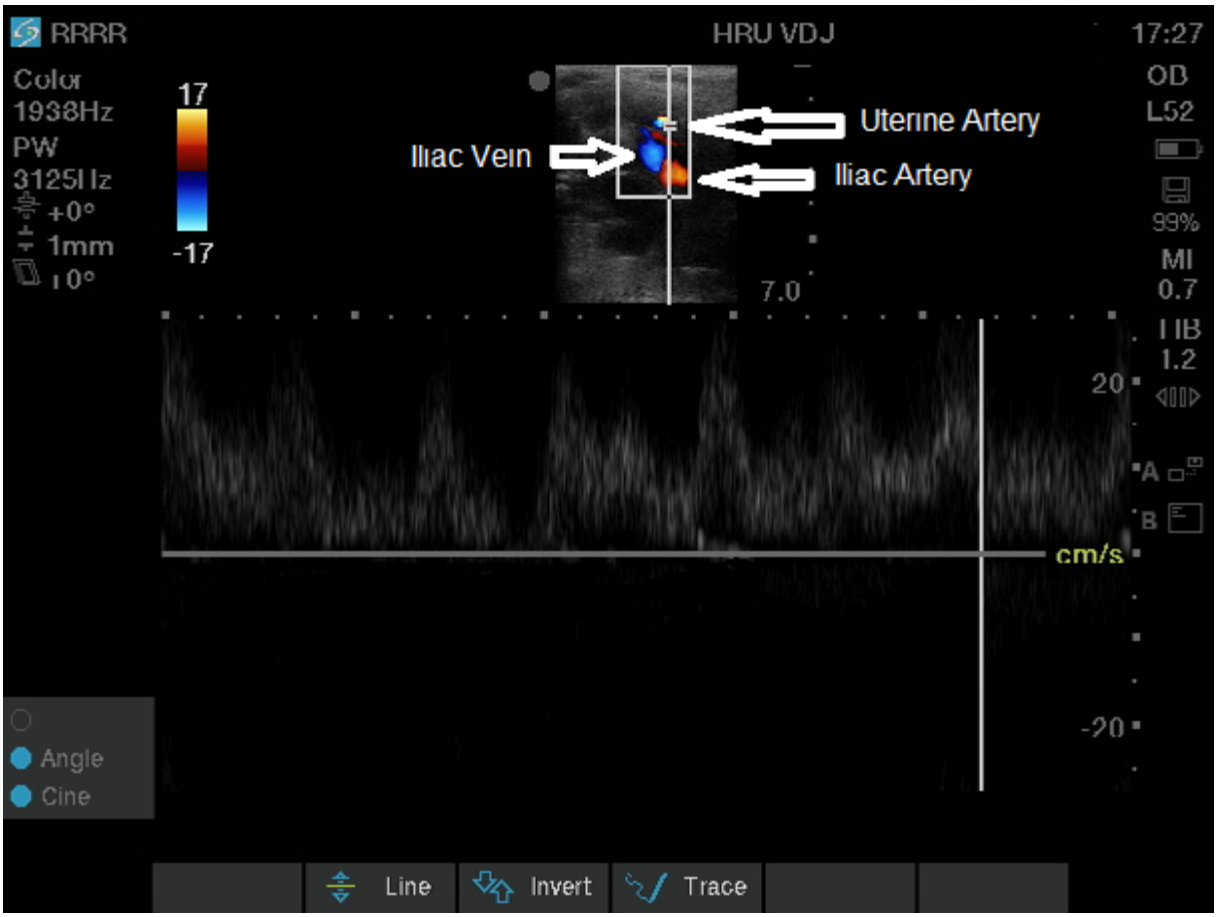

Figure 2. The Pulsed-Doppler image of uterine artery on 90th day.

EDV), TAP: PSV-EDV/PI] (Bollwein et al., 2002; Ginther and Utt, 2004). If physical movement was observed in the mother or fetus, the application was paused and the measurements were repeated after a period. To minimise the thermal and cavity effect which can form during the Doppler ultrasound examination, a pause of approximately 60 seconds was given between the 30-second measurements. All the examinations were completed in 10-15 minutes.

Statistical Analysis

Statistical analysis of the data was performed with the Statistical Package for the Social Sciences (SPSS 26.0, Chicago, IL, USA) package program. Data were examined for normal distribution using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses for variables showing normal distribution were stated as mean ± standard error of values. Time-related changes in the Doppler parameters showing normal distribution were examined using repeated measures ANOVA (variance analysis). When the sphericity assumption was not satisfied, Greenhouse-Geisser correction was performed. Variance homogeneity was determined with the Levene test. A value of P<0.05 was accepted as statistically significant.

Results

There were no pathological conditions affecting the general health of the ewes during pregnancy. Rumination was observed to decrease in the ewes a few days before birth and they remained separate from the flock during that time. These sheep were placed in separate compartments (2x2m) and the births were followed up. The births took place on 150±2 days of pregnancy (151.10±0.11), all vaginally, without assistance, and no maternal problems were encountered in the postpartum period. A total of 9 male and 11 female healthy lambs were born. The mean Doppler parameter values of the groups on days 90, 105, 120, and 135 of the pregnancy are presented in Table 1, and Figures 3 and 4. Throughout the pregnancies, a time-related significant difference was seen between the groups in respect of the PSV, and RI values (P<0.001). In addition, a time-related significant difference was seen between the groups in respect of the EDV, TAP, PI, and S/D values (P<0.01). The PSV value was observed to be significantly higher on the 135th day of the pregnancy compared to the 90th, 105th, and 120th days (P<0.001). No significant difference was determined between the PSV measurements taken on the 90th, 105th, and 120th days (P>0.05). The EDV, and TAP values were observed to be significantly higher on the 135th day of the pregnancy compared to the 90th, 105th, and 120th days (P<0.01). No significant difference was determined between the EDV and TAP measurements taken on the 90th, 105th, and 120th days (P>0.05). The RI value was seen to be significantly lower on the 135th day compared to the 90th, 105th, and 120th days (P<0.001), with no significant difference between the measurements on the 90th, 105th, and 120th days (P>0.05). The PI and S/D values were seen to be significantly lower on the 135th day compared to the 90th, 105th, and 120th days (P<0.01), and no significant difference was determined between the measurements taken on the 90th, 105th, and 120th days (P>0.05).

Table 1. The mean Doppler parameters value during pregnancy (Mean±Standard error).

Days	n	PSV (cm/s)	EDV (cm/s)	TAP (cm/s)	PI	RI	S/D
90th day	20	65.62 ± 1.46^{b}	25.42 ± 0.96^{b}	36.87 ± 1.11^{b}	1.06 ± 0.03^{a}	0.62 ± 0.002^{a}	2.69 ± 0.035^{a}
105th day	20	65.74 ± 1.16^{b}	25.85 ± 0.44^{b}	37.62 ± 1.21^{b}	1.05 ± 0.01^{a}	0.61 ± 0.001^{a}	2.63 ± 0.042^{a}
120th day	20	65.97 ± 1.59^{b}	25.95 ± 0.75^{b}	38.94 ± 1.23^{b}	0.96 ± 0.01^{a}	0.59 ± 0.001^{a}	2.55 ± 0.044^{a}
135th day	20	74.52 ± 1.03^{a}	31.94 ± 1.45^{a}	45.36 ± 1.06^{a}	0.92 ± 0.02^{b}	0.54 ± 0.001^{b}	2.52 ± 0.052^{b}
P value		<0.0001	0.007	0.001	0.008	<0.0001	0.007

^{a,b,c,d}:Different letters in the same column indicate a statistically significant difference. Peak systolic velocity (PSV), end diastolic velocity (EDV), and time-averaged peak (TAP), pulsatilite index (PI), resistance index (RI), and systolic/diastolic ratio (S/D).

Discussion

The results of this study present important data which will contribute to understanding the vascular physiology in the second half of pregnancy in Awassi ewes. Regular provision of nutrients to the fetus throughout the pregnancy is an important factor in the development of fetal growth (Molina-Font, 1998). Fetal nutrition depends on the size of the placenta, morphology, blood flow, and the presence of nutrients (Fowden et al., 2006). The uterine artery shows hemodynamic changes such as the volume and velocity of the blood flow to the uterus in response to the fetal requirements (Hassan et al., 2020).

In a study of ewes and goats, Elmetwally et al. (2016) showed a decrease in uterine artery RI, PI, and S/D ratio, and an increase in the TAP value until birth. Beltrame et al. (2017) conducted a study of ewes throughout pregnancy, and reported that the PI, RI, and S/D ratio decreased after the first trimester, and this decrease continued without any great changes through the other periods of the pregnancy. A corresponding continuous increase in the PSV, EDV, and TAP values was also determined throughout the pregnancy. In another study by Yilmaz et

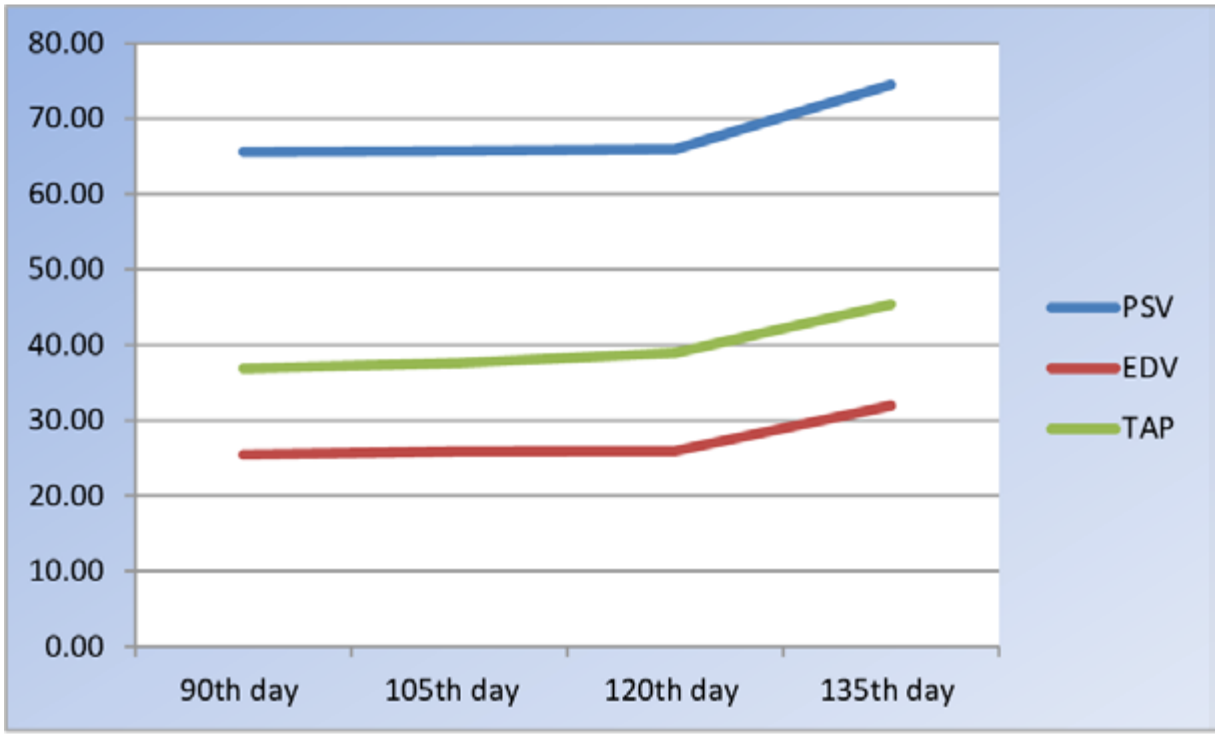

Figure 3. Peak systolic velocity (PSV), end diastolic velocity (EDV), and time-averaged peak (TAP) of the uterine artery on days 90, 105, 120 and, 135 of the pregnancies in ewes.

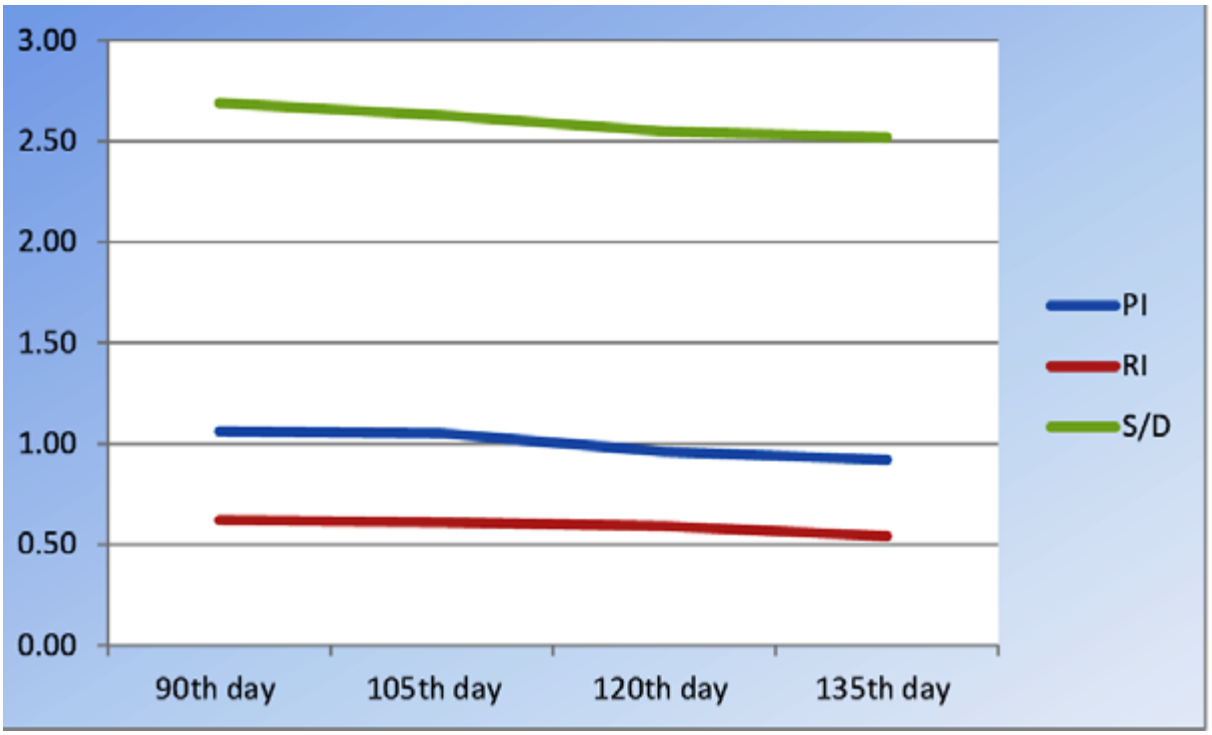

Figure 4. Pulsatilite index (PI), resistance index (RI), and systolic/diastolic ratio (S/D) of the uterine artery on days 90, 105, 120 and, 135 of the pregnancies in ewes.

al. (2017), ewes pregnancies were evaluated at 15-day intervals from the 45th day to the 135th day, and there were stated to be no significant changes in the PI and RI values. Veiga et al. (2018) examined ewes pregnancies on days 60, 90, and 120, and reported a decrease in the uterine artery PI value in parallel with the progress of the pregnancy, and an increase in the PSV, EDV, and TAP values. In an examination of pregnant ewes from the 21st day until birth, Santos et al. (2021) reported that despite an increase in the uterine artery EDV and PSV values in the last 3 weeks of the pregnancy, there was no significant changes throughout the pregnancy, and the RI value was high in the first weeks, then showed a significant fall in the last week of the pregnancy. Consistent with data in literature, our study results showed a continuous decrease in the PI, RI, and S/D ratio in the second half of the pregnancy, and a continuous increase in the PSV, EDV, and TAP values. This was thought to be due to

an increase in blood flow to the fetus as the pregnancy progressed. In our study, the continuous decrease in PI, RI and S/D ratios and the continuous increase in PSV, EDV and TAP values were thought to be due to the negative correlation between resistance and vascular perfusion. It can be interpreted that here the uterine vessels turn into a low-resistance system and increase blood flow to meet the requirements of placentation and fetal development. In our study, uterine artery PI, RI and S/D ratio showed a significant decrease as pregnancy progressed. These changes were thought to be due to the progressive development of the distal vascular bed and changes in the tone of the uterine vessels. The irregular diameter of sinusoidal capillaries on the fetal surface may reduce blood flow resistance and subsequently increase the capacity for trans-placental nutrient exchange (Hafez et al., 2010). Increases in the RI and PI show a decrease in vascular perfusion, while decreases in RI and PI are a sign of increases in vascular perfusion (Gupta et al., 2009; Elmetwally, 2016). The PI and RI values are expected to gradually decrease as the pregnancy progresses, and this shows greater fetal blood flow (Elmetwally et al., 2016). A decrease in the uterine artery PI and RI values during pregnancy and the increases in fetal nutrient demands and placental growth reflect the stage of development of the fetus and increasing diameter of the blood vessels (Rüsse, 1993; Meschia, 2011). These changes are thought to be the mechanism increasing blood flow in the uterine artery to compensate for the increased nutrient need with progression of the pregnancy, which is an important factor in the regulation of fetal growth (Molina-Font, 1998). It is also thought that these changes in vascular perfusion are due to the increased blood flow provided by changing the uterus vessels to a low-resistance system as a response to the metabolic requirements of placentation and fetal development (Herzog and Bollwein, 2007). It was observed that the average PSV, EDV, S/D ratio, PI, RI, and TAP values reported in our study were higher than those in Beltrame et al. (2017). The reason for this situation is likely to be differences in the Doppler insonation angle in the evaluation of blood flow velocity. In addition, it was thought that the location of the arterial flow may differ between studies due to branching and anastomosis in feto-maternal vascularization, making individualization and standardization difficult.

In studies of other species, very little change has been shown throughout pregnancy of the uterine artery PI value in bison (Singh et al., 2018), of the RI value in mares (Bollwein et al., 2003) and cats (Brito et al., 2010), and of both the PI and RI value in rabbits (Akkuş and Erdoğan, 2019), and although not directly related to the current study, these findings provide an idea about uterine artery Doppler follow-up. Starting from the second half of the pregnancy, there has been shown to be a steady decrease throughout pregnancy in the uterine artery PI and RI values of dogs (Nautrup, 1998; Miranda and Domingues, 2010; Blanco et al., 2011), cats (Scotti et al., 2008; Pereira et al., 2012; Blanco et al., 2014), mares (Bollwein et al., 2004; Strübing, 2011; Abdelnaby et al., 2022), cows (Bollwein et al., 2002), and rats (Mu and Adamson, 2006). In a study of pregnant cows, Panarace et al. (2006) determined a decrease in the uterine artery PI and RI values, and an increase in the TAP value as the days of pregnancy increased between days 30 and 270. Hassan et al. (2020) reported that although the uterine artery PI and RI values of pregnant cows at 1, 2, 4, 6, and 8 months were lower at the end of the pregnancy, the EDV and TAP values were higher. In the examinations of small breed dogs at 10-day intervals from the 30th to the 60th day of pregnancy, Batista et al. (2018) determined a continuous increase in PSV and EDV values and a continuous decrease in the RI value. Although not directly related to the our study, these studies of other species also provide information about uterine artery follow-up and are consistent with the current study findings. Decreased vascular resistance in the uterine artery is associated with increasing perfusion of the artery and somatic development of the fetus (Miranda and Domingues, 2010). A decrease in the indexes is a result of the loss of the musculoelastic layer of the arteries and simultaneous invasion of trophoblasts to the endometrium during placentation (Wright and Royston, 1997). Other studies have indicated that this increase in vascular resistance is a sign of retarded intrauterine growth (Owen and Ogston, 1997), maternal hypertension (Gudmundsson and Marsal, 1991), abortus (Blanco et al., 2016), and perinatal death (Dubiel et al., 2003).

Conclusion

In conclusion, the results of this study demonstrated that changes in uterine artery blood flow measured after the second half of pregnancy, when most of the fetal development occurs, were successfully evaluated using Doppler ultrasound. It was concluded that it may benefit a broader understanding of hemodynamic changes in physiologically healthy pregnancies. Our results reflect physiological uterine behavior during healthy pregnancy and therefore additional studies are needed to distinguish physiology from pathology in sheep. With further studies based on these data, it has been concluded that Doppler ultrasound can be used in the diagnosis of specific diseases by examining the uterine artery hemodynamic changes in infectious or metabolic diseases that are frequently encountered after the second half of pregnancy in ewes.

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

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Research Article

Investigation of *In Vitro* Effectiveness of Polymeric Nanoparticles Containing Clinoptilolite on *Cryptosporidium parvum*

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ABSTRACT

Cryptosporidium species, which do not have a definite prevention and treatment protocol, are pathogenic protozoans that cause diarrhoea in humans and many animal species. This study aimed to demonstrate the in vitro efficacy of clinoptilolite polymer nanoparticle, which has known antidiarrheal effects. DNA isolation was made for species identification of *Cryptosporidium* oocysts obtained from faeces samples from naturally infected calves and lambs. RFPL analysis was performed in typing. For this purpose, nested PCR and SrpI, VspI and MboII enzymes were used. The coverslip surface was covered with 80% CaCo-2 cells and infected with $1x10^9$ *C. parvum*. Nanoparticles containing 250, 500, 750 and 1000 µg/mL clinoptilolite were applied to infected cells. Percent of infection rate was calculated by counting under a fluorescent microscope following incubation. While the infection rate was 23.46% in the water-treated control cell group, the percentage infection rates in the clinoptilolite-containing nanoparticle treated group were respectively 15.60%, 8.13%, 10.33% and 13.46%. Inhibition percentages were determined as 33.54%, 65.56%, 55.99% and 42.66%, respectively. As a result, it was observed that the nanoparticle containing clinoptilolite had anticriptocidal activity in infection with *C. parvum* in Caco-2 cells. In addition, it was observed that the efficacy was dose-dependent, and the IC_{50} value was the most appropriate value at 750 and 1000 µg/mL doses.

Keywords: *Cryptosporidium parvum, clinoptilolite, nanoparticle.*

Klinoptilolit İçeren Polimer Nanopartiküllerin *Cryptosporidium Parvum* Üzerine *İn Vitro* Etkinliğinin Araştırılması

ÖZET

Kesin bir korunma ve tedavi protokolü bulunmayan *Crptosporidium* türleri insan ve pek çok hayvan türünde ishale neden olan patojen prozoonlardır. Bu çalışmada antidiyarel etkileri bilinen klinoptilolitin polimer nanopartikülünün in vitro olarak etkinliğinin ortaya konması amaçlanmıştır. Doğal enfekte buzağı ve kuzulardan alınan dışkı örneklerinden elde edilen *Cryptosporidum* ookistlerinin tür tayini için DNA izolasyonu yapılmıştır. Tiplendirmede RFPL analizi gerçekleştirilmiştir. Bu amaçla nestet PCR ve SrpI, VspI ve MboII enzimleri kullanılmıştır. Lamel yüzeyi % 80 CaCo-2 hücresi ile kaplanmış ve $1x10^9$ *C. parvum* ile enfekte edilmiştir. Enfekte hücrelere 250, 500, 750 ve 1000 µg/mL klinoptilolite içeren nanopartikül uygulanmıştır. İnkubasyonu takiben floresan mikroskopta sayım yapılarak yüzde enfeksiyon oranı hesaplanmıştır. Su uygulanan kontrol hücre grubunda enfeksiyon oranı %23,46 iken klinoptilolit içeren nanopartikül uygulanan grupta yüzde enfeksiyon oranları sırasıyla %15,60; %8,13; %10,33 ve %13,46 olarak belirlenmiştir. İnhibisyon yüzdeleriyse sırasıyla %33,54, %65,56, %55,99 ve %42,66 olarak saptanmıştır. Sonuç olarak Caco-2 hücrelerinde *C. parvum* ile enfeksiyonda klinoptilolite içeren nanopartikülün antikriptosidal etkinliğinin olduğu gözlenmiştir. Ayrıca etkinliğin doza bağımlı olduğu ve 750 ve 1000 µg/mL dozlarda IC_{50} değerinin en uygun değerler olduğu görülmüştür.

Anahtar Kelimeler: *Cryptosporidium parvum, klinoptilolit, nanopartikül.*

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Introduction

Diarrhoea is an important problem that causes economic loss and death in lambs, goats, and calves. In these species, *Cryptosporidium spp*. is one of the causes of diarrhoea in the first weeks of life worldwide. It is a zoonotic disease that can be affected by animal, environment, and production practices (Adkins, 2022). *C. parvum, C. hominis, C. canis, C. felis, C. meleagridi*s, and *C. muris* have been reported in diarrheal and gastrointestinal diseases in birds, sheep, horses, cattle, camels and rodents (Ranjbar et al., 2018; Haghi et al., 2020). Despite advances in medicine and technology, diarrhoea is the world's main problem in newborn farm animals. In our country, the extent of direct and indirect economic losses due to diarrhoea is increasing due to the breeders' lack of knowledge and financial situation. Treatment of animals with diarrhoea is time-consuming, labour-intensive and expensive. For this reason, it emphasises the prevention or reduction of the occurrence of diarrhoea. Currently, there is no standard preventive and therapeutic protocol for *C. parvum*. Despite the use of many drugs in preventing and treating *C. parvum* infection, complete success has yet to be achieved. Therefore, there is a need for a new treatment protocol for cryptosporidiosis. Natural products significantly impact human and animal health sectors, mainly through drug discovery and chemical biology.

It is known that aluminium silicates, one of the most important components of zeolites containing clinoptilolite, have beneficial biological properties in reducing diarrhoea associated with intestinal diseases, especially in pigs, calves and rats (Sverko et al., 2004; Papaioannou et al., 2005; Deligianis et al. 2005). Various research results show that aluminium silicates, one of the essential components of zeolites containing clinoptilolite, play an indispensable role in regulating the immune system. Clinoptilolite is a crystalline compound with low solubility in the digestive system, non-absorbable, connected by channels, having smooth pores, and contains ions such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg). It is thought that clinoptilolite can change environmental factors such as moisture and nutrition, which enable it to sporulate and become infective by affecting the hydrostatic pressure of *C. parvum* with its water loss and gain feature. It is predicted that clinoptilolite may play an important role in the prevention and treatment of *C. parvum* infection due to its ability to affect the environmental conditions necessary for the development and survival of the oocyst, as well as its ability to regulate the immune system and be used as an immune stimulant.

It is accepted that nanobiotechnology, which is rapidly entering our lives in fields such as health, food, agriculture and the pharmaceutical industry, will be the most effective technology of our near future and will directly affect our lives and will be a superior technology that can increase our living standards considerably. The nanobiotechnological products to be prepared can radically change human and animal life and economic activities. Therefore, this study aimed to reveal the in vitro efficacy of clinoptilolite-containing polymer nanoparticles on *C. parvum.*

Materials and Methods

Preparation of Cryptosporidium parvum

Cryptosporidium oocysts were collected from naturally infected lambs and calves brought to Adnan Menderes University, Faculty of Veterinary Medicine, Internal Medicine Clinic, as described by Lorenzo et al. (1993). The presence of *E. coli* K99, *Cryptosporidium*, Coronavirus and Rotavirus infections in stool samples taken from animals before the collection was tested using the BoviD-4 Ag test kit (Anigen, Korea). After the faeces collected from lambs and calves with only *Cryptosporidium sp*. infection were diluted with distilled water and passed through a 45 µm perforated strainer, the resulting liquid part was centrifuged at 1000 x g at 4 °C for 5 minutes. The pellet was mixed with 20 mL of distilled water and 20 mL of diethyl ether and centrifuged at 1000 x g at 4°C for 5 minutes, and the upper three layers were removed. This process was repeated until the fat in the stool was wholly removed. The obtained sediment was mixed with 1 mL of distilled water and spread over the Percoll gradient formed from four different densities (1.13, 1.09, 1.05 and 1.01 g/mL, each 2.5 mL). The mixture was centrifuged at 650 x g for 15 minutes at 4°C. The tape containing the oocyst eggs was removed and washed with distilled water by centrifugation at 1000 x g at 4 °C for 5 minutes, and the washing process was repeated three times. The oocysts were stored in 2.5% (w/v) potassium dichromate solution until experimental infection. Figure 1 shows *Cryptosporidium* oocysts collected from feces**.**

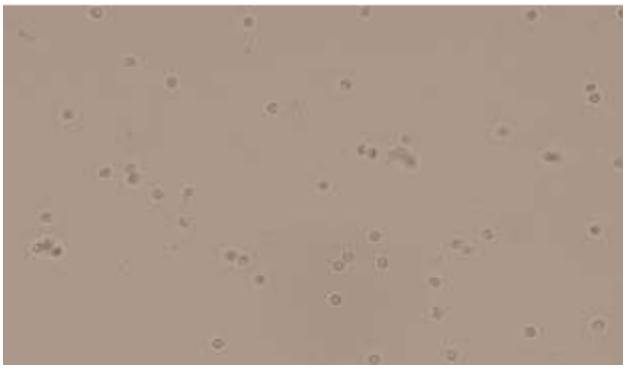

Figure 1. *Cryptosporidium parvum* oocyst (x400)

Determination of the Type of Cryptosporidium Oocysts by PCR and RFLP

DNA isolation was performed in 100 µL suspension using the phenol/chloroform method to determine the type of *Cryptosporidium* oocysts obtained. Nested PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) analysis were performed to determine the type of oocysts obtained from naturally infected lambs. For this purpose, 100 µL of the obtained oocyst suspension was taken, and DNA was isolated by the classical phenol/chloroform method (Sambrook et al., 1989). The *C. parvum* species was determined by RFLP analysis using Nested PCR and SspI, VspI and MboII enz-

ymes. PCR was performed as previously described using primers of the 18S ribosomal RNA gene specific to the Cryptosporidium strain (Xiao et al., 1994; Aysul, 2009).

The first PCR was performed using primers (forward: 5'-TTC TAG AGC TAA TAC ATG CG-3' and reverse: 5'-CCC TAA TCC TTC GAA ACA GGA-3') amplifying the 1325 bp portion of the 18S ribosomal DNA gene of Cryptosporidium. The second PCR was performed using primers (5'-GGA AGG GTT GTA TTT ATT AGA TAA AG-3' and 5'-AAG GAG TAA GGA ACA ACC TCC A-5') amplifying the 826-864 bp portion of the first PCR product. The PCR mix contained 1 x PCR buffer, 6 mM $MgCl_2$, 200 mM deoxynucleoside triphosphate, 100 nM primers, and 2.0 U of HotStartTaq DNA polymerase. The final PCR volume was 25 µl. 2 µl of DNA sample was used in the first PCR, and 1 µl of the first PCR product was used in the second PCR. Both PCRs were performed in 35 cycles (denaturation at 94°C 45 s, hybridisation at 57°C 45 s, and extension at 72°C 1 min). Both PCRs included cycles of 3 minutes at 94°C initially and 7 minutes at the end of 72°C. Analyses of PCR products were performed with Safeview-stained 1.5% agarose gel electrophoresis.

In nested PCR products (approximately 850 bp), RFLP analysis was performed using the enzymes SspI, VspI and MboII as previously described (Feng et al., 2007). These enzymes distinguish *C. parvum, C. bovis, C. andersoni* and *C. range* genotypes. For this purpose, the second PCR product was isolated from agarose gel with the help of the QIAquick gel extraction kit. For each VspI, SspI and MboII enzyme, 15 units of enzyme for 40 µL of the PCR product were mixed with 5 µL of buffer and 2 µL of sterile de-ionized water. It was incubated at 37ºC for 2 hours to complete the cutting process. The mixture was stained with Safeview and visualised by running on a 3% agarose gel. The SspI enzyme cuts *C. parvum* or *C. bovis* at 449, 267 and 108 bp sizes, while it cuts *C. andersoni* at 448 and 397 bp sizes. MboII cuts *C. parvum* at 771 and 76 bp and *C. bovis* at 412, 185, and 162 bp. The VspI restriction enzyme forms 628, 115 and 104 bp fragments in *C. parvum* and 730 and 115 bp in *C. andesroni*. As a result of the RFLP analysis performed in this study, all DNA samples tested were found to be *C. parvum*. Due to the low sensitivity of the agarose gel, products around 100 bp obtained as a result of RFLP analysis were not detected in the gel (Figure 2).

In addition, sequence analysis was performed by cloning the nested PCR product to confirm that the oocysts used in the in vitro experiment were *C. parvum*. DNA samples amplified by nested PCR were visualised under UV light following 2% agarose gel electrophoresis with Safeview. A band of approximately 850 bp was cut from the gel and purified using the QIAquick gel extraction kit. The purified product was then cloned into the pCR4–TOPO plasmid vector and transformed into TOP10 *E. coli* cells, as indicated in the commercial kit. Selected colonies were grown overnight, and plasmids contained in cells were purified using the QIAGEN plasmid purification kit. After cutting with the EcoR1 enzyme, they were electrophoresed in 1.5% agarose gel, and plasmids containing the cloned DNA were determined under UV light. Then, DNA samples of these plasmids were sent to Iontek (Istanbul) company for sequence analysis. The results were compared with sequences stored in GenBank and were found to be 99-100% similar to C. parvum (Figure 3).

Preparation of Polymer Nanoparticle Containing Clinoptilolite

Nanoparticle was prepared using a biopolymer with known biocompatible mucoadhesive properties, and clinoptilolite encapsulation was carried out into the nanoparticle. In this context, syntheses of nanoparticles were carried out in a spray drier device using chitosan (1%), clinoptilolite-containing solution and thiamine pyrophosphate in acetic acid. Optimisation of nanoparticle synthesis was performed by encapsulation at varying flow rates, and syntheses of nanosized particles were performed. In optimising nanoparticle synthesis and characterising nanoparticles prepared under optimum conditions, size measurements were carried out with zetasizers (Malvern Panalytical, Ultra Zetasizer, United Kingdom).

In Vitro Infection

In order to determine the in vitro efficacy of clinoptilolite-containing polymer nanoparticles on *C. parvum* oocysts, the cell culture study was examined in vitro as follows. Human colon adenocarcinoma cells were used to perform experimental in vitro infection. Cells reproduced using 10% Fetal Bovine Serum, 100U/mL penicillin, 100 µg/mL streptomycin added DMEM medium (Sigma D6429) (4500 mg/L Glucose, 110 mg/L sodium pyruvate, L-glutamine) in a 5% CO_2 incubator at 37ºC. In the infection trials, 18 mm sterile round coverslips were placed in 12 well cell culture plates, and Caco-2 cells were seeded on them. After the cells covered 80% of the surface of the coverslips, they were infected with $1x10^6$ C. *parvum oocyst*. The percent inhibition of *C. parvum* for each dilution of the clinoptilolite-containing polymer nanoparticle in vitro culture was calculated according to the following formula.

$$\text{Inhibition (\%)} = \frac{\text{Control oocyst number - Dilution oocyst number}}{\text{Control oocyst number}} \times 100$$

Statistical evaluation of the data mean and standard error values were calculated using the Statistical Package for the Social Sciences (SPSS 19, Chicago, IL, USA) package program.

Results

The per cent effect of polymer nanoparticles containing clinoptilolite on *C. parvum infection* in in vitro tissue culture of the study with Caco-2 cells is shown in Table 1. The appearance of oocysts in the in vitro treatment of clinoptilolite-containing polymer nanoparticles in *Cryptosporidium parvum* infection carried out in Caco-2 cells is shown in Figure 4. The per cent inhibition concentration and IC_{50} values of polymer nanoparticles containing clinoptilolite in *C. parvum* infection carried out in Caco-2 cells are shown in Figures 5 and 6.

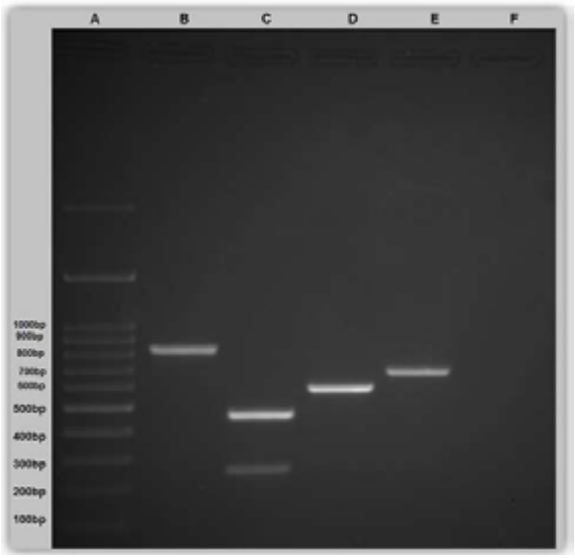

Figure 2. RFLP analysis of the nested PCR product of *Cryptosporidium parvum* using cutting enzymes SspI, VspI, and MboII. A; Molecular Marker (ABM 100bp plus), B; *C. parvum* nested PCR product (847 bp), C; The nested PCR product (449 and 267 bp) cut with SspI, D; The nested PCR product (628 bp) cut with VspI, E; The nested PCR product cut with MboII (771 bp), F; negative control. 3% agarose gel stained with SafeView (ABM).

Figure 3. Sequence analyses of *Cryptosporidium parvum*

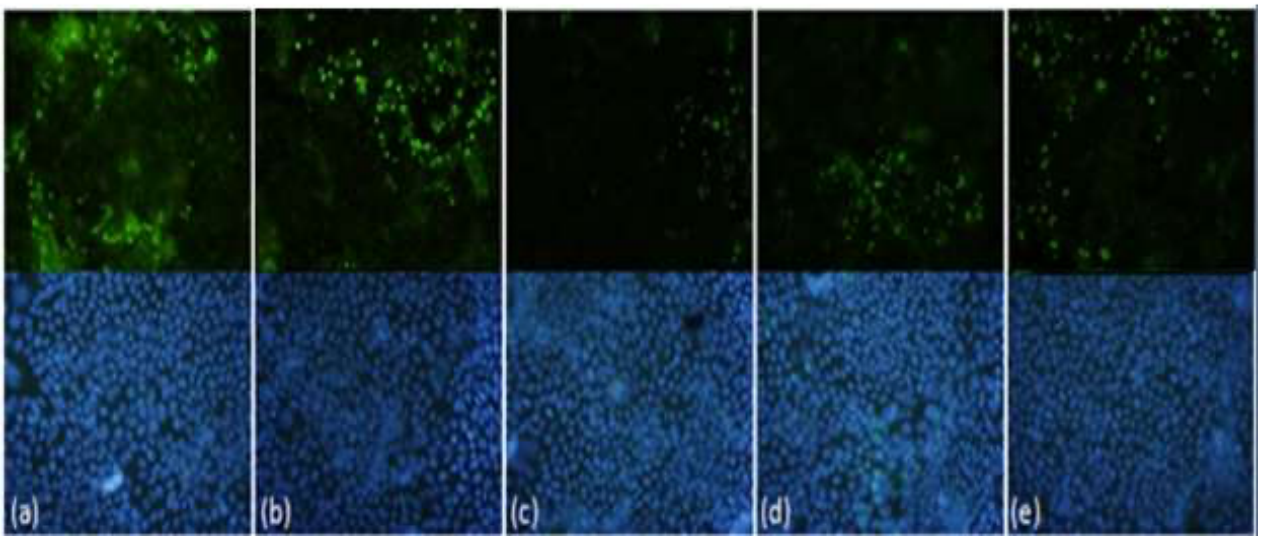

Figure 4. In vitro appearance of *C. parvum* oocysts of Caco-2 cells treated with nanoparticle-containing clinoptilolite. Real-time cell nucleus staining with DAPI(400X)

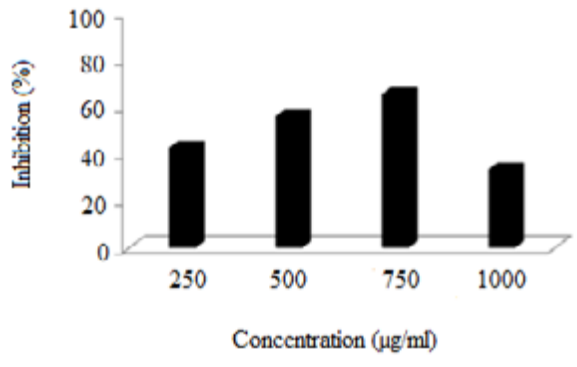

Figure 5. Percentage of infection of nanoparticles containing clinoptilolite

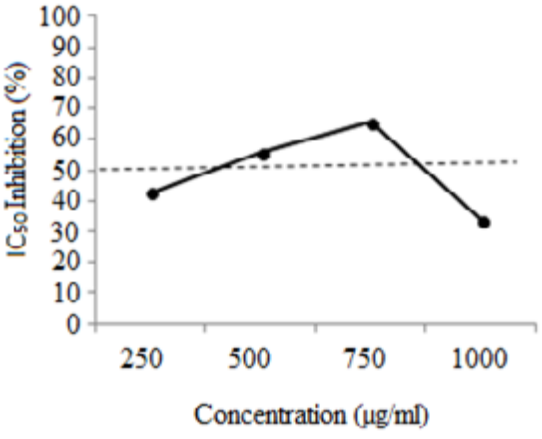

Figure 6. IC_{50} values of polymer nanoparticles containing clinoptilolite

Table 1. Effect of polymer nanoparticle containing clinoptilolite on *C. parvum* in invitro cell culture ($(\bar{X})\pm S_{\bar{x}}$)

Groups	Control	Clinoptilolite dilution			
		1000 µg/mL	750 µg/mL	500 µg/mL	250 µg/mL
Percentage of infection (%)	23.46±5.50	15.60±2.54	8.13±1.60	10.33±1.70	13.46±2.06

Discussion

Diarrhoea in farm animals is more common, especially in the neonatal period, with severe consequences. In addition to *E. coli*, Rotavirus and Coronavirus in the neonatal period in ruminants, another factor that causes diarrhoea and whose importance is increasing today is *C. parvum*, which has a zoonotic character. The number of oocysts required to establish or establish clinical infection with *Cryptosporidium* varies considerably depending on individual susceptibility and host resistance. Some researchers (Viel et al., 2007; Schnyder et al., 2009; Al-Mathal & Alsalem, 2012; Zambriski et al., 2013) have revealed experimental clinical infection in different animal species by administration of *Cryptosporidium parvum* oocyst between $1x10^3$ and $1x10^7$. The factor that facilitates the rapid spread of the infection and becomes a herd problem is that an infected animal sheds millions of oocysts with 1 gram of feces (de Graaf et al., 1999; Hamnes et al., 2006; Divers & Peek, 2008). In vitro studies create infection by applying oocysts at a rate similar to in vivo studies (Santın et al., 2004).

Various ionophore polyether antibiotics such as Lasalocid Na, paromomycin, azithromycin, halofuginone, decoquinate, and Nitazoxanide (NTZ) have been tried in the treatment of *Cryptosporidium parvum* infection. However, there are certain limitations in their use due to their toxic or side effects. In addition, none of the tested substances provided complete control of clinical findings and elimination of infection (Shadiduzzaman & Daugschies, 2012). For this reason, studies on the prophylaxis and treatment of *C. parvum* continue, and many active substances from different groups are still being tested. It is reported that clinoptilolite added to feeds in farm animals prevents diarrhoea. It is reported that adding clinoptilolite at a dose of 2g/kg body weight for treatment purposes in calf diarrhoea and 1g/kg body weight for protection purposes affects preventing diarrhoea. 1g/kg of clinoptilolite added to colostrum or milk reduces the occurrence of diarrhoea in calves. However, it does not affect passive immunity (Sadeghi & Shawrang, 2008). Adding 1g of clinoptilolite per kg body weight to colostrum in postpartum calves reduces respiratory and diarrheal problems and the use of antibiotics (Vrzgula et al., 1988). Lamb ration that includes 3% clinoptilolite to effectively control nematodes (Deligiannis et al., 2005). It has been reported that clinoptilolite affects the morphology of oocysts in vitro coccidiosis in sheep and reduces the excretion of oocysts and environmental contamination in vivo (Alcala-Canto et al., 2011). Ay et al. (2021) demonstrated the prophylactic effect of clinoptilolite in experimentally infected lambs with *C. parvum*. In addition, this study revealed that clinoptilolite changes environmental factors such as humidity, which causes sporulation and infection of oocysts and stimulates the immune system.

Researchers said that aluminium silicates, one of the essential components of zeolites containing clinoptilolite, have beneficial biological properties in reducing diarrhoea associated with intestinal diseases, especially in pigs, calves and rats. It is reported that aluminium silicates have the potential as a new alternative feed supplement in inflammatory conditions, the regulation of the immune system, and the prevention of immunosuppressive diseases (Sverko et al., 2004; Papaioannou et al., 2005). Although the efficacy of clinoptilolite in the prevention and treatment of diarrhoea in farm animals has been reported, there is no study on the effectiveness of clinoptilolite-containing polymer nanoparticles in both diarrhoea and *C. parvum* infection. This study is the first to evaluate the in vitro efficacy of clinoptilolite polymer nanoparticle formulation against *C. parvum*. The absence of a study in the literature assessing the efficacy of clinoptilolite-containing polymer nanoparticles against *C. parvum* limits the discussion of the results of this study. However, investigating the effects of different nanobiotechnological products on *C. parvum* has come to the fore in recent years. Cameron et al. (2016) reported that silver nanoparticles destroy *C. parvum* oocysts and reduce their survival in a dose-dependent manner. Likewise, Fallah et al. (2017) say that nano nitazoxanide is more effective than normal nitazoxanide, and that nano product can treat *Cryptosporidium.*

In this study, in which the effect of clinoptilolite-containing polymer nanoparticles on *C. parvum* oocysts was evaluated in vitro, the inhibition percentage of different concentrations (1000µg/mL, 750µg/mL, 500µg/mL, 250µg/mL) was found to be 33.54%, 65.56%, 55.99%, 42.66%, respectively. It was observed that polymer nanoparticles containing clinoptilolite had anticriptocidal activity in in vitro infection of Caco-2 cells with *C. parvum*, and clinoptilolite-containing polymer nanoparticle concentrations (IC50) that provided 50% inhibition for *Cryptosporidium parvum* were 750µg/mL and 500µg/mL.

It is thought that this effect of the polymer nanoparticle containing clinoptilolite is due to the positive effect of its activity against *C. parvum* in epithelial cells through different mechanisms concerning the prolongation of the intestinal transit time and the increase of the contact time with the intestinal mucosa. The clinoptilolite-con-

taining polymer nanoparticle used in this study, such as paromomycin, halofuginone and azithromycin, also reduces the number of *C. parvum* oocysts with direct and indirect effects. Therefore, it can be used alone or in treating combined *C. parvum* infection.

Conclusion

In conclusion, with these effects, polymer nanoparticles containing clinoptilolite may be an economical alternative to other anticryptocidal agents for prophylactic and treatment purposes in *C. parvum* infection. For this purpose, conducting in vivo studies in which clinical and parasitological improvement can be determined using nanoparticles containing clinoptilolite in farm animals with Cryptosporidiosis would be beneficial.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflict of interest.

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Review

The Relationship Between Microbiota and Alzheimer's Disease

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ABSTRACT

The term microbiota refers to the micro-organisms that interact with the host from birth to death. These interactions can reach the brain via the bloodstream or the gut-brain nervous system. The microbiota performs important beneficial functions, such as contributing to digestive processes, breaking down toxins and creating defense mechanisms against pathogenic bacteria. However, this positive situation only takes place when the microbiota is also positive, i.e. when the probiotics, known as eubiosis, are dominant. Factors such as nutritional habits, age and the use of antibiotics can impair the balance of the microbiota and lead to a situation where harmful microorganisms, known as dysbiosis, are dominant in the gut. In this case, the production of many microbial products that are normally beneficial to the body, such as neurotransmitters and some short-chain fatty acids, reduces and pathogenic metabolites are produced. In the case of dysbiosis, intestinal permeability increases, allowing harmful pathogenic metabolites to enter the bloodstream and even reach the brain via the bloodstream. For these reasons, prolonged dysbiosis is known to pave the way for many diseases such as depression, anxiety, schizophrenia, autism, diabetes, and Alzheimer's disease. Alzheimer's disease is characterized by the death of nerve cells in the brain and loss of cognitive abilities. The disease is associated with amyloid plaques and tau protein. It has been argued that disruption of the intestinal microbiota may contribute to the pathology of Alzheimer's disease and may also have therapeutic potential. Amyloid production may be triggered by the intestinal microbiome, causing a way for the studies on Alzheimer's disease. This review examines the relationship between the intestinal microbiota and Alzheimer's disease.

Keywords: *Alzheimer's, microbiota, probiotics.*

Mikrobiyata ve Alzheimer İlişkisi

ÖZET

Mikrobiyota, doğumdan ölüme kadar konakçı ile etkileşimde bulunan mikroorganizmaları ifade etmektedir. Bu etkileşimler kan dolaşımı veya bağırsak-beyin sinir sistemi yoluyla beyine kadar gidebilmektedir. Mikrobiyota, sindirim süreçlerine katkı sağlama, toksinleri parçalama ve patojen bakterilere karşı savunma mekanizmaları oluşturma gibi önemli faydalı işlevleri yerine getirir. Ancak bu olumlu durum mikrobiyotanın da olumlu yani ösbiyozis adı verilen probiyotiklerin baskın olduğu durum ile gerçekleşmektedir. Beslenme alışkanlıkları, yaş, antibiyotik kullanımı gibi faktörler mikrobiyotanın dengesini bozabilir ve disbiyözis adı verilen olumsuz ve zararlı mikroorganizmaların bağırsaklarda baskın olduğu duruma yol açabilmektedir. Bu durumda normalde vücut için yararlı olan nörotransmitterler, bazı kısa zincirli yağ asitleri gibi birçok mikrobiyal ürünün üretimi azalacak ve patojen metabolitler üretilemeye başlanacaktır. Disbiyozis durumunda bağırsak geçirgenliği artmakta, bu da zararlı patojen metabolitlerin kana karışmasına ve hatta kan dolaşımı yoluyla beyne ulaşmasına yol açmaktadır. Bu sebeplerden dolayı disbiyözis durumunun uzun sürmesi sonucunda, depresyon, anksiyete, şizofreni, otizm, diyabet, Alzheimer gibi birçok hastalığın önü açıldığı bilinmektedir. Alzheimer hastalığı, beyinde bulunan sinir hücrelerinin ölümü ve bilişsel yeteneklerin kaybıyla karakterizedir. Bu hastalığın amiloid plakları ve tau proteini ile ilişkilendirildiği belirtilmektedir. Bağırsak mikrobiyotasının bozulmasının Alzheimer hastalığının patolojisine katkıda bulunabileceği ve aynı zamanda bu konuda tedavi potansiyelinde olabileceği tartışılmaktadır. Özellikle amiloid üretimi bağırsak mikrobiyomu tarafından tetiklenebilmekte ve bu da Alzheimer hastalığıyla ilgili çalışmalar için yeni bir yol açmaktadır. Bu derleme de bağırsak mikrobiyotası ile Alzheimer arasındaki ilişki incelenecektir.

Anahtar Kelimeler: *Alzheimer, mikrobiyota, probiyotikler.*

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Introduction

The word 'microbiota', which was defined in the early 1900s, refers to the community of microorganisms that live in different parts of the human body. These microorganisms have 150 times more genetic information than the human genome and are referred to as "hidden organs". While the term 'microbiota' refers to the living microorganisms, the term "microbiome" includes the genomes, structural elements, and metabolites of these organisms. The concept of microbiome therefore describes a wider scope than microbiota (Hou et al., 2022).

The fact that the microbiota has 10 times the number of cells in the body, and the microbiome has 100 times the number of genes, is a clear indication of how effective these organisms are for the living beings (Dinan and Cryan, 2015). In the past, it was thought that the number of microorganisms comprising the microbiota was quite small, but today it has been reported that research in this field is gaining momentum as more microorganism species are being discovered using cheaper and more efficient culture-independent methods and new-generation sequencing methods, such as 16S rRNA sequencing (Koçak and Şanlıer, 2017). The formation of the microbiota is influenced by many factors, such as race, mode of birth, age, gender, diet, previous diseases, and antibiotic use. While a healthy balance, or eubiosis, is associated with positive microbiota, unhealthy imbalance, or dysbiosis, is associated with negative microbiota (Yılmaz and Altındiş, 2017). There is a symbiotic, pathogenic, and commensal relationship between the microbiota and the human body. Metabolites produced by microbiota can affect brain through gut-brain relations, suggesting important connections and interactions between brain and intestinal microbiota. The Human Microbiome Project was undertaken to better understand this relationship, to find treatments for disease and to maintain human health, and at the end of the project it was reported that the human body is a super-organism (Çetinbaş et al., 2017).

Alzheimer's disease is the primary one of the world's major health problems, especially among the elderly population. Current treatments for the disease are aimed at slowing the progression of the disease or reducing symptoms. In addition to these treatments, exercise programs that can be applied at every stage are used. Exercise can prevent or delay the decline of cognitive signals in the aging brain, because exercise increases the formation of new neurons (Keleş and Özalevli, 2018). Regarding the relationship between the microbiota and Alzheimer's disease, it has been reported that an imbalance in the intestinal microbiota may increase the risk of Alzheimer's disease by causing microbial amyloids to enter the bloodstream and neuroinflammatory processes. Probiotics, made up of bacteria that benefit the health of the host, have been found to have the capacity to stabilize the pH of the digestive system, reduce inflammation and increase levels of neuroprotective molecules, while prebiotics are substances that provide nutrients to the bacteria that make up probiotics (Angelucci et al., 2019). This leads researchers to believe that probiotic and prebiotic supplements could be a potential treatment for Alzheimer's patients today. Therefore, understanding the intestinal microbiota and its relationship with the human body could shed light on the development of treatment methods in the future.

Relationship between Microbiota and Brain

The human body has interacted with microorganisms since its existence. Beneficial, harmless, or harmful microorganisms are found in many tissues of the body, such as the skin, throat, mouth, mucous membranes of organs and the gastrointestinal tract. These microorganisms are called the "microbiota" and the genes they carry are called the "microbiome" (Çetinbaş et al., 2017). Gastrointestinal system is the system that contains the most microbiota due to its large surface area. The community formed by all microorganisms in or passing through this system is called "intestinal microbiota" (Gerritsen et al., 2011). The intestinal microbiota has many important functions and benefits. These include facilitating the absorption of hard-to-digest or indigestible foods by breaking them down, preventing the proliferation of harmful microorganisms by creating an acidic environment, and playing a role in the immune system. In addition, the intestinal microbiome and its metabolites are involved in fundamental neurogenerative processes such as neuroinflammation, formation of blood-brain barrier, myelination, neurogenesis, and microglial maturation (Nazlıkul and Acarkan 2014; Li et al., 2018). The microbiota includes many eukaryotic organisms such as viruses, bacteria, archaea, and fungi, but is predominantly composed of bacteria, which have a significant effect on human health and diseases. The microbiome generally contains two essential bacterial phylotypes: *Bacteriodetes* and *Firmicutes*. In addition, other bacteria such as *Proteobacteria, Actinobacteria, Fusobacteria* and *Verrucomicrobia* are found in smaller rates. The microbiota of older people differs from that of younger people. These age-related changes in the microbiota have been associated with adverse health effects, and in particular, the number of probiotic bifidobacteria has been found to decrease with increasing age (Dinan and Cryan, 2015; Yılmaz and Altındiş, 2017). The intestinal microbiota has an important place in producing neurotransmitters and short-chain fatty acids. *Lactobacillus* and *Bifidobacterium* can produce 70% of the inhibitory neurotransmitter GABA, which contributes to the development of the nervous system and formation of synapse. The 90% of serotonin produced by the microbiota regulates cognitive functions, mood, sleep and appetite. In addition, species such as *Escherichia*, *Bacillus* and *Saccharomyces* produce dopamine and norepinephrine. Short-chain fatty acids produced in the intestine include butyrate, acetate, propionate, and lactic acid. Butyrate is noted for its health benefits and is produced by *Butyricicoccus* and *Clostridium* species, providing a source of energy and increasing ATP production (Li et al., 2018). The microbiota of adult individuals can be divided into permanent and transient microbiota. The permanent microbiota is usually unchanging and can regenerate rapidly; whereas, the transient microbiota consists of microorganisms that

are constantly changing and are usually harmless, but can also be pathogenic (Çetinbaş et al., 2017).

The microbiota that emerges in late pregnancy is the first microbiota to affect infancy and begins in the womb. The type of birth is known to affect the structure of the microbiota: vaginal delivery is dominated by the vaginal microbiota, and caesarean section is dominated by the maternal skin microbiota. Vaginally delivered babies have higher concentrations of *Bacteroides*, *Bifidobacterium* and *Lactobacillus* in the first days of their life and greater microbial change in the following weeks. Caesarean-born babies have been reported to have *Staphylococcus*, *Streptococcus,* and *Clostridium*, with less diversity, except for a microbiota similar to the mother's skin and the hospital setting (Coelho et al., 2021). The initial microbiota is a determinant for the individual in later periods of life and tends to return to a similar structure with age. Breast milk plays a critical role in shaping the microbiota of infants (Dinan and Cryan, 2015). From the prenatal period to old age, the intestinal microbiome and metabolites are involved in neurogenerative conditions such as formation of blood-brain barrier and neuroinflammation (Li et al., 2018). In newborns aged 4 weeks and older, Bifidobacteria were reported to be the most abundant species in both breastfed and formula-fed infants, and in most cases no significant numerical differences were found between breastfed and formula-fed infants (Guaraldi and Salvatori, 2012). In another study, Bezirtzoglou et al. (2011) reported that breastfed infants had higher bacterial cell counts than formula-fed infants.

Nutritional habits and diets, which are an important factor, can change the structure of the microbiota. The studies have reported that diets can change the intestinal microbiota in 3 weeks (Kalip and Nazlı, 2018). Some nutrients, especially diets containing substances such as carbazoles and tryptophan, prevent the formation of some diseases, while diets containing phosphatidylcholine and some fatty acids may increase the risk of disease (Çetinbaş et al., 2017). Imbalance in the intestine can increase the permeability of the intestinal barrier, which leads to the entry of pathogenic substances into the body (Kalip and Nazlı, 2018). Drugs such as antibiotics can cause disturbances in the microbiome (Çetinbaş et al., 2017).

Communication between the brain and intestine is established through the central nervous system, enteric nervous system, and hypothalamic pituitary adrenal axis. This interaction starts in the womb and intestinal microbiota has an important role in this relationship (Nazlıkul and Acarkan, 2014). The central nervous system (CNS) communicates with the enteric nervous system, intestinal muscles, and mucosa via afferent and efferent pathways. Through this communication, intestinal permeability, mucus secretion, motility and immunity are regulated (Pistollato et al., 2016). Gut-brain communication also occurs through the circulatory system. Neurotransmitters and short-chain fatty acids produced by the intestinal microbiota are transmitted to the brain by mixing into the blood (Özer et al., 2019). Immune system modulation, hypothalamic pituitary adrenal (HPA) axis, tryptophan metabolism and bacterial metabolite production can also be added to the mechanisms by which intestinal microbiota affect behavior (Kelly et al., 2016).

Effects of Probiotics, Prebiotics and Diets on Microbiota

Probiotics involve the direct delivery of live bacteria to the host in the form of artificial encapsulation or fermented foods (Green et al., 2020). A microorganism accepted as a probiotic should be non-pathogenic, tolerate gastric acid, be able to secrete antimicrobials, be able to attach to intestinal cells, adapt to the natural intestinal microbiota, and have a positive effect on the health of the individual (Taşdemir, 2017). Probiotics strengthen immunity by interacting with the immune system, support the intestinal barrier, prevent the proliferation of pathogens, and have protective and therapeutic effects in allergic diseases (Çetinbaş et al., 2017). Probiotics can directly affect the gastrointestinal tract. By interacting with the intestinal mucus and epithelium, they can modulate the intestinal barrier and mucosal immune system and affect the systemic immune system and organs such as the liver and brain (Gerritsen et al., 2011).

Prebiotics include nutrients that are utilized by microorganisms living in the intestine and promote their growth (Green et al., 2020). Some prebiotics are found naturally (e.g. in breast milk) (Orel and Trop, 2014), while others are added to foods. Examples of prebiotics include fructooligosaccharides (FOS), inulin, galacto-oligosaccharides (Orel and Trop, 2014; Özer et al., 2019) and soy oligosaccharides (Orel and Trop, 2014). Prebiotics and probiotics can inhibit the growth of potentially pathogenic microorganisms by increasing the production of short-chain fatty acids and lowering colonic pH. These compounds have antimicrobial secretory properties and inhibit bacterial adhesion (Gerritsen et al., 2011; Orel and Trop, 2014).

Diet is effective on mental and physical health; in this sense, stress and obesity play an important role. The Mediterranean diet, for example, is known for its health benefits; it includes healthy ingredients such as vegetables, fruit, nuts, and olive oil, and limits red wine and saturated fats. This diet is associated with longer life and lower rates of cardiovascular disease. On the other hand, a high-fat diet can lead to obesity, anxiety, and cognitive dysfunction. Stress can lead to obesity by triggering overeating behavior, which is associated with changes in neurotransmitters, neuropeptides, and inflammatory factors (Bremner et al., 2020).

Conditions Causing Dysbiosis and Their Effects

The gastrointestinal system forms a complex intestinal barrier to limit the exposure of the host's immune system to the microbiota. This barrier has physical, biochemical, and immunological components. However, an imbalance in the microbiota can weaken the function of this barrier (Thursby and Juge, 2017). The intestinal microbiota has an important role in bidirectional interactions between the intestine and the nervous system and may interact with the CNS by affecting neuroendocrine

systems involved in stress response, anxiety, and memory function (Carabotti et al., 2015). It has been reported that individuals with autism are unable to digest casein and gluten, and this deficiency leads to the formation of peptides that produce opioid effects in the brain. It is also known that a casein- and gluten-free diet reduces the symptoms of autism. In addition, people with autism have more intestinal problems than healthy people, strengthening the possibility that autism may be associated with intestinal health (Doenyas, 2018). Intestinal disorders may also play a role in other neuropsychological disorders. Psychological stress can cause intestinal problems, and antidepressants can make this condition worse. Therefore, in some cases, probiotic supplementation may be a more effective treatment option than antidepressants (Nazlıkul and Acarkan, 2014). Studies in Alzheimer's disease have reported that the intestinal microbiota can produce neurotransmitters and neuromodulators. These neurotransmitters include compounds such as GABA, serotonin, and dopamine. GABA in particular plays an inhibitory role in the brain and when this signaling is disrupted, problems such as anxiety, depression and cognitive impairment can occur (Akbari et al., 2016).

Intestinal Microbiota in Veterinary Medicine

The dominant bacterial phyla in the human intestinal microbiota, such as *Firmicutes* and *Bacteroidetes,* have also been evaluated for suitability in various animal species (Nguyen et al., 2015). The dominant intestinal bacterial phyla in humans are *Firmicutes* and *Bacteroidetes.* Various animal species have also been evaluated for microbiota suitability (Turner, 2018). It has been reported that the microbiota of rats and mice are most like each other, but the microbiota profile of rats is more similar to that of humans than that of mice (Flemer et al., 2017).

Both mini-pigs and traditional pig models contain *Firmicutes* and *Bacteroidetes* species that are present in the human intestinal microbiota. They are also considered as useful models for human gastrointestinal health (Lamendella et al., 2011; Pedersen et al., 2013). The intestinal microbiome of non-human primates, such as macaques, shows significant differences when compared to mice and humans (McKenna et al., 2008).

Although the mouse intestinal microbiome shows similarities to the human intestinal microbiome, it has been reported that human-specific bacterial genera are missing from detailed analyses. While this situation leads to thoughts about how effectively mouse models reflect human intestinal health and diseases, it is also part of the information that the mouse is the commonly used model for general mechanisms of intestinal microbiome and microbiota transfers, as it is more appropriate to genetic manipulation (Turner, 2018). Garcia-Mazcorro et al. (2011) reported that probiotic and prebiotic intake in cats and dogs did not alter the bacterial phylum found in feces, while symbiotic supplementation led to an increase in probiotic bacteria in the feces of healthy cats and dogs.

Alzheimer's Disease

Alzheimer's disease is a progressive, irreversible neurodegenerative disorder that affects cognition, function, and behavior (Porsteinsson et al., 2021). Symptoms of the disease result from a progressive loss of cholinergic function due to neuronal cell death in the hippocampus and cerebral cortex, as well as other regions of the brain that regulate thought and memory processes (Ton et al., 2018). Alzheimer's disease is a progressive condition that can be treated with medication and exercise, but it cannot be stopped completely, only slowed down. Between 2012 and 2017, the prevalence rate of Alzheimer's disease in the elderly population in Turkey increased by 17.1%. In addition to mental and behavioral differences and memory-related symptoms, sleep problems, dependence on daily tasks and depression are observed in people with Alzheimer's disease (Keleş and Özalevli, 2018). Alzheimer's disease is known to progress over a long period of time, usually such as 15-25 years. During this time, patients may not manifest any symptoms, including mild cognitive impairment, until the symptoms of dementia are clear. The onset of dementia may be a consequence of the long persistence of pathology of Alzheimer's disease. However, it is known that this process is not the same for every patient (Scheltens et al., 2021). The main pathological manifestations of Alzheimer's disease are amyloid plaques, tau protein and loss of neurons and synapses (Li et al., 2018). The ApoE gene used as a marker is recognized by three different allelic polymorphisms: ApoE2 is considered as protective, ApoE3 as neutral and ApoE4 as high risk (Ton et al., 2018). Amyloid β, the culprit in Alzheimer's disease, also normally forms in healthy individuals, due to proteolysis of APP. The disruption of the healthy system is the beginning of the pathology of Alzheimer's disease. The enzymes α-secretase, β-secretase and γ-secretase are involved in this process; α-secretase and β-secretase cleave APP at different sites to form α-APP and β-APP, respectively. Then, cleavage by γ-secretase produces p3 and Aβ fragments. This normal Aβ formation is a pathological precursor of the Aβ plaques that are the onset of Alzheimer's disease. The tau protein is involved in the organization of microtubules and is a gene located on chromosome 17. Hyperphosphorylation of tau causes the pathology of Alzheimer's disease. Hyperphosphorylated tau cannot bind to microtubules and forms structures that are the main pathological features of Alzheimer's disease (Saka, 2010).

Alzheimer's Disease and Intestinal Microbiota

Alzheimer's disease is a complex neurodegenerative disorder and the most common form of dementia. Its main pathological symptoms are amyloid plaques and neurofibrils. It is also recognized that the disease may be associated with intestinal dysbiosis. This theory is strengthened by the connection of the intestinal microbiota with the brain via the vagus nerve. The vagus nerve is an important interface between the intestine and the central nervous system. It connects to nuclei in the brainstem and exchanges signals via afferent (sensory) and efferent (motor) fibers. This allows the brainstem to control in-

testinal functions and communicate with other brain regions (thalamus, cortical areas, etc.). This multidirectional communication between the intestinal microbiota and the central nervous system occurs through many different mechanisms and pathways (Angelucci et al., 2019). Microbial amyloids fulfil many functions in the gut. For example, some bacteria, such as *Escherichia coli,* can increase the formation of amyloid protein fibrils, which has been accepted to be effective in Alzheimer's disease (Pistollato et al., 2016). Neuroinflammation is a long-term inflammatory process associated with some neurodegenerative diseases, including AD. Neuroinflammation describes the inflammation of neurons and the secretion of substances that trigger the immune response. The microbiota influences neuroinflammation, contributing to the modulation of microglia and astrocytes (Li et al., 2018). Microglial activation is considered to be an important factor in the pathogenesis of Alzheimer's disease. It can be activated in response to the accumulation of amyloid beta (Angelucci et al., 2019). Increased intestinal permeability caused by altered intestinal microbiome leads to the leakage of harmful bacterial metabolites into the bloodstream. These metabolites interact with the brain and influence signaling that contributes to Alzheimer's pathogenesis and neurodegeneration. Furthermore, the intestinal microbiota can enhance the inflammatory response that triggers amyloid beta deposition (Ton et al., 2018). Escherichia coli in the microbiome is the source of curli protein, a bacterial amyloid (Kowalski and Mulak, 2019). Chen et al. (2016) reported increased neuronal alpha-synuclein accumulation, increased microgliosis and astrogliosis, and increased expression of TLR2, IL-6 and TNF, which play a role in neuroinflammation, in rats exposed to *Escherichia coli* producing curli protein. High levels of bacterial lipopolysaccharides have been observed in the brains of patients with Alzheimer's diseases, particularly in the hippocampal and temporal lobe regions. In addition, in blood analyses in patients with amyloid deposition in the brain and cognitive impairment, the number of proinflammatory *Escherichia/Shigella* increased, anti-inflammatory *Escherichia rectale* decreased and proinflammatory cytokine levels increased (Angelucci et al., 2019). It has been reported that the number of Firmicutes and Actinobacteria decreased in the microbiota of patients of with Alzheimer's disease, the number of Bacteroidetes increased, and Ruminococcaceae, Turicibacteraceae, Clostridiaceae and Clostridium sensu stricto families in Firmicutes were at lower levels (Li et al., 2018). Based on all this information, impaired intestinal barrier in patients with Alzheimer's disease has led to new approaches to the formation and treatment of the disease. However, it is still a matter of curiosity whether the impaired intestinal microbiota is the culprit or consequence of the disease.

While antibiotics are used to treat bacterial infections, they can also have a negative effect on the intestinal microbiota. This effect varies depending on whether or not the antibiotic is broad-band spectrum or narrow-band spectrum. Because broad-spectrum antibiotics are effective against many types of bacteria, they can also reduce the number of beneficial bacteria in the gut. The use of such antibiotics can reduce the diversity of the microbiota, and this imbalance persists for a long time after use. While this imbalance can be corrected over time with short-term use of antibiotics, long-term and frequent use of antibiotics can lead to permanent imbalances. Antibiotic use may adversely affect the course of Alzheimer's disease by increasing neuroinflammatory responses. Antibiotics thought to trigger Alzheimer's disease include streptozotocin and ampicillin. In addition to causing an imbalance in the intestinal microbiota, streptozotocin has been used to induce cognitive impairment that mimics Alzheimer's disease in animal models (Angelucci et al., 2019).

It has been noted that diet has an important role to play in the treatment and prevention of Alzheimer's disease. Tully et al. (2003) found that levels of docosahexaenoic acid, an omega-3 fatty acid important for brain function, were low in the serum of patients with Alzheimer's disease. In the "Cardiovascular Risk Factors, Ageing and Dementia" study conducted at the University of Eastern Finland, 1409 randomly selected people aged 65-79 were followed for an average of 21 years. They were examined under 3 groups: 0-2 cups (low), 3-5 cups (medium) and >5 cups (high) daily coffee drinkers. It was reported that the risk of dementia was lower at the rate of 65-70% and the risk of Alzheimer's disease was lower at the rate of 62-64% in moderate coffee drinkers compared to low coffee drinkers (Eskelinen et al., 2009). In the Chicago Health and Aging Project, it was reported that 815 people aged 65 years and older without a history of heart attack, stroke or diabetes were followed for 6 years and there was a positive correlation between saturated and trans-fat intake and the risk of developing Alzheimer's disease (Morris et al., 2004). These findings suggest that saturated and trans fats may increase the risk of Alzheimer's disease (Barnard et al., 2014). In particular, diets containing antioxidant compounds may reduce the risk of Alzheimer's disease, while high-fat diets may increase the risk. It has been found that people who eat a low-fat diet have a lower risk of Alzheimer's disease (Ton et al., 2018). The study on rats reported that amyloid beta-induced learning difficulties could be treated with probiotics. Probiotic supplements containing Lactobacillus and Bifidobacterium were found to have positive effects on learning and memory. Although probiotics are known to be effective in the treatment of Alzheimer's disease, antibiotic treatment remains the preferred treatment for many patients due to its low cost and prevalence (Rezaeiasl et al., 2019).

Conclusion

Consequently, it is understood that the intestinal microbiota is closely related to the body's health and disease states. Healthy lifestyles support eubiosis, while negative factors lead to dysbiosis. Dysbiosis can be the cause of many diseases, and Alzheimer's in particular. Evidence has been found that Alzheimer's disease may be associated with the intestinal microbiota, with reduced neurotransmitter production in the intestine and microbial amyloid production. This association has led to the idea that probiotics may have a potential role in the treat-

ment of Alzheimer's disease. Due to the negative effects of antibiotics on the microbiota, probiotic-based therapies are attracting increasing attention. Interest in this area is growing by the day. It is thought that probiotic supplements may reduce inflammation, strengthen the intestinal barrier and even have neuroprotective effects. However, further studies are needed to understand exactly the functionality of these effects and their clinical effectiveness.

Conflict of Interest

The authors declare no conflict of interests.

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Animal Health Prod and Hyg (2023) 12 (2) : 63 - 68

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Case Report

Successful Treatment with Dexamethasone of a Cat with Suspected Feline Atopic Skin Syndrome Not Responding to Prednisolone

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ABSTRACT

A 6-year-old client-owned indoor, female neutered Persian-exotic shorthair cat was presented for further investigation of severe itchy lesions on the head and neck to our polyclinic. The patient had previously been applied selamectin and a cleansing, soothing, and protecting shampoo, but did not respond positively to the treatment. After the exclusion of ectoparasite infestation, retroviral infection, neoplastic and psychogenic causes, and food allergy, a suspect diagnosis of FASS in the cat was made. Although skin lesions clearly improved, the intensity of pruritus did not reduce during the 4-week prednisolone therapy. Based on these results, the case was considered to be resistant to prednisolone. Treatment with dexamethasone provided a fast and complete recovery of clinical signs and the cat has been clinically healthy maintained for over 2-years. This study suggests that oral dexamethasone can be a good alternative for treating cats with suspected FASS that has not responded positively to prednisolone therapy.

Keywords: *FASS, therapy, prednisolone, resistance, dexamethasone.*

Prednizolona Yanıt Vermeyen Atopik Deri Sendromu Şüpheli Bir Kedinin Deksametazon ile Başarılı Tedavisi

ÖZET

Altı yaşlı, ev ortamında barındırılan, kısırlaştırılmış dişi İran egzotik ırkı kedi kafa ve boyunda şiddetli kaşıntılı deri lezyonlarının ileri değerlendirmesi için kliniğimize getirildi. Bu hastada önceki salemektin ve temizleyici, yatıştırıcı ve koruyucu içeren bir şampuan uygulamasından olumlu sonuç alınmadığı bildirildi. Ektoparazit enfestasyonu, retroviral enfeksiyon, neoplastik ve psikojenik nedenler ile gıda alerjisi dışlandıktan sonra kediye FASS şüpheli tanısı konuldu. Dört haftalık prednizolon tedavisi sırasında deri lezyonlarında belirgin bir düzelme olmasına karşın kaşıntının şiddeti azalmadı. Bu sonuçlara göre olgunun prednizolona dirençli olduğu düşünüldü. Deksametazon tedavisi, klinik belirtilerin hızlı ve tam olarak iyileşmesini sağladı ve kedi, iki yılı aşkın bir süredir klinik olarak sağlıklı bir şekilde korunuyor. Bu çalışma, oral deksametazonun, FASS şüphesi olan ve prednizolon tedavisine olumlu yanıt vermeyen kedileri tedavi etmek için iyi bir alternatif olabileceğini düşündürmektedir.

Anahtar Kelimeler*: FASS, tedavi, prednizolon, direnç, deksametazon.*

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Introduction

Feline Atopic Skin Syndrome (FASS), previously defined as feline atopy or "non-flea, non-food hypersensitivity dermatitis (NFNFHS)," is an inflammatory and pruritic skin syndrome manifested by a series of reaction patterns associated with IgE antibodies against environmental allergens (Favrot et al., 2012; Halliwell et al.,2021a; Halliwell et al., 2021b; Santoro et al., 2021). These reaction patterns include miliary dermatitis, self-induced alopecia/hypotrichosis, eosinophilic granuloma complex, and/or excoriation-ulcers in the head and neck (Santoro et al., 2021).

The syndrome is one of the most skin diseases among cats with a prevalence of 20% (Hobi et al., 2011), and its management can be challenging (Scott et al., 2001; Favrot et al., 2012; Mueller et al., 2021; Santoro et al.,2021). In comparison with dogs and humans, intradermal skin tests and serological allergy tests used for the diagnosis of FASS in cats are currently not sufficient due to the undefinition of the types and concentrations of allergens (Miller et al.,2013; Santoro et al.,2021). Allergy tests only support the clinical diagnosis and guide for Allergen Specific Immunotherapy (ASIT) treatment. Therefore, the definitive diagnosis of FASS is based mainly on history, clinical signs, and the exclusion of appropriate differential diagnoses for each case (Miller et al.,2013; Santoro et al.,2021). Treatment of FASS consists of managing pruritus while identifying and addressing aetiological factors. A patient-specific "Multimodal Therapy" is often planned and the severity of the syndrome, the seasonality, the owner and the patient's compliance with the treatment, the cost, and potential side effects should be considered (Mueller et al., 2021). In this context, there are therapy options such as systemic and/or topical glucocorticoids, cyclosporine, oclacitinib, antihistamines, maropitant citrate, essential fatty acids, and palmitoylethanolamide (Mueller et al., 2021).

This report highlights the potential usefulness of oral dexamethasone in a cat suspected FASS resistant to prednisolone therapy.

Case

A 6-year-old, client-owned indoor, vaccinated female neutered Persian-exotic shorthair cat was presented for further investigation of severe itchy lesions on the head and neck. The cat was eating a tuna-based dry food for sterilized cats. No other cat/animal was living in the house. Selamectin topical solution (Stronghold; Zoetis) was applied to the cat every two months for primarily ectoparasite control. According to the owner, severe pruritic lesions, which started with only redness, itching, and hair loss around the ear/eyes and bilateral face area, then spread to the neck, were observed about two months ago. The cat has never experienced any dermatological problems previously. The complaints reported occurred for the first time and did not respond to selamectin (Stronghold; Zoetis) and a cleansing, soothing, and protecting shampoo (Allermyl; Virbac) application in the referred clinic. On physical examination, the patient was awake and sensitive to the environment, and bilateral ulcers and erosions, erythema, epithelial excoriation, and alopecia in the face and neck were observed (Figure 2 A-C). The itching score observed by the owner was noted as 9/10. The cat was normal except for the skin lesions. Additionally, complete blood count and routine serum biochemistry profile were within the reference ranges. Screening for both feline immunodeficiency virus (FIV) antibody and feline leukemia virus (FeLV) antigen with the rapid test kit (Anigen Rapid FIV Ab/FeLV Ag Assay; Bionote Inc) were negative.

Microscopic examination of multiple swab samples taken from the ear canals and superficial/deep scraping specimens taken from the lesions did not reveal any parasitic organisms. Cellophane tape samples taken from all lesions were also negative for ectoparasites (fleas or flea excreta, lice). Large numbers of *Malassezia* spp. were found in a cytological examination of the ear canal and skin samples stained with Giemsa Wright (Figure 1). In skin cytology, intracellular bacteria were identified in neutrophils. There was no evidence of thymoma following the chest radiograph, and no abnormalities were detected on the abdominal ultrasound.

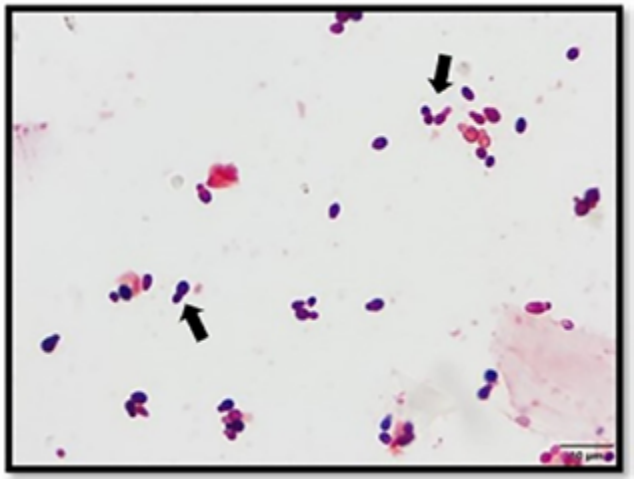

Figure 1: *Malassezia* spp. identified in sterile swab samples taken from the ear canal (arrows).

Although an ectoparasite was not observed in all skin scraping samples, an imidacloprid and moxidectin combination (Advantage Multi; Bayer) was recommended to be applied two weeks apart due to the risk of false negative results and not to ignore the flea allergy dermatitis.

A diagnosis of concurrent bacterial and yeast pyoderma was made based on the clinical and laboratory findings. Cephalexin monohydrate (Maksipor oral suspension; Actavis) at a dose of 22 mg/kg twice a day for 15 days; Saniotic (Richterpharma) ear drop containing miconazole nitrate, polymyxin B, prednisolone acetate, two drops in both ears two times a day for ten days, and Specialist shampoo (Vet Expert) containing ketoconazole and chlorhexidine once a week for a total of 2 applications were

Figure 2: Bilateral erosion and epithelial excoriation of the face (A, B) and alopecia with erosion and epithelial excoriation of the neck (C) at initial presentation. Self-induced alopecia in the right lateral neck (D). Significant improvement in lesions (E, F) after 15 days of treatment. Regrese of hot spots and completion of skin integrity after approximately one month with Elizabethan collar (G, H, I). General clinical appearance and complete resolution of lesions six months after initiation of oral dexamethasone therapy (J).

suggested. Considerable improvement in lesions on the head and neck (Figures 2D-F) was confirmed in the control examination after 15 days of the treatment, whereas no change in the level of itching was observed. The patient continued to have self-induced alopecia in the head and neck, and new lesions were also emerging. Control cytology revealed that the ears are clean and bacterial dermatitis on the skin has regressed noticeably. Oral application of cephalexin was continued for one week, and Elizabethan collar was advised to prevent the licking, chewing, or scratching of wounds. In the control examination, regression of skin lesions on the head and neck continued, but the pruritus in similar intensity lasted as soon as the Elizabethan collar was removed.

Upon this observation, both food allergy and/or atopy could be the cause of pruritis. An elimination diet containing a hydrolyzed protein source (Royal Canin Hypoallergenic for cats), which would last 8-12 weeks, was started. At the end of this trial, however, no regression in self-induced alopecia and no change in the pruritus score were recorded. In this way, food allergy was eliminated as the cause of itching, and the history, clinical findings, and diagnostic testing made the diagnosis of FASS most likely. Considering the severity of attacks and reaction patterns, methylprednisolone (Prednol tablet; Gensenta at dose of 1 mg/kg PO q12h) was administrated concurrently with essential fatty acids (Bio Pet Active Opti Biomega Omega 3-6; 500 mg/cat of Omega 6 fatty acids, and 250 mg/cat of Omega 3 fatty acids) and topical Sudocrem (Teva Pharmaceutical Industries) containing zinc oxide, sodium benzoate, and butylated hydroxy anisole until the lesions and pruritus reduce. The dose of methylprednisolone gradually was decreased and discontinued over a 4-week period. At the end of this period, the lesions on head and neck clearly improved, but self-scratching was still manageable with just an Elizabethan collar (Figures 2G-I). Based on this treatment result, we supposed that the syndrome is resistant to prednisolone treatment and oral dexamethasone at a daily dose of 1.5 mg/cat (approximately 0.4 mg/kg) initial treatment was started. The owner reported the complete disappearance of itch in the cat within three days of starting the treatment. After seven days, the dose was tapered to 1 mg/cat per day (approximately 0.3 mg/kg) for 7 days, to 0.75 mg/cat (approximately 0.2 mg/kg) daily for the next three months, and 0.5 mg/cat was continued for six months every other day as previously reported (Rzeszutek, 2020). The cat was clinically healthy (Figure 2J), and routine haematological and biochemical parameters analyzed six months after the beginning of dexamethasone therapy were within the reference ranges, except for stress leukogram and mild hyperglycaemia. No recurrence was observed in the two-year follow-up at six-month intervals. The cat's clinical views before and during treatment were presented in Figure 2.

Discussion

Diagnosis in patients with suspected FASS is based on history, clinical signs, and exclusion of appropriate differential diagnoses for each case. The primary dermatological problem of the cat presented here was pruritus; therefore, the most likely differential diagnoses included: fleas or flea allergy dermatitis, cheyletiellosis, demodicosis, dermatophytosis, neoplastic and psychogenic causes, food allergy, and atopy (Favrot et al., 2012; Favrot, 2013). Skin diseases associated with both two immunosuppressive feline viruses, FIV and FeLV, enclose bacterial skin infections, nonhealing wounds, exfoliative dermatitis, generalized pruritus, dermatophytosis, demodicosis, and others (de Mello et al., 2023). Because the cat in this study was FIV and FeLV negative, immunosuppression related to both feline viruses did not come in question. The possibility of parasitic infestations and flea allergy dermatitis was ruled out due to multiple negative skin scrapes and a lack of response to empirical treatment with selamectin for fleas and/or mites. Similarly, dermatophytosis can be eliminated based on negative results of impression smears. Chest radiography, abdominal ultrasound, and blood analysis helped also to rule out paraneoplastic cutaneous syndrome that may occur due to thymoma, pancreatic neoplasia, and/or gallbladder carcinoma (Pascal-Tenorio et al., 1997; Turek, 2003; Rottenberg et al., 2004). There were no environmental changes, such as construction, remodeling, moving, or introduction of a new pet or person into the household for the presumed cause of psychogenic pruritus in our case. Yeast and bacterial pyoderma are possible causes of pruritus, but they are most likely to be secondary to another underlying disease (Hobi et al., 2011; Santora et al., 2021). The topical and systemic therapy for *Malassezia* and bacterial pyoderma in the cat presented here resulted in a noticeably regression of skin lesions on head and neck, but pruritus lasted without change in its intensity. For this reason, concurrent yeast and bacterial pyoderma can most likely be evaluated as secondary to another underlying disease. There was also no regression in self-induced alopecia and the pruritus score during the elimination diet so food allergy as the cause of the complaints in our case was ruled out.

Based on all the results of the case mentioned above, FASS was suspected. The treatment of FASS can take a long time, and management can be complicated. While planning treatment and management, the severity of the syndrome, seasonality, patient and patient adherence to treatment, cost, and potential side effects should be assessed (Mueller et al., 2021). The treatment with systemic methylprednisolone as recommended first option for FASS has been started under consideration of these conditions. The treatment trial with methylprednisolone in our case did not provide full resolution of skin lesions, and self-scratching was still persistent. As previously reported in a case resistant to prednisolone by Rzeszutek (2020), drug resistance was considered due to the inability to obtain a satisfactory response. In this context, cyclosporine or oclacitinib could be primarily considered as an alternative treatment option (Mueller et al., 2021).

Nevertheless, an oclacitinib-containing drug is not available in our country, and cyclosporine drugs are expensive. Therefore, dexamethasone due to its easy availability and the lower cost was tried to treat presumed FASS in the cat. In accordance with a previous case report on resistant to prednisolone but responsive to dexamethasone treatment (Rzeszutek, 2020), a fast and long-time complete improvement without significant adverse effects and recurrence in a two-year observation period was achieved in our case. The response difference between methylprednisolone treatments versus dexamethasone for FASS in our case can firstly be explained by differences in glucocorticoid activity and biological half-life. Dexamethasone has a more potent glucocorticoid activity and a longer biological half-life (24-72 h) as compared to 12-36 h of methylprednisolone (Lowe et al., 2008). Therefore, dexamethasone can be expected to have a greater anti-inflammatory and immunosuppressive effect as compared to methylprednisolone for FASS treatment. Studies showed that dexamethasone may lead to more diabetogenic side effects than methylprednisolone in cats (Lowe et al., 2009). However, except for stress leukogram and mild hyperglycaemia, oral dexamethasone therapy with the above-mentioned protocol applied to the patient did not result in significant changes in clinical, haematological, and serum biochemical profiles, and none of the adverse effects warranted intervention. This condition can be explained by the fact that cats appear to tolerate glucocorticoids well (Lowe et al., 2008).

Conclusion

In conclusion, dexamethasone can be used successfully in FASS that does not respond to prednisolone therapy.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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ANIMAL HEALTH, PRODUCTION AND HYGIENE

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