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CONTENTS

Internal and external parasites prevalence of domestic cats in Konya province

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Abstract
Parasitic diseases caused by helminths, protozoa and ectoparasites endanger both animal and human health by causing clinical changes and transmitting potentially infectious pathogens. The aim of this study was to evaluate the parasitic diseases in domestic cats brought to Selçuk University Veterinary Faculty Animal Hospital (SUVFAH) between 2015 and 2021. In this context, a total of 828 samples, including 743 cat faeces and 85 cat skin samples sent to the laboratory of the Department of Parasitology were examined. In the study, 37.56% (311/828) of domestic cats were infected with at least one parasite species and single (43.67%), double (2.81%) and triple (0.27%) mixed internal parasite infections were determined. This study revealed that *Giardia duodenalis* (34.72%, 258/743), *Isospora* spp. (3.36%, 25/743), and *Entamoeba histolytica* (0.27%, 2/743) as the most common protozoan parasites, while *Toxocara* spp. (1.62%, 12/743), *Joyeuxiella* spp. (1.08%, 8/743), and *Dipylidium caninum* (0.27%, 2/743) were the predominant helminths among the sampled cats. *Otodectes cynotis*, the only ectoparasite species, was detected in 1.7% (2/85). Although the cats enrolled in the study were owned house cats, it was observed that the rates of internal and external parasites were high. Pet owners should take responsibility to prevent parasite infections/infestations in pets. Especially indoor cats with access to the street can be a source of many parasitic agents with zoonotic properties. Domestic cats should be regularly examined by a veterinarian and antiparasitic applications should be made regularly.

Keywords: *Cat, ectoparasites, helminths, protozoa, zoonoses*

INTRODUCTION

Cats are often seen as popular and loved pets due to their apparent ease of care and potential for domestication; however, despite these benefits, they can pose a danger to humans. Internal and external parasites pose a significant health risk to domestic cats, affecting their well-being and, in some cases, causing serious illness or even death. The risk of transmission of parasitic diseases among cats and from cats to humans might be increased by the possibility of occasional contact between domestic cats and stray cats, and between stray cats and wild cats (Karakuş & Denizhan, 2021). In particular, parasites such as *Ancylostoma* sp., *Echinococcus* sp., *Giardia* spp., *Toxocara canis* and *Toxocara cati*, are significant zoonotic agents causing human infections. Control of these parasites is therefore important not only for the health of cats and dogs, but also for public health and national economies. Understanding the prevalence of these parasites in specific regions is essential for developing effective prevention and control strategies. To date, 68 parasite species have been identified in cats in Türkiye, comprising 13 ectoparasites, 33 helminths, and 22 protozoan species. Parasites and parasitic diseases are a public health concern, as some have zoonotic potential (Barılı et al., 2023). Many studies have been carried out in different regions of Türkiye, mostly in large cities, highlighting the helminths harbored by carnivores including cats, their prevalences, and their role in animal and human health (Altaş &Taşan, 1999; Burgu et al.,1985; Dinçer et al., 1980; Doğanay, 1992; Durukan, 1995).

Konya Province, located in central Türkiye, is a region with diverse climatic conditions, ranging from semi-arid to temperate, which can influence the distribution and prevalence of parasitic infections in animals. Despite the importance of parasitic infections in domestic animals, there is a paucity of data regarding the internal and external parasites prevalence of domestic cats in this region.

The goal of the current study was to investigate the prevalence of internal and external parasites in domestic cats in the province of Konya. By identifying the most common parasites affecting cats in this area, the research aims to provide valuable information that could inform veterinary practice and public health policy, ultimately improving the health and welfare of both cats and the wider community.

MATERIALS AND METHODS

The study examined a total of 828 samples, including 743 cat faeces and 85 cat skin samples, sent to the Selcuk University Veterinary Faculty Parasitology Department Laboratory between 2015 and 2021. To determine gastrointestinal helminth fauna in owned cats, fecal samples were collected from 743 cats and analyzed using Native, Fulleborn flotation, and Benedek sedimentation methods (Umur et al., 2006). Initially, all fecal samples were macroscopically screened in terms of the presence of nematodes and proglottids of cestodes. 0.9% isotonic saline solution was used for the Native fecal examination method, saturated salt water for the flotation method, and distilled water for the Benedek sedimentation method. According to the literature, oocysts, cysts, and eggs were identified based on morphological characteristics (Umur et al., 2006; Zajac et al., 2021). Skin samples of 85 cats were collected in sterile petri dishes and sent to

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the laboratory for analysis. The debris is then placed on a microscope slide, coverslipped, and inspected with a 10× microscope objective. The material was put on a slide, then 10% potassium hydroxide was added in five drops. After placing a cover slip over the sample, it was examined under a microscope to check for the presence of mites, larvae, or ova (Zajac et al., 2021).

RESULTS

As a result of the study ecto and endoparasites were detected in 311 of 828 (37.56%) cats. During the study period, 37.56% (311/828) of the cats tested positive for at least one parasite, including single (43.67%), double (2.81%) and triple (0.27%) internal parasite infections. The prevalence of *Giardia duodenalis* 34.72% (258/743), *Isospora* spp. 3.36% (25/743), and *Entamoeba histolitica* was 0.27% (2/743); the prevalence of *Toxocara* spp. 1.62% (12/743) and cestodes such as *Joyeuxiella* spp. 1.08% (8/743) and *D. caninum* was determined to be 0.27% (2/743). *Otodectes cynotis* was detected at a rate of 1.7 % (2/85). The parasites detected in the study are shown in Table 1 and Table 2.

DISCUSSION

Cats are an essential part of social life, sharing the same environment and sometimes the same home with humans. They may pose a risk to the health of both humans and animals due to the parasites that they can carry (Bowman et al., 2002; Schnieder, 2006). Intestinal parasites harbored by cats include protozoa like *Giardia* spp., *Entamoeba histolytica, Isospora* (*I.*) *felis,* and *I. rivolta,* and some cestodes and nematodes such as *Dipylidium caninum, Joyeuxiella pasqualei*, and *Toxocara cati*. Some of the infected cats with these parasites in close contact with humans are of zoonotic significance (Bowman et al., 2002).

Giardia intestinalis is a common and important zoonotic protozoan parasite that causes diarrhea and infects the human and animal gastrointestinal tract**.** Human infection by this protozoan parasite can occur through consuming contaminated food or water, as well as by indirect or direct interaction with infected animals or people (Kar et al., 2015). *Giardia intestinalis*, which has highly variable host specificity, is a multi-complex species including eight (A-H) unique assemblages. The primary zoonotic genotypes Assemblages A and B are the most typically recognized human genotypes but can also be encountered in various hosts. Throughout the world, canine and feline hosts are typically infected with *Giardia* assemblages that have adapted to their respective hosts, of which assemblages C and D are prevalent in dogs and assemblage F in cats. However, some other *G. intestinalis* assemblages have also been reported, including Assemblages C, D, and E in cats, and assemblages A and B in both dogs and cats (Enemark et al., 2020). Feline giardiasis by *Giardia intestinalis* has been recorded in many countries between the rates of 3.6-11.1% (Enemark et al., 2020; Li et al., 2019; Paoletti et al., 2011; Sotiriadou et al., 2013; Tangtrongsup et al., 2020; Yang et al., 2015). *Giardia* spp. have been detected in cats in the range of 4-68.6% in studies based on faecal examination and molecular methods in Türkiye (Burgu et al., 1985; Önder et al., 2021; Sürsal et al., 2020). In our study infection rate in cats was found 34.72%; higher than the other countries and most of the studies conducted in Türkiye. A recent study revealed the molecular prevalence of *G. intestinalis* as 68.6% in diarrhoeic cats in the Central Anatolian region of Türkiye (Sürsal et al., 2020). The prevalence determined in this investigation (34.72%) was lower than the prior report. The prior study's diagnostic method may have been more specific, which could account for this. While the incidence and molecular characterization of *Giardia* spp. in felids have been studied internationally, there is an obvious need for more information on the existence of these protozoans in cats in Türkiye.

Table 1. *Single parasitic infection rates in cats between 2015-2021*

Helminths	(n:743)	Positive	Prevalence %
Nematod			
Toxocara spp.		12	1.62
Cestod			
Joyeuxiella spp.		8	1.08
Dipylidium caninum		2	0.27
TOTAL		**22**	**2.97**
Protozoans			
Giardia spp.		258	34.72
Isospora spp.		25	3.36
Entamoeba spp.		2	0.27
TOTAL		**285**	**38.35**
Ectoparasites	(n:85)		
Otodectes cynotis		2	1.7
TOTAL		**2**	**1.7**

Table 2. *Mixed parasitic infection rates in cats between 2015-2021*

Parasites	n:743	Positive	Prevalence %
Toxascaris spp.+ *Isospora* spp.		1	0.13
Toxocara spp.+ *Giardia* spp.		5	0.67
Isospora spp. + *Giardia* spp.		12	1.62
Giardia spp.+ *Dipylidium* spp.		1	0.13
Toxocara spp.+ *Toxascaris* spp		1	0.13
Giardia spp.+ Cestod egg.		1	0.13
Total dual infection		**21**	**2.81**
Isospora spp.+ *Toxocara* spp. + *Giardia* spp.		2	0.27
Total triple infection		**2**	**0.27**

Two *Isospora* species*, I. rivolta* and *I. felis*, cause infections in cats. Isosporosis, particularly common in young animals, is generally unnoticed even in mild infections, but when combined with other infections, the cat may show symptoms characterized by loss of appetite, anorexia, stagnation, diarrhea, and apathy (Gates & Nolan, 2009; Lappin, 2010; Schnieder, 2006; Tzannes et al., 2008). *Isospora* spp. prevalence of cats in Türkiye has been reported to be 2.8-65.9% (Burgu et al., 1985; Doğanay, 1992). Two studies conducted in Van and Kırıkkale reported that 43.28% (Karakuş & Denizhan, 2021) and 65.9% (Korkmaz et al., 2016) of *Isospora* spp. oocysts were found in cats. In this study, the prevalence of *Isospora* spp. in cats was determined as 3.36%, which is in line with most of the previous studies conducted in Türkiye. However, it was found to be much lower than in the Van and Kırıkkale studies. This may be due to the fact that in both studies the majority of the study material were shelter and street cats.

Toxocara cati is a parasite transmitted between cats through breast milk or by feeding on the parasite host (Lee et al., 2010; Schnieder, 2006). This parasite is recognized as one of the causative agents of human visceral larva migrans (Bowman et.al., 2002; Lee et al., 2010; Schnieder 2006). Although the parasite is widespread in many geographical regions (Beugnet et al., 2015; Villeneuve et al., 2014; Yang and Liang, 2015), the actual prevalence of *Toxocara* spp. larva migrans is unknown in Türkiye (Taylan Özkan, 2020). In Türkiye, *Toxocara* spp. eggs have been reported in cats at a rate of 3.0-62.5% (Ayaz et al., 2001; Doğanay, 1992; Gürler et al., 2015; Karakavuk et al., 2021; Korkmaz et al., 2016; Öge et al., 2014; Yaman et al., 2006). *Toxocara* spp. eggs were found in 1.62 % of the cats sampled in this study. The rate found was lower than other studies in Türkiye due to the policy of replacing sand pits in children's playgrounds with rubber and the fact that the animals studied were domestic cats.

Data concerning the *Entamoeba* spp. prevalence in cats and dogs is still sporadic and constricted (Shimada et al., 1992), and there are no studies on *Entamoeba histolitica* in cats in Türkiye. A study conducted in Spain reported an *Entamoeba* spp. prevalence of 0.4% in dogs and cats sampled (Gracenea et al., 2009), and another study conducted in Malaysia reported a prevalence of *Entamoeba* spp. of 12.4% in the same animal species (Ngui et al., 2014). In our study, the prevalence of *E. histolytica* in cats was 0.27%. Nonetheless, there appears to be little risk to public health from the zoonotic spread of *Entamoeba* species, particularly the harmful *E. histolytica*. Contrary to the notion that dogs and cats may pose a significant risk of transmitting *E. histolytica* to humans, the available evidence suggests this reverse zoonosis is unlikely to cause major environmental pollution (Eyles et al., 1954). Only the motile and fragile trophozoite, which is not infectious, is shed by infected dogs and cats. However, the infected humans shed extremely resistant and highly infectious cysts (Eyles et al., 1954).

Joyeuxiella pasqualei is a cestode that lives in the duodenum of cats and has a rostellum surrounded by spines on its scolex. There are some studies reporting the *J. pasqualei* prevalence in cats in various parts of the world (Calvete et al., 1998; Ngui et al., 2014; Waap et al., 2014). *Joyeuxiella pasqualei* has been reported in Türkiye between 4.2-50% (Burgu et al., 1985; Dinçer et al., 1980; Doğanay, 1992; Karakuş & Denizhan, 2014; Korkmaz et al., 2016; Öter et al., 2011; Palaz, 2008; Tüzer et al., 2010; Yaman et al., 2006). *Joyeuxiella pasqualei* eggs were detected in cat feces at a rate of 1.08 % in the present study. This rate is lower than the results of all other studies conducted in Türkiye. The reason for this is that the other studies conducted in Türkiye mostly focused on shelter and stray cats, whereas in our study only house cats were used. It may also be due to the decrease in contact between house cats living with humans and the intermediate host coprophagous insect as a result of the use of pesticides in houses.

Dipilidiosis is a cestode infection caused by the species *Dipylidium caninum*. Fleas and, less commonly, lice act as intermediate hosts and for an infection to occur, they must be consumed. It is a zoonotic disease, with the majority of human cases occurring in childrens, even though the disease mostly affects domestic and wild animals. Dipilidiosis in humans is an uncommon condition. *D. caninum* is thought to be the most frequent tapeworm infection in pets. (Rousseau et al., 2022). There are reports on the prevalence of *D. caninum* in different parts of the world. *D. caninum* has been found in 0.1% in Germany (Barutzki and Schaper, 2003), 49.5% in Iran (Zibaei et al., 2007), and 0.7% in shelter cats in the Netherlands (Robben et al., 2004), 4.8% in Malaysia (Ngui et al., 2014). In Türkiye, *D. caninum* has been reported in 0.21-65% (Altaş & Taşan, 1999; Burgu et al., 1985; Dinçer et al., 1980; Güralp, 1966; Karakavuk et al., 2021; Karakuş & Denizhan 2021; Palaz, 2008; Yaman et al., 2006). This study revealed that *D. caninum* prevalence was 0.27% in cats from Konya province. This prevalence is compatible with the results of studies conducted worldwide and in Türkiye.

Otodectes cynotis, a member of the Psoroptidae family, is the causative agent of external ear infections in cats, other carnivores (dogs, ferrets, foxes) and rarely in humans (Blot et al., 2003; Otrando et al., 2004; Shanks et al., 2000). Ear infections due to *O. cynotis* have been reported to be quite common in cats and this pathogen is responsible for 50-84% of cases of otitis externa in cats (Blot et al., 2003; Lefkaditis et al., 2007; Otrando et al., 2004; Roy et al., 2011). The disease is more common in young animals and is transmitted from animal to animal by contact (Blot et al., 2003; Otrando et al., 2004). Although many studies (Blot et al., 2003; Lefkaditis et al., 2007; Otrando et al., 2004; Roy et al., 2011) have been conducted worldwide on *O. cynotis* in cats, no prevalence study has been found in Türkiye. *O. cynotis* was detected at a rate of 1.7 % (2/85) in our study. The reason why this rate was detected in owned cats was thought to be the result of contact of owned cats with infected house or stray cats. It can be observed that further studies regarding O. cynotis in cats are required in Türkiye.

CONCLUSION

In conclusion, both internal and external parasites in cats, including fleas, ticks and worms, not only threaten cat health but also pose zoonotic risks to humans. Therefore, regular preventive treatment, good hygiene and routine veterinary check-ups are essential to protect both cats and their owners from these threats.

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Conflict of interest

The authors declare no conflict of interest.

Ethical statement

In our study, we did not examine live animals. We examined faeces and skin samples, which were sent to the laboratory for diagnostic purposes.

Author contributions

The initial draft, preparation, conceptualization, technique, and study were conceived and written by CC and MI. The tests were carried out by CC, OC, AE, ŞY, and DSY, who also edited and amended the manuscript. Each author accepted the submitted version of the paper and made contributions to it.

Availability of data and materials

All data generated or analyzed during the current study are included in this article. The data that support the findings of this study are available from the corresponding author, CC, upon reasonable request.

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Investigation of pollen analysis and antimicrobial effects of honey from Posof (Ardahan) district

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Abstract
This study aimed to determine the pollen composition and antimicrobial activity of honey samples from Posof District, Ardahan Province, located in the Eastern Anatolia region of Türkiye. A total of 29 honey samples were collected from various villages in Posof. As a result of melissopalynological analysis in these honey samples, 19 different plant families were identified. A total of 18 honey samples were identified as monofloral, while the remaining 11 were classified as multifloral. One sample was dominated by pollen from the Cistaceae family, while the dominant pollen in other monofloral honey samples belonged to the Fabaceae family. The most abundant pollen types in the honey samples were Fabaceae (40.5%), Rosaceae (11.7%), Lamiaceae (9.3%), Boraginaceae (7.5%), and Cistaceae (7.2%). Antimicrobial activity of the honey samples was tested at different concentrations (0.50%, 0.25%, 0.125%) against eight bacterial strains, including Gram-positive bacteria *Staphylococcus aureus* (ATCC 29213), *Staphylococcus aureus* (ATCC 25925), and *Enterococcus faecalis* (ATCC 29219), and Gram-negative bacteria *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (O157:H7 RSSK 09007), *Escherichia coli* (ATCC 25922), *Escherichia coli* (ATCC 35218), and *Pseudomonas aeruginosa* (ATCC 27853) using the disc diffusion method.

Keywords: *Antibacterial activity, honey, pollen analysis, Posof*

INTRODUCTION

Türkiye, with approximately 12,165 plant species, is one of the richest countries in the world in terms of flora diversity, owing to its varied ecosystems, geographical location, and climate diversity. This richness is a result of the country being at the intersection of three floristic regions, along with its diverse climatic conditions (Dülgeroğlu and Aksoy, 2018; Savci et al., 2018; Şenkul and Kaya, 2017). Türkiye's rich vegetation and geographical diversity have positioned it as a globally significant player in honey production. This richness enhances the pollen diversity in Turkish honey, thereby increasing its nutritional value (Apan et al., 2021).

Throughout history, honey has been used both as a food product and as a natural remedy. Its rich content of nutrients and bioactive components reinforces the positive health effects of honey (Alvarez-Suarez et al., 2009; Pranskuniene et al., 2022; Zubair and Aziz, 2015). Türkiye, with its vast flora diversity, holds a significant position in global honey production. The country's rich variety of plant species creates distinct differences in the pollen profiles of regionally produced honeys (Külekçi and Bulut, 2016; Tel et al., 2019). In this context, pollen analysis is a crucial tool for determining the origin of honey and ensuring quality control. Pollen analyses reveal not only the botanical and geographical origin of honey but also its purity and quality. The high pollen diversity in Turkish honey contributes to its unique aromatic and nutritional properties (Keskin et al., 2021; Mısır et al., 2020).

Honeys obtained from various regions of Türkiye exhibit diverse pollen profiles, and this diversity can also influence the antimicrobial effects of the honey (Keskin et al., 2020; Özkök and Bayram, 2021; Şenkul and Kaya, 2017). The pollen content of honey is a significant factor that affects its antimicrobial properties (Kösoğlu et al., 2019; Onbaşlı, 2019). Pollen derived from different plant species diversify the antimicrobial characteristics of honey, thereby enhancing its positive health effects (Kunat-Budzyńska et al., 2023). Particularly, honeys produced in regions with rich plant diversity, such as Türkiye, stand out not only for their nutritional value but also for their pollen diversity and antimicrobial effects (Mercan et al., 2007). The antimicrobial effects of honey are closely related to pollen diversity, as pollen from various plant species contain different bioactive components that influence the biological activity of the honey (Acar, 2021; Özkök and Bayram, 2021).

The Posof region, a district of Ardahan Province, is notable for its rich vegetation, diverse climatic conditions, and high altitude. In the floristic study of Posof, Damal, and Hanak districts of Ardahan Province, a total of 1,225 taxa belonging to 411 genera and 95 families were identified, with the highest numbers of taxa found in the families Asteraceae (190), Fabaceae (78), Lamiaceae (70) Rosaceae (70), and Caryophyllaceae (65) (Esen, 2010). The Caucasian honey bee subspecies (*Apis mellifera caucasica*) is commonly found in northeastern Türkiye, particularly in the Ardahan region and its surroundings, with Posof recognized as a gene center for this subspecies. Research conducted in the Ardahan region on the genetic diversity and adaptability of *Apis mellifera caucasica* reveals the contribution and importance of these bees to local ecosystems (Kambur and Kekeçoğlu, 2020). These characteristics make Posof honey comparable to other regional honeys in terms of both pollen diversity and antimicrobial activities. Particularly, pollen analyses of Posof honeys reveal the presence of pollen from endemic and rare plant species, highlighting their significant role in geographical indication and quality assessments (Şık et al., 2017).

Previous studies on Posof honeys have focused on pollen analysis, but this study is the first to determine both pollen composition and antimicrobial activity at a local

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level (Sorkun et al., 2014; Şık et al., 2017). In this study, the pollen profiles of honeys produced in the Posof region were examined, and the obtained data were compared with honeys from other regions of Türkiye. Additionally, the antimicrobial effects of these honeys were evaluated.

MATERIALS AND METHODS

The 29 honey samples used in this study were collected from local beekeepers in 22 villages of the Posof District in Ardahan Province during August and September 2020. The list of villages from which the samples were collected, along with the altitude and coordinates of these villages, is provided in Table 1. The samples were placed in 500-gram glass jars, with the region's name, the date of collection from the hive, and the producer's name recorded on each jar. All honey samples were stored at room temperature in a dry, dark room throughout the research period.

Palynological analysis

To determine the pollen diversity in 10 grams of honey, the samples were prepared using a standard method accepted and employed by international beekeeping institutes (Lieux, 1972; Louveaux et al., 1978; Maurizio and Hodges, 1951). Honey samples for pollen analysis were prepared as follows: Initially, crystallized or solidified honey samples were softened in a water bath at 40-45°C. The honey was homogenized using a sterile glass rod. A 10-gram portion of this homogenized honey was transferred to a test tube, and 20 ml of distilled water was added. The tubes were placed in a water bath at 45°C for 10-15 minutes to dissolve the honey. The solution was then centrifuged at 3500-4000 rpm for 45 minutes. After the supernatant was discarded, the tubes were inverted and allowed to dry. The pollen residues at the bottom of the tubes were transferred onto a microscope slide using a basic fuchsin glycerin-gelatin mixture, and the mixture was covered with a cover slip while still warm at 30-40°C. The preparations were left to dry, inverted for 12 hours. The place of honey collection and the sample number were written on the label, making the samples ready for microscopic examination. Pollen preparations were examined using an Olympus CX21 microscope with a 40X objective. Pollen grains were identified by scanning the 18x18 mm slide area. Relevant literature and the pollen preparation collection of the Department of Biology at Kafkas University were utilized during this process (Erdtman, 1969; Faegri and Iversen, 1989; Sorkun, 2008). For each honey sample, two tubes were prepared, with two preparations from each tube, resulting in a total of four pollen preparations. In each preparation, 200 pollen grains were counted using the 40X objective. The percentages of the counting results were calculated, and the pollen was classified according to the dominant (≥45%), secondary (16-44%), minor (3-15%), and trace (<3%) proportions found in Posof honeys (Barbattini et al., 1991; Warakomska and Jaroszynska, 1992). In the tables, dominant pollen are shown in red, secondary pollen in green, minor pollen in blue, and trace pollen in yellow (Table 2 and Table 3)

Determination of antimicrobial effect

For the antimicrobial efficacy test, strains of *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*, obtained from the Microbiology Division of the Department of Biology, Faculty of Arts and Sciences, Kafkas University, were used. The antimicrobial properties of the honey samples were tested using the disk diffusion method, which was modified from the method of Anand et al. (2019). The test bacteria were incubated overnight in Nutrient Broth and then homogenized using a vortex. Colonies were transferred to 3-5 ml of Mueller-Hinton Broth (MHB) and adjusted to the 0.5 McFarland standard. Bacterial suspensions were spread onto Mueller-Hinton Agar plates in 100 µl volumes and allowed to dry at room temperature for 10 minutes. Sterile 6 mm disks were impregnated with 15 µl of honey dilutions (0.5 ml/ml, 0.25 ml/ml, and 0.125 ml/ml) and placed onto the dried plates. DMSO was used as the negative control, and netilmicin, ofloxacin, and cefoperazone/sulbactam antibiotics were used as positive controls. The plates were incubated at 37°C for 24 hours. After incubation, the diameters of the inhibition zones were measured, and honey samples showing inhibition zones of 5 mm or greater were considered effective.

Table 1. *Coordinates and altitudes of the villages where honey samples were taken*

Village	Number of samples	Coordinate	Altitude (m)
Alabalık	2	41°25'17"N 42°37'01"E	2044
Balgözü	1	41°26'52"N 42°54'24"E	2044
Baykent	2	41°25'14"N 42°38'50"E	1820
Binbaşıeminbey	1	41°32'55"N 42°47'25"E	1532
Çambeli	1	41°29'33"N 42°47'02"E	1361
Çamyazı	1	41°28'34"N 42°44'24"E	2052
Derindere	1	41°26'11"N 42°55'54"E	2300
Gönülaçan	1	41°34'01"N 42°43'24"E	1788
Günbatan	2	41°28'20"N 42°36'38"E	2044
Günlüce	1	41°31'18"N 42°39'57"E	1802
Gürarmut	1	41°30'29"N 42°39'18"E	1596
Kaleönü	2	41°26'14"N 42°38'04"E	1788
Kolköy	2	41°26'39"N 42°36'44"E	1815
Kopuzlu	1	41°30'13"N 42°38'35"E	1570
Kumlukoz	1	41°34'12"N 42°47'10"E	1532
Kurşunçavuş	1	41°31'28"N 42°37'33"E	1788
Özbaşı	1	41°29'53"N 42°41'33"E	1532
Söğütlükaya	1	41°28'30"N 42°41'20"E	2044
Süngülü	1	41°28'22"N 42°52'13"E	1802
Taşkıran	2	41°33'43"N 42°45'39"E	1587
Türkgözü	1	41°34'49"N 42°49'18"E	1276
Yeniköy	2	41°28'40"N 42°49'10"E	1625

Table 2. *Distribution of pollen seen in monofloral honeys in Posof district (%)*

Family/Samples Number	3	10	11	12	14	17	18	19	20	27	29
Amaranthaceae					2						
Apiaceae		2		0.5	2	2	3		2	4	5.5
Asteraceae	3.5	9	1		8	10	4	1	3	6.5	4.5
Boraginaceae	12.5	8	6.5	31.5		9.5	8	1		8	4
Brassicaceae	1.5	1	1		2	2.5		0.5	9	3	3
Campanulaceae	1				2	3	3	1.5	2	2	
Caryophyllaceae						0.5					
Cistaceae	0.5	5	3.5	2.5	7	4.5	5	47	6	3.5	2.5
Dipsacaceae			1	1		2	2.5	0.5	4	2	1.5
Ericaceae					1			10		0.5	
Euphorbiaceae	1	2	1	2		0.5			2		
Fabaceae	70	53	65	46.5	56	45	45.5	29.5	61	46	58
Hypericaceae	0.5		1.5	1	1						
Lamiaceae	3.5	8	2.5	9.5	11	8	9	1.5	3	8.5	8.5
Onagraceae	1			1		1.5		0.5		1	
Pinaceae								0.5			
Plantaginaceae		1		0.5				1			
Poaceae	1.5	6				0.5	3.5				2
Poligonaceae		1				1	4		1	2	
Rosaceae	1.5	4	9.5	2	8	8	7.5	5.5	7	11	9.5
Salicaceae	1.5			0.5		1.5				1	1
Scrophulariaceae											
Solanaceae											
Urticaceae	0.5		7.5	1.5			5			1	

Statistical analysis

The antimicrobial effects of Posof honey samples were determined by repeating all measurements three times, and the zone diameters were presented as mean ± SD. Tukey's Multiple Comparison Test was used for the statistical evaluation of the results ($p < 0.05$). The IBM SPSS Statistics 20 statistical package program was used to perform statistical analyses. Different letters displayed on the columns indicate that the differences between the antimicrobial effect values of the honey samples are statistically significant.

RESULTS

Pollen analysis of the 29 honey samples was conducted, and the results are presented in Tables 2 and 3. According to microscopic analyses, pollen from a total of 19 families, including Apiaceae, Amaranthaceae, Asteraceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Cistaceae, Dipsacaceae, Ericaceae, Euphorbiaceae, Fabaceae, Hypericaceae, Lamiaceae, Onagraceae, Pinaceae, Plantaginaceae, Poaceae, Polygonaceae, Rosaceae, Salicaceae, Scrophulariaceae, Solanaceae, and Urticaceae, was identified in the honey samples collected from Posof. The samples with the lowest number of families (11 families) were Samples 11, 13, 14, 20, and 29, while the highest number of families (19 families) was found in Sample 2. Among the 29 samples, 11 were identified as monofloral (Samples 3, 10, 11, 12, 14, 17, 18, 19, 20, 27, and 29), and 18 were identified as multifloral. In Sample 18, pollen from the Cistaceae was predominant, whereas in the other monofloral honey samples, pollen from the Fabaceae was dominant (Tables 2 and 3). According to the data in the tables, pollen from various families is present in varying proportions in Posof honey samples. The distribution percentages of the families identified in Posof honey samples are shown in Figure 1. Upon evaluation, it was determined that the most abundant pollen in the analyzed honey samples belonged to Fabaceae (40.5%), Rosaceae (11.7%), Lamiaceae (9.3%), Boraginaceae (7.5%), and Cistaceae (7.2%). Pollen from Fabaceae, Rosaceae, and Lamiaceae was found in all samples, while pollen from Amaranthaceae, Solanaceae, and Scrophulariaceae was detected in only three samples (Figure 1).

The antimicrobial effects of the honey samples were determined using eight bacterial strains, including Gram-positive bacteria *S. aureus* (ATCC 29213), *S. aureus* (ATCC 25925), and *E. faecalis* (ATCC 29219), as well as Gram-negative bacteria *K. pneumoniae* (ATCC 700603), *E. coli* (O157:H7 RSKK 09007), *E. coli* (ATCC 25922), *E. coli* (ATCC 35218), and *P. aeruginosa* (ATCC 27853). The inhibition zone diameters (measured in millimeters) for different concentrations of honey (0.50%, 0.25%, 0.125%) were measured for each bacterial strain, and the results are presented in Tables 4 and 5.

The antimicrobial effects of the honey samples against Gram-positive bacteria are shown in Table 3. According to these results, antimicrobial effects were observed in honey samples 1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 14, 17, 18, 26, 27, 28, and 29 against *S aureus* (ATCC 29213), while no effect was observed in the other samples. The strongest effect was detected in honey sample 27. Against *S.*

Table 3. *Distribution of pollen seen in multifloral honeys in Posof district (%)*

Family/Samples Number	1	2	4	5	6	7	8	9	13	15	16	21	22	23	24	25	26	28
Amaranthaceae		1			0.5													
Apiaceae		4.5	7	2.5	4.5	1	6			1		2.5			3	4.5	1.5	1.5
Asteraceae	9	6.5	5.5	5.5	6	1	4.5	5	9.5		1.5	1	2	6	4.5	7.5	9.5	4.5
Boraginaceae	1.5	8	9	3.5	9.5	4.5	3.5	0.5	7	10	8	10.5	7	11	6	7	12	9
Brassicaceae		1	6.5	1	2		2.5	2	3	4		6			4	2.5	2.5	2
Campanulaceae	7.5	7.5	2.5	7	1.5		1	4.5		1	1	1	4	4.5	3.5	3	1	1.5
Caryophyllaceae		1.5	1							1								10
Cistaceae	2.5	0.5	0.5		13.5	18	14	7	6.5	2	8.5	3	14	1.5	10	10.5		9
Dipsacaceae	3.5	5	4	4.5	2		1	2	4				1	1	4	4.5	2.5	2
Ericaceae		0.5				0.5		0.5		1	1	0.5		5.5				
Euphorbiaceae	7.5	1	3	0.5	0.5	0.5		1	0.5	4	5	1.5	1		1.5			
Fabaceae	12	37.5	16.5	36.5	38.5	44	33	29	40	34	40	14.5	36	39	41	36	39	32.5
Hypericaceae	2.5					2.5				2	2	4	1		1			
Lamiaceae	1.5	11	4	33.5	9.5	3.5	8	31.5	7	12	10.5	3	14	14	5.5	5.5	15.5	6
Onagraceae	1	1	3.5	0.5	1	0.5	1.5								2.5	1	2	
Pinaceae			0.5		0.5											0.5		
Plantaginaceae		0.5													0.5	1		
Poaceae	0.5		0.5		0.5	1		1		1	7	1	1	2		1.5	1	1
Poligonaceae		0.5	3		1.5		0.5	2.5	1.5	4	2	0.5	4	0.5	1	0.5	2	1.5
Rosaceae	36	11.5	28.5	3	6	4.5	22	12.5	20	15	10	28.5	11	11.5	11	9.5	9.5	15.5
Salicaceae	10	0.5	4	2	1.5	5	2.5	1	1	4	1	20		2.5	0.5	2.5	1	4
Scrophulariaceae			0.5			0.5				1								
Solanaceae		0.5			1	1.5												
Urticaceae	5					11.5				3	2.5	2.5	4	1	0.5	2.5	1	

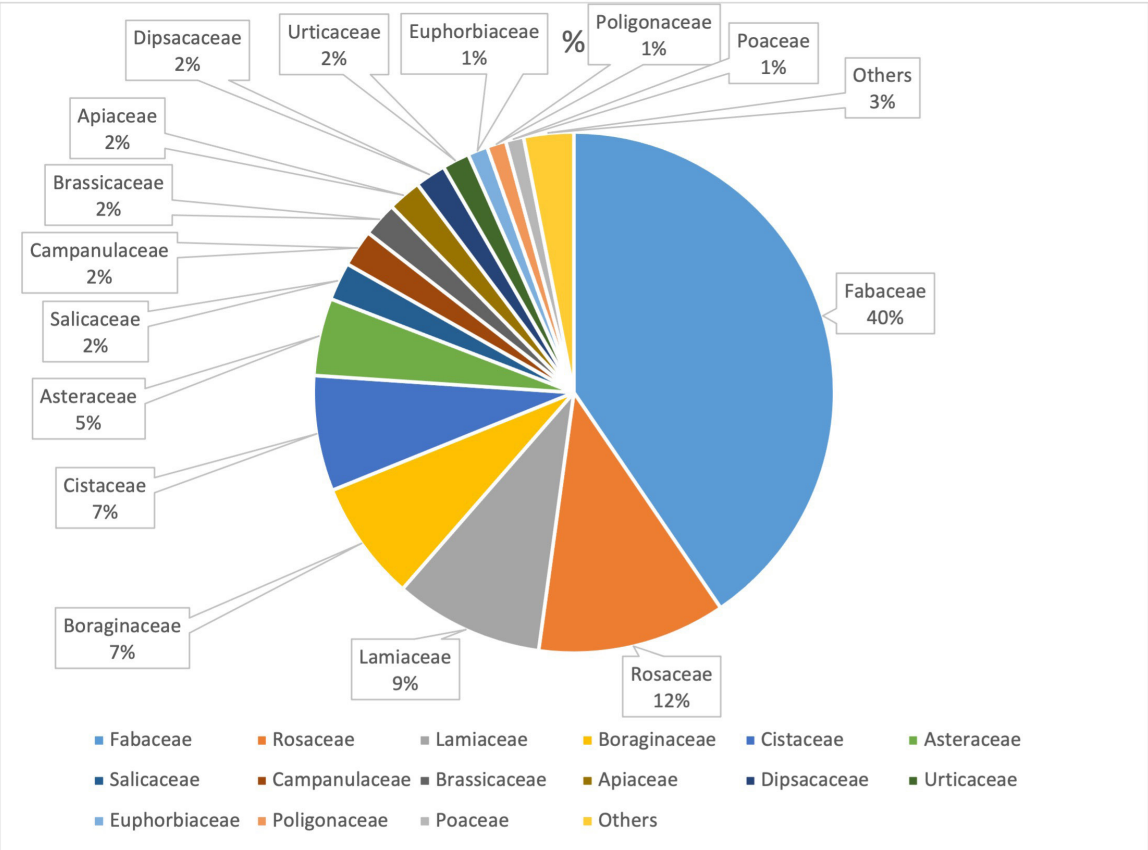

Figure 1. *Distribution percentages of plant taxa seen in Posof honeys (%)*

aureus (ATCC 25925), antimicrobial activity was observed only in honey samples 2, 27, and 28. For *E. faecalis* (ATCC 29219), antimicrobial effects were found in 10 honey samples (7, 8, 11, 13, 14, 22, 24, 25, 26, and 27), with the most significant effects observed in samples 14, 25, and 27 (Table 4).

The antimicrobial effects of the honey samples against Gram-negative bacteria are presented in Table 4. According to these results, antimicrobial effects were observed in honey samples 1, 2, 3, 12, 13, 14, 16, 17, 19, 20, 22, 23, 24, 25, 26, 27, and 28 against *K. pneumoniae* (ATCC 700603), while no effect was found in the other samples. The most effective result was seen in honey sample 17. Against *E. coli* (O157:H7 RSKK 09007), antimicrobial activity was observed in honey samples 1, 2, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 25, 26, and 27. For *E. coli* (ATCC 25922), only samples 6, 8, and 17 showed activity. Antimicrobial effects against *E. coli* (ATCC 35218) were observed in honey samples 1, 3, 8, 9, 10, 12, 16, 22, 24, 25, 26, and 27. Finally, against *P. aeruginosa* (ATCC 27853), honey samples 1, 2, 6, 7, 11, 13, 14, 15, 16, 17, 18, 20, 22, 24, 26, 27, 28, and 29 were found to be effective (Table 5).

The data in Tables 4 and 5 reveal that the antimicrobial efficacy of different honey samples against various bacteria varies depending on both the concentration and the honey sample. For *K. pneumoniae* (ATCC 700603), honey sample 17 exhibited the highest inhibition zone diameters across all concentrations. For *E. coli* (O157:H7 RSKK 09007), the highest antimicrobial activity at all concentrations was observed in honey sample 18. In the case of *E. coli* (ATCC 25922), the highest inhibition zone diameter was recorded at a 0.25% concentration, with sample 26 showing the strongest effect. For *E. coli* (ATCC 35218), sample 13 demonstrated the highest inhibition zone diameters, particularly at 0.25% and 0.125% concentrations. For *P. aeruginosa* (ATCC 27853), sample 18 stood out as the most effective honey sample, showing the highest inhibition zone diameters at all concentrations. These findings suggest that certain honey samples possess stronger antimicrobial properties against specific bacteria (Tables 4 and 5).

Table 4. *Antimicrobial effects of honey samples against gram positive bacteria*

	***S.aereus* (ATCC 29213)**			***S.aereus* (ATCC 25925)**			***E.faecalis* (ATCC 29219)**		
Samples Number	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**
1	8.27 ± 0.24^{cd}	6.70 ± 0.18^{bcd}	6.68 ± 0.16^{d}						
2	8.65 ± 0.13^{bc}	6.65 ± 0.13^{d}	6.65 ± 0.15^{d}	8.27 ± 0.24^{a}					
3	8.22 ± 0.20^{cd}	7.68 ± 0.16^{a}	6.62 ± 0.10^{d}						
4									
5	8.23 ± 0.23^{cd}	7.67 ± 0.21^{a}	6.75 ± 0.22^{cd}						
6									
7	9.20 ± 0.18^{ab}	7.67 ± 0.15^{a}	6.62 ± 0.13^{d}				9.27 ± 0.24^{a}	6.70 ± 0.18^{cd}	6.75 ± 0.22^{b}
8	9.23 ± 0.23^{ab}	7.23 ± 0.23^{ab}	6.70 ± 0.20^{cd}				7.70 ± 0.18^{cd}	6.68 ± 0.16^{cd}	6.75 ± 0.22^{b}
9	7.70 ± 0.18^{de}	7.20 ± 0.18^{abcd}	6.68 ± 0.16^{d}						
10	8.27 ± 0.24^{cd}	7.25 ± 0.23^{ab}	6.60 ± 0.13^{d}						
11	7.20 ± 0.18^{e}	6.67 ± 0.15^{cd}	6.75 ± 0.22^{cd}				8.67 ± 0.14^{ab}	7.70 ± 0.18^{a}	7.25 ± 0.23^{ab}
12	7.22 ± 0.23^{e}								
13							7.72 ± 0.20^{cd}	6.65 ± 0.13^{d}	6.72 ± 0.19^{b}
14	7.17 ± 0.18^{e}	6.70 ± 0.18^{bcd}	6.58 ± 0.10^{d}				8.27 ± 0.24^{bc}	7.70 ± 0.18^{a}	7.27 ± 0.24^{ab}
15									
16									
17	9.27 ± 0.24^{ab}	7.63 ± 0.13^{a}	6.68 ± 0.18^{d}						
18	8.70 ± 0.18^{abc}	7.22 ± 0.2^{abc}	7.20 ± 0.18^{bc}						
19									
20									
21									
22							7.25 ± 0.23^{d}	7.23 ± 0.23^{ab}	6.70 ± 0.18^{b}
23									
24							8.22 ± 0.2^{bc}	7.70 ± 0.18^{a}	7.23 ± 0.23^{ab}
25							8.20 ± 0.23^{bc}	7.72 ± 0.20^{a}	7.70 ± 0.18^{a}
26	7.70 ± 0.18^{de}	7.70 ± 0.18^{a}	7.70 ± 0.18^{ab}				7.20 ± 0.2^{d}	7.18 ± 0.16^{bc}	6.68 ± 0.18^{b}
27	9.28 ± 0.25^{a}	7.72 ± 0.23^{a}	7.72 ± 0.23^{a}	6.70 ± 0.18^{c}	6.70 ± 0.18^{a}	6.61 ± 0.72^{a}	8.22 ± 0.2^{bc}	7.68 ± 0.16^{ab}	7.75 ± 0.23^{a}
28	7.23 ± 0.23^{e}	7.20 ± 0.18^{abcd}	6.68 ± 0.18^{d}	7.70 ± 0.18^{b}	6.72 ± 0.19^{a}	6.69 ± 0.10^{a}			
29	9.23 ± 0.23^{ab}								

Table 5. Antimicrobial effects of honey samples against gram negative bacteria

	K.pneumoniae **(ATCC 00603)**			***E.coli*** **(O157:H7 RSKK 09007)**			***E.coli*** **(ATCC 2522)**			***E.coli*** **(ATCC 3521)**			***P.auriginosa*** **(ATCC 253)**		
Samples Number	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**
1	7.10±0.10^{ab}	6.62±0.1^{b}	6.52±0.76^{b}	7.12±0.13^{efg}	6.70±0.18^{e}	6.65±0.13^{d}	-	-	-	6.73±0.23^{c}	6.70±0.18^{c}	6.65±0.13^{c}	10.27±0.24^{b}	7.70±0.18^{de}	7.70±0.18^{c}
2	6.53±0.15^{c}	6.53±0.1^{b}	6.47±0.06^{b}	6.63±0.13^{g}	6.60±0.10^{e}	6.52±0.08^{d}	-	-	-	-	-	-	7.78±0.25^{fg}	-	-
3	6.62±0.13^{bc}	6.53±0.15^{b}	6.52±0.08^{b}	-	-	-		-	-	7.75±0.22^{ab}	7.70±0.18^{ab}	6.70±0.18^{c}	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	6.70±0.18^{b}	6.70±0.18^{b}	6.62±0.06^{a}	-	-	-	7.28±0.25^{gh}	6.70±0.18^{fg}	6.68±0.18^{d}
7	-	-	-	-	-	-	-	-	-	-	-	-	6.77±0.24^{h}	6.77±0.24^{fg}	6.73±0.21^{d}
8	-	-	-	8.68±0.18^{b}	8.23±0.23^{b}	6.70±0.18^{d}	6.72±0.2^{b}	6.68±0.16^{b}	6.67±0.88^{a}	6.77±0.24^{c}	6.73±0.21^{c}	-	-	-	-
9	-	-	-	6.72±0.19^{fg}	6.67±0.14^{e}	6.65±0.13^{d}	-	-	-	6.75±0.22^{c}	6.72±0.19^{c}	6.68±0.16^{c}	-	-	-
10	-	-	-	8.05±0.13^{cd}	7.70±0.18^{cd}	7.20±0.18^{c}	-	-	-	6.75±0.22^{c}	6.70±0.17^{c}	6.68±0.16^{c}	-	-	-
11	-	-	-	6.70±0.18^{g}	6.67±0.14^{e}	6.62±0.1^{d}	-	-	-	-	-	-	6.72±0.19^{h}	6.70±0.17^{fg}	6.67±0.14^{d}
12	7.13±0.15^{ab}	6.57±0.76^{b}	6.52±0.08^{b}	-	-	-	-	-	-	6.73±0.20^{c}	6.72±0.19^{c}	6.72±0.19^{bc}	-	-	-
13	7.05±0.18^{abc}	6.60±0.13^{b}	6.50±0.1^{b}	6.70±0.18^{g}	6.65±0.15^{e}	6.60±0.1^{d}	-	-	-	-	-	-	8.27±0.24^{ef}	7.30±0.26^{ef}	-
14	6.65±0.18^{bc}	6.57±0.16^{b}	6.52±0.13^{b}	8.23±0.23^{bc}	8.17±0.18^{bc}	7.27±0.24^{c}	-	-	-	-	-	-	8.73±0.23^{de}	6.73±0.2^{fg}	6.70±0.17^{d}
15	-	-	-	6.62±0.16^{g}	6.58±0.14^{e}	6.52±0.08^{d}	-	-	-	-	-	-	7.27±0.24^{gh}	6.73±0.2^{fg}	6.70±0.17^{d}
16	7.25±0.22^{a}	6.57±0.76^{b}	6.52±0.08^{b}	7.63±0.13^{de}	6.65±0.13^{e}	6.52±0.08^{d}	-	-	-	6.73±0.20^{c}	6.70±0.20^{c}	6.68±0.16^{c}	8.73±0.2^{de}	6.73±0.2^{fg}	6.67±0.14^{d}
17	7.23±0.23^{a}	7.13±0.13^{a}	7.07±0.06^{a}	6.65±0.18^{g}	6.60±0.13^{e}	6.55±0.09^{d}	9.33±0.29^{a}	7.27±0.28^{a}	6.70±0.10^{a}	-	-	-	9.77±0.24^{bc}	9.27±0.24^{b}	8.70±0.18^{b}
18	-	-	-	9.23±0.23^{a}	9.15±0.18^{a}	9.08±0.1^{a}	-	-	-	-	-	-	12.27±0.24^{a}	10.23±0.23^{a}	9.3±0.28^{a}
19	6.67±0.18^{bc}	6.57±0.08^{b}	-	8.53±0.15^{bc}	8.30±0.28^{b}	8.27±0.28^{b}	-	-	-	-	-	-	-	-	-
20	6.62±0.13^{bc}	-	-	6.63±0.15^{g}	6.58±0.1^{e}	6.55±0.05^{d}	-	-	-	-	-	-	9.27±0.24^{cd}	8.27±0.24^{cd}	7.72±0.19^{c}
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	6.68±0.18^{bc}	6.60±0.10^{b}	6.58±0.08^{b}	7.23±0.23^{ef}	7.23±0.23^{d}	6.63±0.19^{d}	-	-	-	6.68±0.16^{c}	6.63±0.12^{c}	6.60±0.1^{c}	6.67±0.15^{h}	6.62±0.1^{g}	6.58±0.08^{d}
23	6.77±0.24^{abc}	6.70±0.17^{b}	6.55±0.05^{b}	-	-	-	-	-	-	-	-	-	-	-	-
24	6.57±0.08^{c}	6.57±0.16^{b}	6.52±0.03^{b}	6.67±0.18^{g}	6.63±0.15^{e}	-	-	-	-	8.27±0.24^{a}	8.20±0.17^{a}	7.28±0.25^{a}	6.63^{h}±0.13	6.60±0.1^{g}	6.60±0.10^{d}
25	6.73±0.20^{abc}	6.65±0.13^{b}	6.58±0.08^{b}	7.57±0.16^{de}	7.55±0.13^{d}	7.30±0.28^{c}	-	-	-	6.67±0.14^{c}	6.58±0.08^{c}	6.53±0.06^{c}	-	-	-
26	7.13±0.13^{ab}	6.52±0.03^{b}	6.47±0.03^{b}	6.67±0.18^{g}	6.65±0.15^{e}	6.63±0.13^{d}	-	-	-	7.27±0.24^{bc}	7.27±0.24^{b}	7.17±0.14^{ab}	8.30±0.26^{ef}	7.67±0.14^{de}	7.67±0.14^{c}
27	6.73±0.20^{abc}	6.70±0.17^{b}	6.65±0.15^{b}	7.55±0.13^{de}	6.68±0.20^{e}	6.67±0.18^{d}	-	-	-	6.70±0.18^{c}	6.63±0.12^{c}	6.60±0.09^{c}	6.72±0.19^{h}	6.68±0.16^{fg}	6.67±0.14^{d}
28	6.67±0.18^{bc}	6.65±0.13^{b}	6.53±0.06^{b}	-	-	-	-	-	-	-	-	-	6.73±0.21^{h}	6.73±0.21^{fg}	6.68±0.16^{d}
29	-	-	-	-	-	-	-	-	-	-	-	-	8.65±0.13^{de}	8.43±0.38^{c}	7.70±0.18^{c}

DISCUSSION

The primary purpose of bees visiting plant flowers is to collect nectar and pollen, which serve as essential food sources that fulfill their protein, vitamin, and mineral needs (Burgut et al., 2023; Cengiz, 2018; Genç and Dodoloğlu, 2017; Özbakir and Alişiroğlu, 2019). The pollen-collecting behavior of bees varies depending on factors such as the flowering periods of plants, nectar production, and pollen quantities. In Türkiye, the plant families most preferred by bees include Asteraceae, Lamiaceae, and Rosaceae (Cengiz, 2018; Öztürk and Görhan, 2021).

Pollen analyses provide significant insights into the pollen diversity of Posof honeys from Ardahan province. The analyses revealed that the most prevalent pollen types in these honeys are from the Fabaceae (40.5%), Rosaceae (11.7%), Lamiaceae (9.3%), Boraginaceae (7.5%), Cistaceae (7.2%), and Asteraceae (4.8%). Eleven honey samples were identified as monofloral honeys. The pollen content varies depending on the source of the collected pollen and nectar, and this variation is influenced by the plant flora, geographical features, and climatic conditions of the region (Anklam, 1998). Pollen diversity in honey and the flora of the region were generally similar, except for the Asteraceae family. Pollen belonging to the families Fabaceae, Rosaceae and Lamiaceae were observed in high amounts in honey samples. Also Among the plant families most preferred by bees for pollen, Fabaceae, Asteraceae, Brassicaceae, and Rosaceae are prominent. Fabaceae family, particularly the flowers of legumes, serves as a rich pollen source for bees, providing essential proteins, vitamins, and minerals necessary for their nutrition (Mısır et al., 2023). Plants that bloom in the spring months increase pollen collection activities among bees. During this period, plants from the Fabaceae and Asteraceae families are among the most frequently visited species by bees (Şimşek, 2023).

In the pollen analysis studies of honeys conducted in the Ardahan region by Sorkun et al. (2014) and Şık et al. (2017), various plant families were identified. Sorkun et al. (2014) detected pollen from Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Cistaceae, Dipsacaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Pinaceae, Poaceae, Polygonaceae, Rosaceae, and Salicaceae. Şık et al. (2017) identified pollen from Amaranthaceae, Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Fabaceae, Hypericaceae, Lamiaceae, Salicaceae, and Scrophulariaceae. These results show similarities with the plant families found in our study.

Türkiye is notable for its rich plant diversity, which is attributed to several key factors, including its geographical location, climate diversity, and phytogeographic characteristics. Positioned at the intersection of the Irano-Turanian, Mediterranean, and Euro-Siberian phytogeographic regions, Türkiye hosts a wide range of plant species with varying climatic and soil requirements (Karaköse et al., 2018; Savcı et al., 2018; Tel et al., 2019). This makes Türkiye a significant location globally in terms of plant diversity.

Regional similarities and differences in the plant species preferred by bees have been observed. For instance, in Şırnak honey, Fabaceae, Lamiaceae, and Rosaceae are secondary, while Boraginaceae is dominant (Gürbüz et al., 2019). In the Adapazarı districts of Hendek, Akyazı, and Kocaali, Asteraceae, Fabaceae, Lamiaceae, Rosaceae, and Cistaceae are prominently found (Erdoğan et al., 2008). In Sinop, Fabaceae is predominant (Özler, 2015). In Kars, which has a similar climate, the most common pollen types are from Fabaceae, Boraginaceae, and Asteraceae (Gençay et al., 2018). In Anzer honey, Fabaceae, Asteraceae, Boraginaceae, and Rosaceae are prominently present (Sorkun and Doğan, 1995). In Posof honey, while Fabaceae and Asteraceae pollen overlap with Anzer honey, Boraginaceae pollen is less prevalent in Posof. This variation reflects the diversity of regional vegetation and the different pollen compositions resulting from regional flora.

The antimicrobial effects of the honey samples examined in this study against Gram-positive and Gram-negative bacteria are consistent with other research on the antibacterial properties of honey. The literature indicates that the antibacterial effects of honey are attributed to several factors, including low pH, high sugar concentration, and hydrogen peroxide production through the glucose oxidase enzyme (Bhushanam et al., 2021; Saxena et al., 2010). In our study, specific honey samples were found to exhibit a stronger antibacterial effect, particularly against the Gram-positive bacteria *S. aureus* and *E. faecalis*.

The observation of the best antimicrobial effect against *S. aureus* (ATCC 29213) in honey sample 27 suggests that this sample may contain potentially more potent bioactive compounds. The literature indicates that honey is effective against Gram-positive bacteria, with hydrogen peroxide production playing a significant role in this effect (Almasaudi, 2021; Bhushanam et al., 2021). However, the antimicrobial activity observed only in honey samples 2, 27, and 28 against *S. aureus* (ATCC 25925) suggests that these samples may have different biochemical profiles.

In addition to honey samples effective against Gram-positive bacteria, significant antimicrobial effects have also been observed against Gram-negative bacteria. Specifically, the effectiveness of many honey samples against *K. pneumoniae* (ATCC 700603) and *E. coli* strains suggests that the phenolic compounds and defensins in these honeys may have the potential to disrupt the cell membranes of these bacteria (Oliveira et al., 2018; Stavropoulou et al., 2022). The honey sample 17, which demonstrated the best effect, is particularly effective against *K. pneumoniae*, highlighting its potential for clinical applications.

It is known that the antibacterial effect of honey is not limited to hydrogen peroxide production alone, but also involves various phytochemicals and antimicrobial peptides (Almasaudi, 2021; Kwakman et al., 2011). This study demonstrates that different honey samples exhibit varying levels of antibacterial activity against various bacterial strains, and this activity may be influenced by the botanical source, geographical origin, and the biologically active compounds present in the honey.

In conclusion other studies conducted throughout Türkiye also show parallels with our findings (Bayram et al., 2019; Güneş, 2021; Karagözoğlu and Kıran, 2022; Mercan et al., 2007; Yalazi and Zorba, 2020). Also the

findings of our study suggest that honey's antibacterial potential spans a wide spectrum, indicating its potential use as an alternative therapeutic agent against bacteria. Future research should involve a more detailed analysis of the chemical components of these honey samples and testing their clinical efficacy, which will help us better understand the medicinal potential of honey.

CONCLUSION

Twenty-nine honey samples were collected from local beekeepers during the 2020 honey harvest season in the Posof District of Ardahan Province. These samples underwent palynological and microbiological analyses. According to the pollen analysis results, 11 honey samples were identified as monofloral, while 18 were identified as multifloral. The predominant pollen types in the honey samples were from Fabaceae, Rosaceae, Lamiaceae, Boraginaceae, and Cistaceae. Considering the region's geographic structure and rich vegetation, it can be concluded that the honey produced in this area has a highly diverse botanical content. Posof honey is derived from various plant species, highlighting the richness of the region's flora in terms of honey plants. Additionally, the antimicrobial effects of the honey were assessed. To accurately identify the precise botanical sources of honey produced in Posof, further pollen analysis at the genus level with additional samples is required. Furthermore, beyond pollen analysis and antimicrobial activity, it is important to examine physical and chemical properties such as proline, water-insoluble solids, moisture (Brix), free acidity, electrical conductivity, pH, HMF, fructose, glucose, sucrose, and maltose, as these are critical in determining honey quality.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Statement

The authors declare that the approval of an ethics committee is not required for the scope of this study.

Author Contributions

The research idea, material acquisition, various laboratory processes, and result evaluation were carried out by SA and AGU.

Availability of data and materials

All data and materials of the study are available in contact with the corresponsible author.

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Serum IgG threshold values associated with increased risk of diseases in preweaned lambs

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Abstract
Serum IgG cut-off points associated with increased risk of septicemia, fatigue anorexi syndrome (FAS), diarrhea and pneumonia in preweaned lambs was investigated in this study. The study involved 347 Akkaraman crossbred lambs born on two farms in Kars, Turkey. Blood samples were collected 24±1 h after birth and serum IgG concentration was measured by ELISA assay and cut-off values for each disease were determined. Neonatal lambs with diarrhea, FAS and septicaemia had statistically significantly lower IgG concentrations compared to healthy lambs (P<0.05). Critical SIgGC-24 cut-off values for increased risk of diarrhea, FAS and septicaemia in neonatal lambs were <800, <1000 and <200 mg/dl. In post-neonatal period, SIgGC-24 (mg/dL) was lower in only lambs with pneumonia compared to healthy lambs (P<0.05). The risk of developing septicaemia (IgG<200 mg/dL vs IgG>200 mg/dL), diarrhoe (IgG<800 mg/dL vs IgG>800 mg/dL), FAS (IgG<1000 mg/dL vs IgG>1000 mg/dL) and pneumonia ((IgG<1000 mg/dL vs IgG>1000 mg/dL) was 203, 6, 18 and 12 times higher, respectively. A threshold vaule of IgG<998 mg/dL and IgG<193 mg/dL were determined for neonatal morbidity and mortality, respectively. An appropriate colostrum management may help to maintain the health of pre-weaning lambs, thereby improving the productivity and profitability of sheep farms..

Keywords: *Colostrum, IgG cut-offs, lamb health, passive immunity*

INTRODUCTION

The first three months of life (before weaning), which includes the neonatal period, is the most important period, when morbidity and mortality in lambs is highest, resulting in a loss of future production capacity, since the main objectives of lamb rearing are health, growth, and profitability (Dwyer et al., 2016; Gokce and Erdogan, 2009). Transfer of passive immunity to preweaning lambs via colostrum is an important predictor of lamb health and should be evaluated regularly (Aganbeg et al., 2021; Demis et al., 2020; Sawyer et al., 1977).

Despite significant advances in genetics, nutrition and management, high preweaning morbidity (20-50 %) and mortality (15-20%) remain a global problem affecting lamb welfare and farm productivity (Berge et al., 2016; Dwyer, 2008, Gokce et al., 2013b). Hypothermia, starvation, diarrhoea, septicaemia and pneumonia have been reported to be the most common diseases in pre-weaning lambs (Gokce and Erdogan, 2008; Gokce and Erdogan, 2009; Herndon et al., 2011). Many of these diseases are preventable and the primary predisposing factor is inadequate amount of good quality colostrum (Berge et al., 2016; Demis et al., 2020). Therefore, the first step in addressing these problems is to ensure adequate passive transfer of immunity as lambs are born agammaglobulinemic due to synepitheliochorial placentation (Gokce et al., 2013a; Gokce et al., 2013b; Massimini et al., 2006a). Colostrum is the main food that protects lambs against diseases and this is defined as passive transfer of colostral immunity (PTCI) (Campion et al., 2019). The peak concentration of IgG in the serum of newborn ruminants is reached at 24 hours after birth. For adequate immunity, colostrum should be given as soon as possible after birth, as the concentration of colostral IgG decreases by 3.3 mg/kg/hour after birth (Bond, 2020; Kessler et al., 2019).

Lambs receiving 30g/L of IgG in colostrum are considered to have adequate PTCI, and below this value is regarded as failure of passive transfer (FPT) (Alves et al., 2015). However there is no internationally accepted threshold value for IgG associated with PTCI or FPT. In neonatal calves, serum IgG levels below 1,000 mg/dL have been associated with an increased risk of morbidity and mortality but a cut-off point between hypogammaglobulinemia and normal serum IgG levels in neonatal lambs has not been universally accepted. Therefore, there is no single IgG cut-off point for FPT and research-based recommendations range from 6 to 16 IgG mg /mL in lambs (Britti et al., 2005).

FPT has been associated with several diseases in ruminants, including respiratory diseases, diarrhea, septicemia, and omphalophlebitis in the first few months of life (Andres et al., 2007; Gokce et al., 2013a; Herndon et al., 2011; Turquino et al., 2011). FPT has been associ-

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ated with an increased risk of morbidity and mortality in lambs (Dwyer et al., 2016; Kessler et al., 2019) but the postcolostral IgG threshold values in lambs that increases or predicts the risk of selected diseases such as fatigue-anorexia syndrome, suspected septicemia, pneumonia, and diarrhea is unknown. This may be because disease detection, health examination, and observation require longer periods of time. Studies on passive immunity in lambs are usually related to risk factors affecting colostrum quality (Alves et al., 2015; Campion et al., 2019; Castro et al., 2011). For a profitable lamb rearing, there needs to be a better understanding of the relationship between common early life diseases and IgG concentrations, particularly disease-related cut-off values so that appropriate measures can be taken to prevent losses in preweaned lambs.

The aim of this longitudinal study was to investigate the relationship between post-colostral [24th hour (±1)] IgG concentrations and selected diseases such as suspected septicemia, fatigue-anorexia syndrome, diarrhea and pneumonia in preweaning lambs. This will also identify SIgGC-24 cut-off values that increase the risk of diseases of concern.

MATERIALS and METHODS

The study was approved by the Kafkas University Institutional Ethics Committee for Animal Experimentation and Use (KAU HADYEK code:2008-23)

Animals

The details of the study design are described elsewhere (Gokce et al., 2014, Gokce et al., 2024). This was a longitudinal study that covered 301 ewes and 347 intensively reared Akkaraman cross lambs born to them on two neighbouring farms in Kars, Turkiye, with similar management practices and feeding regimes, agreed to take part in the study. Ewes were only dewormed and vaccinated against clostridial disease, and no drugs or other compounds were administered during pregnancy. Lambing took place under farmer observation in winter (December to February) or spring (March to May). Only lambs determined as healthy at 24 hours after birth were included in the study. Plastic ear tags were attached to both ears of the lambs shortly after birth and the lambs were then allowed to suckle their mothers naturally. Lambs and their mothers were kept in individual pens for seven days and then moved to groups. Lambs were allowed to suckle twice a day (in the morning and evening) and were fed hay only for three weeks after the first week of life and straw and commercial growth feed (Bayramoglu AS, Turkey) in addition to hay for three months. No vaccines, drugs or other compounds were administered to the lambs during the study period.

Sampling

Blood samples were collected from all healthy lambs at 24 ± 1 h after birth by jugular vein puncture into an 8.5-mL serum clot tube (BD Vacutainer, BD, Franklin Lakes, NJ). Serum was harvested by centrifugation at 4000 rpm for 30 minutes and stored at -20°C until analysed.

Serum IgG Assays

Serum IgG concentrations in lambs were measured directly using a commercially available ELISA kit (Bio-X Competitive ELISA Kit for Ovine blood serum IgG Assay-BIO K 350, Bio-X Diagnostics, Belgium). The assays were performed and the result were interpreted according to the manufacturer's instructions.

Clinical Examination

The health of each lamb was monitored daily during the neonatal period and every two days after the neonatal period until weaning. A routine clinical examination was used to describe each clinical entity. Lambs with one or more of the following signs on clinical examination: poor suckling reflex, anorexia, depression, lethargy, fever, nasal discharge, abnormal lung sounds and high respiratory and heart rates, coughing, diarrhea or watery faeces, at least two signs of dehydration (skin elasticity, sunken eyes etc) and died from causes other than trauma at necropsy were considered sick. Disease groups were classified as diarrhea (watery stools for more then 12 hours, dehydration requiring treatment, weakness, fever, sudden death, bloodstained stools), pneumonia (abnormal lung sounds, dyspnoea, labored breathing, nasal discharge, fever or no fever, anorexia, weakness), suspected septicemia (absent or weak suck reflex, impaired ability to stand or recumbency, full sclera, marked or severe hyperemia, cyanosis or anemia, mucosal or subscleral hemorhage/petechie or presence of hypopyon, prolonged capillary refill time or low urine output, and weakness or depression), fatigue-anorexia syndrome (difficulty in standing, anorexia, partial or complete loss of sucking reflex, depression, incoordination, staggering, pain and starvation at necropsy), and others or unknown according to the previously reported procedure (Gokce and Erdogan, 2009).

Statistical Analysis

Data were entered into a database (Microsoft Access). Data were first tested for normality using the Kolmogrov Smirnov test. Mean ± SE values for serum IgG concentrations were calculated, since the data show a normal distribution. The results of clinical examination were categorized according to the period of life as the neonatal (the first four weeks of life after birth) and postneonatal (the period from 5 to 12 weeks of life after birth). Lambs were categorized as healthy, sick, and dead based on the clinical examination. Independent samples T-test was used to compare SIgGC-24 and different categories of healthy status in both periods. Disease-associated IgG cut-off values were based on the formula: number of lambs diagnosed with a particular disease at that cut-off value/ (total number of lambs at particular threshold value - total number of lambs with other diseases). Chi-square for trend test was used to determine the critical SIgGC-24 cut-off associated with increased risk of diarrhea, pneumonia, FAS, and suspected septicemia. Receiver operating characteristic (ROC) curve analysis was used to find the optimal cut-off point associated with diseases so that to understand the relationships between sensitivity and specificity of measurements. In this study, the Area Under Curve (AUC), Positive Predictive Value (PPV), Negative Predictive Value (PPV), sensitivity, specificity, and diagnostic accuracy statistics were used to evaluate the diagnostic performance of the cut-off values obtained. The AUC can be considered as an index of discriminatory power of a test. An AUC value of 0.50 or less is considered as unsuccessful, 0.6-0.7 is adequate,

0.7-0.8 is good, 0.8-0.9 is very good and 0.9-1.0 is excellent (Simundic, 2009). A value of $p < 0.05$ was considered significant. In this study, IBM SPSS Statistics 26 (academic license) and R 4.4.1 with RStudio were used for statistical analyses and diagnostic test evaluations.

RESULTS

Health Status

A total of 347 lambs were evaluated in this study. The proportion of lambs that developed disease and died in the neonatal period was 17.3% (60/347) and 3.7% (13/347) respectively. Diseases diagnosed in lambs during the neonatal period were diarrhea (9.2%, 32/347), pneumonia (1.7%, 6/347), suspected septicemia (3.2%, 11/347) and fatigue-anorexia syndrome (FAS) (3.2%, 11/347). In lambs with FAS, mistmothering (n=7), starvation (n=2) and hypothermia (n=2) were observed. Nine of the neonatal deaths were due to suspected septicemia in the first week of life. The proportion of diseased and dead lambs during the period of 5-12 weeks were 32.4% (109/334) and 3.9% (13/334) respectively. The most common health problems in lambs in the post-neonatal period were diarhoea (18.6%, 62/334), pneumonia (7.5%, 25/334), suspected septicemia (1.2%, 4/334) and others/unknown causes (5.4%, 18/334) and the number of lambs that died during this period was 15. Of the 47 lambs that were neonatal patients, 26 became ill again in the post-neonatal period and 6 of these died.

The mean SIgGC-24 in all lambs studied was 2162±167 mg/dl. The distribution of SIgGC-24 in different diseases is shown in Table 1. Lambs with suspected septicaemia (354 mg/dL), diarrhea (1890 mg/dL) and fatigue-anorexia syndrome/FAS (1006mg/dL) had significantly lower SIgGC-24 than healthy (2338 mg/dL) lambs in the neonatal period ($P<0.001$, $P<0.05$ and $P<0.01$, respectively). In the postneonatal period, only lambs with pneumonia (1655 mg/dL) had significantly lower SIgGC-24 ($P<0.05$) than healthy lambs (2294 mg/dL) (Table 1). Mean serum IgG concentration was significantly lower in lambs that died in the neonatal period (n=13, 318±186.9 mg/dL) compared to healthy lambs (n=287, 2337±64.2 mg/dL) ($P<0.001$). However, no significant difference ($P>0.05$) was found between the healthy (n=225, 2409±74.2 mg/dL) and deceased lambs (n=16, 2311±326.8 mg/dL) in the postneonatal period.

It was found that an IgG level <800 mg/dL at 24 hours after birth in the neonatal period increased the risk of diarrhea in lambs by approximately six times. In addition, the risk of exposure to FAS (mismothering, starvation, hypoglycaemia) in the neonatal period was found to be approximately 18 times higher in lambs with a postcolostral IgG level <1000 mg/dl than in lambs with a higher level. A postcolostral IgG level <200 mg/dl was found to dramatically increase the risk of sepsis in newborn lambs by 203-fold (Tables 2 and 4). The appropriate cut-off points for the postcolostral (24 hours after birth) IgG in predicting postneonatal pneumonia appeared to be <1000 mg/dL. Lambs with low IgG levels were approximately 12 times more likely to develop postneonatal pneumonia than lambs with higher IgG concentrations (Tables 3 and 4).

Table 1: *Serum IgG concentration (mg/dL) in relation to diseases diagnosed in neonatal and postneonatal period.*

Clinical Diagnosis	Period			
	Neonatal (first 4 weeks after birth)		5-12 weeks after birth	
	SIgGC-24	Morbidity (%)	IgG	Morbidity (%)
Diarrhoea	1890 ±179 *	9.2% (32/347)	2055 ± 141	18.6% (62/334)
Suspected Septicaemia	354 ± 213***	3.2% (11/347)	1833 ± 564	1.2% (4/334)
Pneumonia	2031 ± 637	1.7% (6/347)	1655 ± 211*	7.5% (25/334)
FAS*	1006 ±156**	3.2% (11/347)	-	-
Other	-	-	2212 ± 203	5.4% (18/334)
Healthy (n)	2337 ± 64 (n=287)	-	2294 ± 77 (n=238)	-

FAS*=Fatigue-Anorexia Syndrome (Mismothering, hypotermia and starvation) * $P<0.05$, ** $P<0.01$, *** $P<0.001$ significantly different from healthy lambs.

Figure 1 shows the ROC curve for neonatal diarrhoea, FAS, suspected sepsis, and post-neonatal pneumonia. In addition to this figure, Table 5 provides descriptive information on confusion matrices, AUC, PPV, NPV, sensitivity, specificity, and diagnostic accuracy results based on threshold values.

For neonatal diarrhoea, the AUC value was 0.540, indicating that the model had low capability in distinguishing between diseased and healthy individuals. The Positive Predictive Value (PPV) was 81.3%, while the Negative Predictive Value (NPV) was 94.6%, demonstrating the model's effectiveness in identifying negative cases. The sensitivity was 18.7%, reflecting the low ability to correctly identify positive cases, and the specificity was 94.6%, showing strong performance in recognizing negative cases The overall diagnostic accuracy was 93.4%. In the confusion matrix, for <800 mg/dL, there were 6 true positives (correctly identified as Diarrhoea) and 17 false negatives (Diarrhoea cases incorrectly identified as non-Diarrhoea), while for >800 mg/dL, there were 26 false positives (non-diarrhoea cases incorrectly iden-tified as Diarrhoea) and 298 true negatives (correctly identified as non-diarrhoea) (Table 5).

The AUC value was 0.932, indicating high discrimination ability of the model for neonatal FAS. The PPV was

Table 2. *Morbidity and mortality due to various conditions associated with defined categories of serum IgG concentrations at 24 hours after birth in neonatal lambs*

IgG	Diarrhoea				Pneumonia				Suspected Septicemia				FAS*				Total			
	Morbidity		Mortality		Morbidity		Mortality		Morbidity		Mortality		Morbidity		Mortality		Morbidity		Mortality	
Cut-off (mg/dl)	n1/n2	%	n1/n2	%	n1/n2	%	n1/n2	%	n1/n2	%	n1/n2	%	n1/n2	%	n1/n2	%	n3/n4	%	n3/n4	%
<200	1/1	100	1/1	100	0/0	0	0/0	0	9/9	100	9/9	100	1/1	100	1/1	100	11/11	100	11/11	100
201-500	4/4	100	0/4	0	1/1	100	0/0	0	0/0	0	0/0	0	1/1	100	0/1	0	6/6	100	0/6	0,00
501-600	1/2	50	0/2	0	0/1	0	0/1	0	0/1	0	0/1	0	1/2	50	0/1	0	2/3	66,7	0/3	0,00
601-800	0/3	0	0/3	0	0/3	0	0/3	0	0/3	0	0/3	0	0/3	0	0/3	0	0/3	0,0	0/3	0,00
801-1000	0/11	0	0/11	0	0/11	0	0/11	0	0/11	0	0/11	0	3/14	21,4	0/14	0	3/14	21,4	0/14	0,00
1000-1500	4/60	6,7	0/60	0	3/59	5,1	1/59	1,7	1/57	1,8	0/63	0	3/59	5,1	0/59	0	11/67	16,4	1/67	1,49
1501-2000	9/85	10,6	0/85	0	0/76	0	0/76	0	0/76	0	0/76	0	2/78	2,6	0/78	0	11/87	12,6	0/87	0,00
2001-2500	4/41	9,8	0/41	0	0/37	0	0/37	0	1/38	2,6	1/38	2,6	0/37	0	0/37	0	5/42	11,9	1/42	2,38
>2501	9/112	8	0/112	0	2/105	1,9	0/105	0	0/103	0	0/103	0	0/103	0	0/103	0	11/114	9,6	0/114	0,00

n^1= Number of lambs sick or dead, n^2= Total number of lambs - number of lambs in other diseases, n^3= Total number of lambs sick or dead n^4=Total number of lambs. FAS*=Fatigue-Anorexia Syndrome (Mismothering, hypotermia and starvation).

Table 3. *Morbidity and mortality in lambs due to various diseases associated with defined categories of serum IgG concentrations at 24 hours after birth in the post-neonatal period.*

IgG	Diarrhoea				Pneumonia				Suspected Septicemia				Other or Unclasssified				Total			
	Morbidity		Mortality		Morbidity		Mortality		Morbidity		Mortality		Morbidity		Mortality		Morbidity		Mortality	
Cut-off (mg/dL)	n1/n2	%	n1/n2	%	n1/n2	%	n1/n2	%	n1/n2	%	n1/n2	%	n1/n2	%	n1/n2	%	n3/n4	%	n3/n4	%
<200	0/0	0,00	0/0	0,00	0/0	0,00	0/0	0,00	0/0	0,00	0/0	0,00	0/0	0,00	0/0	0,00	0/0	0,00	0/0	0,00
201-500	0/2	0,00	0/2	0,00	4/6	66,7	0/4	0,00	0/2	0,00	0/2	0,00	0/2	0,00	0/2	0,00	4/6	66,7	0/6	0,00
501-600	1/2	50	0/3	0,00	0/1	0,00	0/0	0,00	1/2	50	1/3	33,3	0/1	0,00	0/2	0,00	2/3	66,7	1/4	25,0
601-800	2/3	66,7	0/3	0,00	0/1	0,00	0/1	0.00	0/1	0,00	0/1	0,00	0/1	0,00	0/1	0,00	2/3	66,7	0/3	25,0
801-1000	3/11	27,3	0/13	0,00	3/11	27,3	2/13	15,4	0/8	0,00	0/10	0,00	0/8	0,00	0/10	0,00	6/14	42,9	2/14	14,3
1001-1500	17/55	30,9	0/57	0,00	8/46	17,4	2/48	4,2	1/39	2,6	1/41	2,4	1/39	2,6	3/41	7,3	29/67	43,3	3/67	4,5
1501-2000	1/79	21,5	1/81	1,2	2/64	3,1	0/66	0,00	0/62	0,00	0/64	0,00	6/68	8,8	1/69	1,4	25/87	28,7	2/87	2,3
2001-2500	7/34	20,6	0/35	0,00	4/31	13	0/32	0,00	1/28	3,6	1/29	3,4	3/30	10	1/29	3,4	15/42	35,7	2/42	4,8
>2501	15/101	14,9	1/107	0,9	4/90	4,4	1/97	1,0	1/87	1,1	1/97	1,0	6/94	6.4	2/98	2,0	26/112	23,2	5/114	4,4

n^1= Number of lambs sick or dead, n^2= Total number of lambs - number of lambs in other diseases, n^3= Total number of lambs sick or dead n^4=Total number of lambs. FAS*=Fatigue-Anorexia Syndrome (Mismothering, hypotermia and starvation)

***Table 4**. Postcolostral (24 hours after birth) IgG (mg/dl) cut-off values that increase the risk of various diseases*

Clinical Diagnosis	Period	Grouping (IgG Cut-off)	Morbidity Rate	X2	P value	OR	95%CI
Diarrhoea	Neonatal	IgG>800	11.1% (26/324)	6.35	0.011	4.05	1.46-11.14
		IgG<800	26.1% (6/23)				
	Post-Neonatal	IgG>800	18.3% (59/322)	0.042	0.84	1.48	0.39-5.65
		IgG<800	25.0% (3/12)				
Pneumonia	Neonatal	IgG>1000	1.6% (5/310)	0.00	1.00	1.69	0.19-14.91
		IgG<1000	2.7% (1/37)				
	Post-Neonatal	IgG>1000	5.8% (18/308)	12.49	0.000	5.93	2.20-15.95
		IgG<1000	26.9% (7/26)				
FAS*	Neonatal	IgG>1000	1.6% (5/310)	18.45	0.000	11.80	3.40-40.92
		IgG<1000	16.2% (6/37)				
	Post-Neonatal	-	-	-	-	-	-
Suspected Septicemia	Neonatal	IgG>200	0.60% (2/336)	203.2	0.000	751.5	94.9-5948.4
		IgG<200	81.8% (9/11)				
	Post-Neonatal	IgG>600	0.98% (3/325)	1.48	0.22	13.42	1.25-143.43
		IgG<600	0.92% (1/9)				

FAS* (mismothering, starvation, hypoglycaemia), OR: Odds Ratio, CI: Confidence Interval

low at 45.5%, but the NPV was high at 90.8%, showing good performance in identifying negative cases. The sensitivity was 54.5%, indicating moderate performance in detecting positive cases, and the specificity was 90.8%, demonstrating strong negative case identification. The overall diagnostic accuracy was 89.2%. The confusion matrix shown 6 true positives and 31 false negatives for <1000 mg/dL, and 5 false positives and 305 true negati-ves for >1000 mg/dL (Table 5).

Neonatal suspected epticemic lambs had the AUC of 0.851, showing good model performance. The PPV was 81.8%, NPV is 99.4%, and diagnostic accuracy was 98.8%, indicating high accuracy in distinguishing both positive and negative cases, with excellent performance particularly in negative predictions. The sensitivity was 81.8%, reflecting the strong ability to detect positive cases, and the specificity was 99.4%, showing the outstanding ability to identify negative cases. The confusion matrix shown 9 true positives and 2 false negatives for <200 mg/dL, and 2 false positives and 334 true negatives for >200 mg/dL (Table 5).

For post-neonatal pneumonia, the AUC value was 0.663, indicating moderate discrimination ability. The PPV was 83.3%, NPV is 89.4%, and overall diagnostic accuracy was 89.4%, reflecting high accuracy in positive predi-ctions and effective identification of negative cases, de-monstrating overall good performance. The sensitivity was 16.7%, showing a lower ability to detect positive cases, while the specificity was 89.5%, indicating strong negative case identification. The confusion matrix shown 1 true positive and 36 false negatives for <1000 mg/dL, and 5 false positives and 305 true negatives for >1000 mg/dL (Table 5).

AUC 75%, PPV 37.7%, NPV 95.1%, and diagnostic accuracy 85.1% were determined (P<0, 05) for the threshold value of IgG <998 mg/dL that increases the risk of neonatal disease in lambs (Figure 2A). As for the threshold value of IgG <193 mg/dL that increases the risk of neonatal mortality in lambs, AUC 91.5%, PPV 84.62%, NPV 100%, and diagnostic accuracy 96.72% were determined (P<0.01) (Figure 2B).

DISCUSSION

The first few months of a lambs' life are critical due to inability to overcome unfaviourable conditions and incomplete immunity to fight infectious diseases when the risk of exposure to disease is relatively high. Therefore, good farm management parctices, including colostrum management, are of paramount importance in the pre-weaning period (Gokce et al., 2013b; Gokce et al., 2021).

Colostrum is a vital source of nutrients and immunoglobulins for the neonate, and an adequate supply of colostrum significantly increases the chance of survival to weaning (Agenbeg et al., 2021; Banchero et al., 2004; Dwyer et al., 2016). It has been reported that lambs with FPT or low antibody titers are at high risk of disease (Altiner et al., 2005; Herndon et al., 2011). Defining the success or failure of passive transfer is not a clear-cut process. It requires the identification of a threshold that addresses multiple objectives: a threshold above which increasing serum IgG concentrations no longer increase the risk of morbidity or mortality, or a threshold that predicts the greatest effect on a particular outcome, or a statistically defined threshold. The optimal threshold of serum IgG concentration defined for FPT has been recommended as <6 mg/ml (Gokce et al., 2019), <8 mg/ml (Sawyer et al., 1977), <15 mg/ml (Turquino et al., 2011; Alves et al., 2015), <16 mg/ml (Massimini et al., 2006a). In our study, low IgG was specifically associated with increased susceptibility to diarrhoea, pneumonia, FAS and suspected septicaemia in young lambs, and critical SIgGC-24 threshold values that increased the risk of developing neonatal diarrhea, FAS, septicemia and post-neonatal pneumonia were determined as <800, <1000, <200 and <1000 mg/dL, respectively. The AUC

Table 5. *Performance results by threshold values for neonatal diarrhoea, neonatal fas, neonatal septicemia, and post-neonatal pneumonia*

Neonatal Diarrhoea (Figure 1A)									
IgG (mg/dL)	Positive	Negative	Total	AUC	PPV	NPV	Sens.	Spec.	Diagnostic accuracy
<800	6	17	23						
>800	26	298	324	0.540	0.813	0.946	0.187	0.946	93.4%
Total	32	315	347						
Neonatal FAS (Figure 1B)									
IgG (mg/dL)	Positive	Negative	Total	AUC	PPV	NPV	Sens.	Spec.	Diagnostic accuracy
<1000	6	31	37						
>1000	5	305	310	0.932	0.455	0.908	0.545	0.908	89.2%
Total	11	336	347						
Neonatal Septicemia (Figure 1C)									
IgG (mg/dL)	Positive	Negative	Total	AUC	PPV	NPV	Sens.	Spec.	Diagnostic accuracy
<200	9	2	11						
>200	2	334	336	0.851	0.818	0.994	0.818	0.994	98.8%
Total	11	336	347						
Post-Neonatal Pneumonia (Figure 1D)									
IgG (mg/dL)	Positive	Negative	Total	AUC	PPV	NPV	Sens.	Spec.	Diagnostic accuracy
<1000	1	36	37						
>1000	5	305	310	0.663	0.833	0.894	0.167	0.895	89.4%
Total	6	341	347						

PPV: positive predictive value, NPV: Negative predictive value, Sens.: Sensitivity, Spec.: Specificity

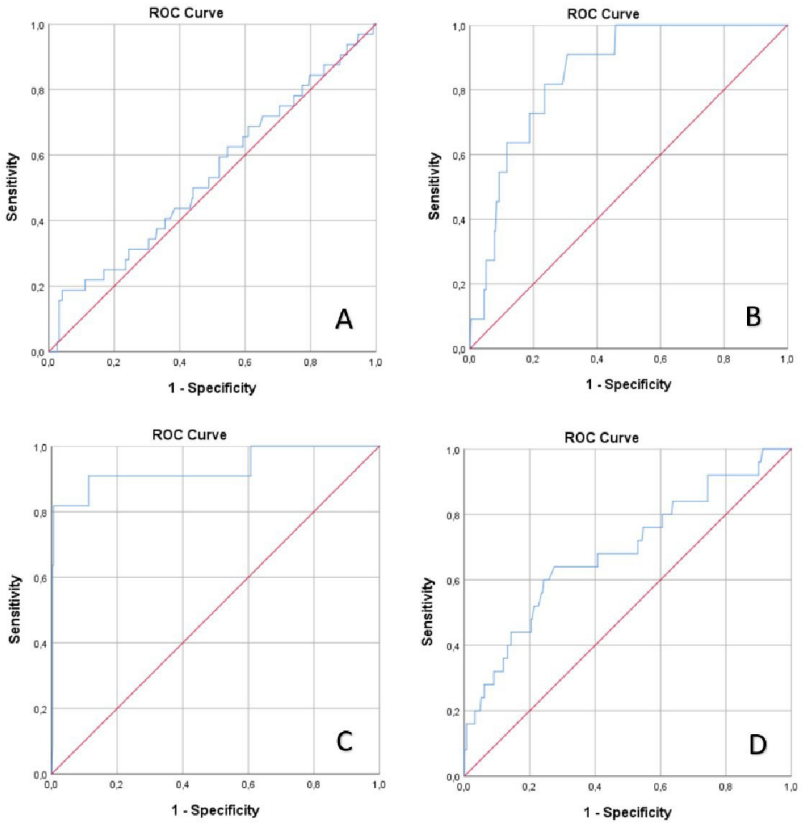

Figure 1. *ROC Curve for A) Diarrhoea, B) FAS, C) Septicemia and D) Pneumonia*

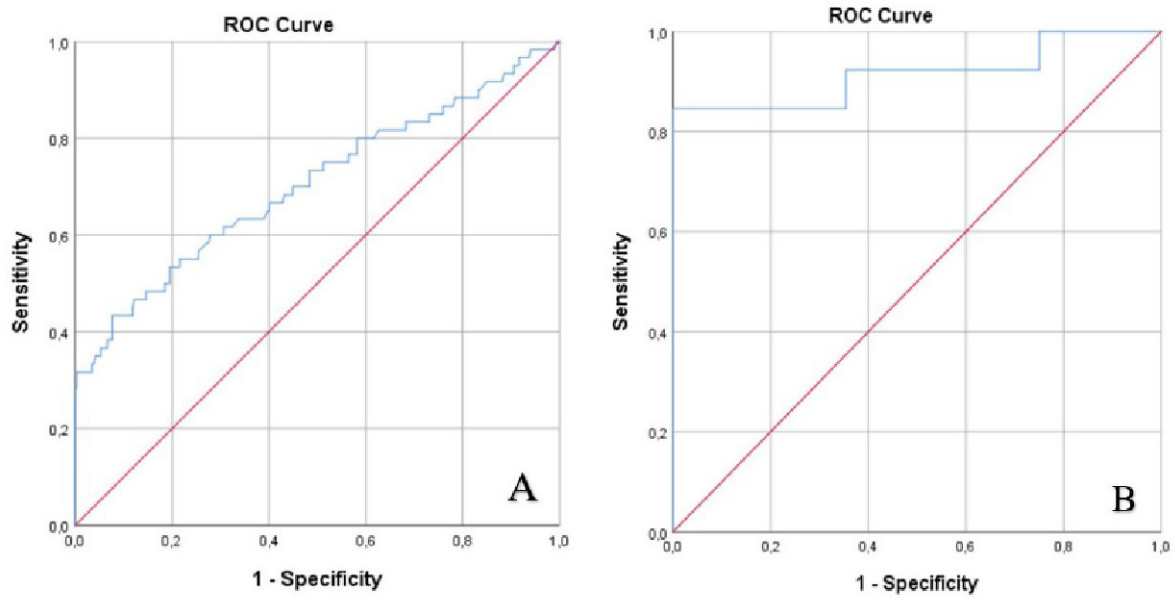

Figure 2. *ROC Curve for A) neonatal morbidity, B) neonatal mortality*

for the respective cut-offs were 56.7%, 72.7%, 90.6% and 60.9%, while the diagnostic accuracy was 87.61%, 89.63%, 98.85% and 88.92%, respectively. The cut off value of IgG <998 mg/dL and the value of IgG <193 mg/dL correctly identified neonatal morbidity and neonatal mortality as the AUC was 75% and 91.5%, respectively. However, it is difficult to compare our results as there are no studies investigating the relationship between SIgGC-24 threshold values and selected diseases in lambs.

In our study, about half of the lambs with neonatal disease relapsed in the postneonatal period, suggesting that precautions should be taken in advance. Identifying these lambs in advance, based on their post-colostral IgG levels, will allow the caretaker to provide adequate passive transfer or desing a therapeutic protocol in advance (Pesca et al., 2020). This timely management of such lambs will lead to rational use of antimicrobials, which is an important concern.

Colostrum is rich in energy sources such as glucose. Therefore, inadequate intake due to mismothering can lead to starvation and hypoglycaemia (Dwyer et al., 2016), namely FAS. Consumption of 100 ml/kg of colostrum within the first 6 hours is recommended to prevent starvation (Bond, 2020) and consequently FAS. Adequate post-colostrum IgG levels also indicate adequate energy intake from colostrum (Dwyer et al., 2016). In our study, lambs with <1000mg/dl SIgGC-24 were 18 times more likely to develop FAS than those with >1000mg/dl. This finding supports the fact that good quality colostrum reduces the risk of FAS as mentioned above. In cases of FAS, adequate colostrum intake can be ensured by management measures such as feeding with another mother or pre-frozen colostrum, and ensuring the mother-offspring relationship (Dwyer, 2008; Dwyer et al., 2016).

An important factor in the susceptibility of the newborn to pathogens is the permeability of the immature gut, which results in the passage of initial immunoglobulins as well as an increased risk of pathogen entry. FPT has been associated with disease in the first two weeks in ruminants. Gram-negative septicaemia is an important cause of mortality and FPT has been positively correlated with sepsis (Altiner et al., 2005). However, colostrum ingestion itself accelerates the process of intestinal closure; thereby it is also preventing the route of neonatal infection (Agenbag et al., 2021; Dwyer, 2008; Fischer et al., 2019). Therefore, the risk of diarrhea and septicemia is high in the first week of the neonatal period. This was also the case in our study where lambs with suspected septicemia died in the first week of life and had FPT, serum IgG below IgG<200mg/ml. Timely administration of colostrum with adequate IgG and low bacterial burden is necessary to prevent these diseases. Monitoring maternal immune and nutritional status and the success of PTCI is also important to prevent diarrhoea (Andres et al., 2007; Gokce et al., 2019).

Respiratory diseases account for 5.6% of all diseases in small ruminants and are an important economic issue in the industry (Kumar et al., 2014; Pesca et al., 2020). In calves, low postcolostral IgG and total protein levels and feeding of mastitic colostrum were found to be associated with a higher risk of pneumonia (Pardon et al., 2015). It has also been reported that preventing pneumonia increases daily gain (Virtala et al., 1996). Our study showed that a low post-colostral IgG concentration was associated with increased risk of pneumonia in lambs, and the cut-off value was determined to be a post-colostral IgG level <1000 mg/dl, as those lambs with a level below this value had approximately 12 times risk when compared to those with IgG >1000 mg/dl. The low proportion of respiratory disease cases observed in the neonatal period was not surprising, as calves and lambs under 30 days of age typically suffer from diarrhea, while respiratory disease is more prevelant in older animals (Gokce and Erdogan, 2008; Lora et al., 2018). This is mainly due to the presence of sufficient antibodies in colostrum against bacterial and viral pathogens in newborn lambs. Interestingly, Smith et al., (1976) also proposed that a small amount of colostrum IgG, once absorbed, diffuses into the nasal and lacrimal secretions of lambs and this may play a role in preventing respiratory infections prior to

local production of IgA and IgM at the 2-3 weeks of age (Smith et al., 1976)

This study demonstrated the critical role of sufficient IgG uptake in protecting lambs from pneumonia, diarrhoea, septicaemia and FAS. This effect is maintained until weaning. Lambs born to vaccinated ewes may have protective antibodies for the first 1-2 months. After this period, the lamb's passive immunity appears to decline dangerously, which can lead to the development of infectious diseases (Pesca et al., 2020). Considering the significant economic losses due to disease In preweaning lambs, it is necessary to adopt useful preventive tools for the benefit of the whole sheep industry. Passive immunisation or immunoglobulin products have a clear role to play in modern animal production as a means of controlling infectious diseases, especially with a very low risk of causing the development of bacterial resistance, thus constituting a real and widely applicable alternative to antibiotics.

In conclusion, the effect of IgG concentration on several diseases during the first 3 months in lambs has been demonstrated. We identified the post-colostral IgG thresholds that increase the risk of neonatal diarrhoea, FAS, septicaemia and post-neonatal pneumonia to be <800, <1000, <200 and <1000 mg/dL respectively. These findings may suggest that appropriate colostrum management may help to maintain the health of pre-weaning lambs, thereby improving the productivity and profitability of sheep farms. Lambs at risk of morbidity and mortality based on postcolostral IgG levels can be carefully monitored and treated early. Further studies are also required to use these results as epidemiological data in details.

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Conflict of Interest

Authors declare no conflicy of interest

Ethical Statement

The study was approved by the Kafkas University Institutional Ethics Committee for Animal Experimentation and Use (Protocol number:2008-23).

Author Contributions

EG: Collection of data, writing – original draft, PC: Writing – original draft, statistical analysis, visualizing, OA: Biochemical analysis, AHK: Collection of data, HME: Collection of data, writing – original draft and editing. All authors have read and agreed to the published version of the paper.

Availability of Data and Materıal

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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Determination of hematological and biochemical parameters in healthy Arabian and thoroughbred racehorses

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Abstract
In this study, it was aimed to determine the differences in terms of race, age, and gender by examining the hemogram and biochemical parameters of healthy thoroughbred racehorses brought to the equine hospital for routine examination. The study material comprised 86 healthy horses (51 Thoroughbred and 35 Arabian Horses) of both genders (31 female and 55 male). The horses were divided into three groups according to their ages (3 years and younger, 4 years, and 5 years and older). Blood samples were analyzed on the hemogram and biochemistry devices after sampling. There was no statistically significant difference in the parameters examined between the breeds ($p>0.05$). Hematologically, it was determined that white blood cell (WBC), lymphocyte (LYM), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW) values were at the highest level in those aged 3 and younger, and neutrophil (NEU), mean corpuscular volume (MCV), and MCH values were at the highest level in 5 years and older. Biochemically, it was determined that aspartate transferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), albumin (ALB), and phosphors (P) values were at the highest level in those aged 3 and younger, and total protein (TP) values were at the highest level in those aged 5 and older. In terms of gender, WBC, red blood cell (RBC), Mean corpuscular hemoglobin concentration (MCHC), RDW, AST, ALP, gamma-glutamyl transferase (GGT), LDH, Creatine kinase (CK), creatinine (CREA), ALB, and P values were higher in males compared to females, while MCV, MCH, Mean platelet volume (MPV), and TP values were higher in females compared to males. As a result, although the Thoroughbred and Arabian Racehorses examined in the study were healthy, there might be significant differences in hemogram and biochemistry values in terms of age and gender.

Keywords: *Age, biochemistry, breed, gender, hemogram, racehorse*

INTRODUCTION

Thoroughbreds have been bred in different geographies and in countries around the world for many years (Milosevic et al., 2020). In addition, Thoroughbred and Arabian horses are bred for racing around the world and in Türkiye with great effort, and meticulous attention is paid to the health of these economically important horses. The health status of these horses, which have high economic value, affects their racing performance, and they are regularly kept under control. Because the illness of horses negatively affects racing performance and investments (Paksoy & Ünal, 2010).

For horses to exhibit better racing performance, they must also have superior performance characteristics such as large lung volume, high hemoglobin concentration, and cardiac output, the ratio of large muscle mass to body weight, high skeletal muscle density, and oxidative enzyme activity (Essen-Gustavsson & Lindholm, 1985; Hinchcliff & Geor, 2008; Munoz et al. 2017; Fails, 2020; Mukai et al.2023).

Although blood values differ in animals according to species, they also show a wide distribution within the same species depending on age, race, gender, region of breeding, and diet (Alilovic et al., 2022; da Conceição et al., 2022; Shawaf et al., 2018; Turgut, 2000). It is reported that revealing the differences in serum biochemistry parameters depending on race will provide a more accurate determination of diseases or metabolic problems that may occur (Akyüz et al., 2020). In addition, training or exercise status, pregnancy, circadian rhythm, health status, and the blood collection process affect hematological and biochemical parameters in horses (McGowan & Hodgson, 2014; Satue et al., 2012).

The number of studies on the hematological and biochemical parameters of blood in thoroughbred racehorses in Türkiye is limited. In studies conducted on horses, data on the effect of exercise on hematological and/or biochemical parameters have usually been presented (Allaam et al., 2014; Burlikowska et al., 2015; Demirel et al., 2022; Güzelbektaş et al., 2006; Klobučar et al., 2019; Kocaman & Fidancı, 2016; Oruç et al., 2017; Pourmohammed et al., 2019; Tepeoğlu, 2018). In addition, the effect of certain active ingredients on race performance in specific horse breeds has been investigated (de Oliveira et al., 2015; Fenger et al., 2014; Harking et al., 1992). However, comparative and detailed research on different races, ages, and genders could not be found in Türkiye. In the previous studies, hematological and/or biochemical parameters were evaluated only in terms of race (Bilal & Meral, 2002), age (Gürgöze & İçen, 2010), the relationship with element concentrations (Yipel et al., 2022), race

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and gender in a certain age group (Demirtaş, 2018), and gender and age in a certain race (Altınsaat, 2008; Ayhan & Gürgöze, 2024; Uluışık et al., 2013). For this reason, the presented study aims to reveal the hematological and biochemical parameters of healthy Thoroughbred and Arabian Horses in terms of race, age, and gender, respectively.

MATERIAL and METHODS

This manuscript was written without the assistance of any artificial intelligence programs.

Animals and experimental design

The study was carried out on 86 racehorses of different breeds, ages, and genders brought to the Turkish Jockey Club Adana Yeşiloba Hippodrome Equine Hospital for routine control. According to pedigree information, 35 horses were grouped as purebred Arabian and 51 as Thoroughbred. A systematic physical examination of the horses was performed. Within this scope, heart frequency, body temperature, number of breaths, lymph nodes examination, skin turgor and examination, digestive system examination (including stool examination), and urinary system examination were performed. According to the results of this examination, horses that did not find an abnormal physical examination were included in the study. The horses in the study were housed individually in concrete stables (4 m x 4 m) at the Turkish Jockey Club Adana Yeşiloba Hippodrome. They were fed with 4 kg of oats, 3 kg of barley, 7 kg of dried grass, 4 pieces of apples, and 4 pieces of carrots as 3 meals per day. The water needs of the horses were met ad libitum. Sawdust was preferred as a substrate.

Sampling method

The study included 86 horses that were found to be healthy according to anamnesis, clinical examination, and then no abnormalities in the reference values were detected according to the species in the laboratory findings. Blood samples were taken from all horses ,4 mL, duly from the *vena jugularis* into tubes with EDTA (BD Vacutainer® Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for hemogram analysis and into tubes without anticoagulant (BD Vacutainer® Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for biochemical analyses in the morning, when on the horses were hungry and before training.

Laboratory analysis

Hemogram analyses were performed with a hematology analyzer (Symex XT-1800i ®, Sysmex Corporation, Kobe, Japan), and WBC, LYM, NEU, monocyte (MONO), RBC, hemoglobin (HBG), hematocrit (HCT), MCV, MCH, MCHC, RDW, Platelet Count (PLT), and MPV values were determined. Blood samples in anticoagulant-free tubes were centrifuged at 4.000 rpm for 5 min, and serum samples were obtained. Biochemical parameters were determined with an automatic biochemistry analyzer (Siemens Dimension® Xpand®, Siemens Healthcare Diagnostics, Tarrytown, NY, USA), and values of AST, ALP, GGT, LDH, CK, lactic acid (LA), TP, ALB, CREA, blood urea nitrogen (BUN), calcium (Ca), magnesium (Mg), and P were determined.

Statistical analysis

In the research, the statistical package program SPSS V22 (IBM, Ehningen, Germany) was used to analyze the averages, standard errors, and data belonging to the groups. General Linear Model "UNIVARIATE" test was used to evaluate the results of the analyses according to race, age, and gender group. A correlation analysis was also performed between the analyses. The model Yijk = μ + ai + bj + eijk was used in the statistical analysis of the blood analyses. In this model; Yijk: Value of the examined trait of any individual; μ: the overall mean; ai: is the effect of gender (i: male and female); bj: effect of age group (j: 3,4,5+): and eijk: experimental error.

RESULTS

The age range of the horses was between 2 and 7 years, and the gender distribution was 31 females and 55 males. The horses included in the study were divided into 3 groups: 39 horses aged 2-3 years, which was the age of starting racing life; 26 horses aged 4 years; and 21 horses aged 5 years and older who were considered mature.

There was no significant difference between Arabian Horses and Thoroughbreds in haemogram and biochemistry values according to breed ($p>0.05$).

A statistically significant difference was found in hemogram values according to the age factor; WBC (p=0,010), LYM (p=0,000), NEU (p=0,000), MCV (p=0,001), MCH (p=0,000), and RDW (p=0,014) values ($p<0.05$). NEU, MCV, MCH, and RDW values increased with age, whereas WBC and LYM values decreased. Moreover, WBC and LYM values were found to be the highest in the age group of 3 years and younger and the lowest in the age group of 5 years and older (Table 1).

Considering the biochemistry values according to age, a statistically significant difference was found in AST, ALP, LDH, TP, ALB, and P values ($p<0.05$). According to age, Ca and P concentrations in horses were in the range of reference values. Blood concentrations (mg d/L) were ranked as Ca>P>Mg for macroelements. AST, ALP, LDH, ALB, and P values decreased with advancing age, whereas TP values increased. Moreover, AST, ALP, LDH, ALB, and P values reached the highest level in those aged 3 years and younger, whereas TP values decreased to the lowest level in those aged 5 years and older (Table 2).

A statistically significant difference was found in the WBC, RBC, MCV, MCH, MCHC, and MPV values from hemogram parameters according to gender factor ($p<0.05$). Of these values, WBC, RBC, and MCHC values were higher in males than females, while MCV, MCH, and MPV values were higher in females than males (Table 1).

From the point of view of gender, AST, ALP, GGT, LDH, CK, TP, ALB, CREA, and P values of biochemical parameters were statistically significantly different between males and females ($p<0.05$). Of these values, it was determined that AST, ALP, GGT, LDH, CK, ALB, CREA, and P values were high in males compared to females, while the TP value was high in females compared to males (Table 2).

It was determined that the values of WBC, MCV, and

Table 1. *Hemogram values of healthy horses according to breed, age, and gender (Mean ± Standard deviation)*

Parameter (Unit)	Reference Ranges	3 years and younger	4 years old	5 years and over	F	P	Female	Male	F	P	Thoroughbred	Arabian	F	P
		X±S	X±S	X±S			X±S	X±S			X±S	X±S		
WBC (x10^3/µL)	5.4-14.3	7.95±0.15	7.26±0.17	7.48±0.22	4.877	0.010*	7.29±0.16	7.82±0.13	6.388	0.013*	7.56±0.13	7.72±0.16	0.554	0.459
Lymphocyte (x10^3/µL)	17-68	42.33±1.17	40.62±1.22	34.51±1.20	9.858	0.000*	38.60±1.41	40.64±0.93	1.589	0.211	40.09±1.11	39.63±1.04	0.084	0.773
Neutrophil (10^3/L)	22-72	51.04±1.20	52.58±1.23	58.71±1.32	8.848	0.000*	54.70±1.43	52.63±0.96	1.542	0.218	52.93±1.15	54.03±1.06	0.453	0.503
Monocyte (10^3/L)	0-14	0.40±0.01	0.39±0.02	0.36±0.02	1.275	0.285	0.38±0.15	0.40±0.12	0.725	0.397	0.40±0.013	0.37±0.14	1.707	0.195
RBC (x10^9/µL)	6.8-12.9	9.89±0.15	9.86±0.19	9.38±0.14	2.449	0.093	9.33±0.14	9.99±0.12	11.479	0.001*	9.86±0.13	9.59±0.15	1.795	0.184
Hemoglobin (g/dL)	11.0-19	14.35±0.26	14.90±0.28	14.40±0.20	1.310	0.275	14.20±0.21	14.71±0.21	2.640	0.108	14.68±0.20	14.31±0.23	1.349	0.249
Hematocrit (%)	32-53	37.65±0.60	39.22±0.61	38.06±0.50	1.842	0.165	37.74±0.49	38.51±0.48	1.078	0.302	38.58±0.49	37.71±0.51	1.434	0.234
MCV (fL)	37-59	38.16±0.42	39.92±0.46	40.69±0.57	7.883	0.001*	40.55±0.48	38.61±0.34	11.115	0.001*	39.23±0.39	39.43±0.45	0.114	0.736
MCH (pg)	12-20	14.51±0.13	15.13±0.13	15.39±0.18	10.180	0.000*	15.24±0.16	14.73±0.11	7.592	0.007*	14.90±0.13	14.93±0.14	0.033	0.857
MCHC (g/dL)	31-39	38.07±0.18	37.96±0.25	37.86±0.19	0.247	0.781	37.62±0.17	38.19±0.16	5.410	0.022*	38.02±0.14	37.93±0.22	0.119	0.731
RDW (%)	24-27	33.77±0.29	34.63±0.22	34.85±0.25	4.502	0.014*	34.67±0.24	34.09±0.22	2.834	0.096	34.42±0.22	34.12±0.25	0.762	0.385
PLT (10^9/L)	100-350	152.08±4.98	148.31±4.52	138.24±7.54	1.480	0.234	150.35±5.43	145.82±4.05	0.510	0.477	148.10±4.40	146.77±4.78	0.040	0.842
MPV (fL)	5.6-8.3	3.29±0.58	4.55±0.67	5.45±0.69	2.907	0.060	5.91±0.48	3.23±0.48	13.246	0.000*	3.98±0.49	4.52±0.60	0.489	0.486

Abbrevetion: WBC: White Blood Count, RBC: Red Blood Count, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW: Red Cell Distribution Width, PLT: Platelet, MPV: Mean Platelet Volume (Weiss & Wardrop, 2011).

*: $p<0.05$, Differences between groups are statistically significant.

Table 2. *Biochemistry values of healthy horses according to breed, age, and gender (Mean ± Standard deviation)*

Parameter (Unit)	Reference Ranges	3 years and younger	4 years old	5 years and over	F	P	Female	Male	F	P	Thoroughb-red	Arabian	F	P
		X±S	X±S	X±S			X±S	X±S			X±S	X±S		
AST (U/L)	226-336	322.26±10.75	296.69±12.03	257.67±11.98	7.281	0.001*	250.77±8.64	325.80±8.24	34.593	0.000*	289.63±9.86	312.06±10.22	2.357	0.128
ALP (U/L)	143-395	169.95±6.65	131.92±4.91	137.95±18.92	4.847	0.010*	133.94±11.80	160.05±6.23	4.638	0.034*	143.06±8.70	161.69±7.00	2.408	0.125
GGT (U/L)	4.5–32.5	31.26±2.21	30.81±2.57	27.00±2.33	0.801	0.452	25.10±1.67	32.89±1.86	7.847	0.006*	30.55±1.85	29.40±2.11	0.164	0.687
LDH (U/L)	162-412	252.95±8.84	212.69±10.87	185.05±9.61	12.199	0.000*	181.97±6.21	247.81±7.74	34.585	0.000*	217.42±8.14	232.47±10.29	1.337	0.251
CK (U/L)	60-330 (Merck)	189.44±5.27	222.65±63.30	137.86±9.67	1.298	0.278	131.55±5.49	218.07±29.55	4.758	0.032*	166.77±6.54	216.20±46.82	1.568	0.214
LA (mg/dL)	1,11-1,78	0.94±0.06	0.90±0.07	0.82±0.06	0.812	0.448	0.81±0.05	0.94±0.05	3.192	0.078	0.92±0.46	0.86±0.60	0.642	0.425
TP (g/L)	52,0-79,0	59.44±0.46	60.69±0.77	62.55±0.86	5.442	0.006*	62.29±0.67	59.61±0.44	12.030	0.001*	60.81±0.50	60.27±0.65	0.448	0.505
ALB (g/L)	26-37	37.13±0.44	36.63±0.39	35.24±0.62	3.731	0.028*	35.01±0.44	37.41±0.31	20.560	0.000*	36.46±0.39	36.62±0.41	0.071	0.791
CREA (mg/dL)	1,2-1,9	1.56±0.04	1.48±0.04	1.47±0.06	1.355	0.264	1.43±0.04	1.56±0.03	6.025	0.016*	1.55±0.04	1.47±0.04	2.280	0.135
BUN (mg/dL)	10-24	14.79±0.48	14.12±0.50	13.81±0.65	0.920	0.403	13.81±0.52	14.65±0.38	1.741	0.191	14.84±0.40	13.63±0.48	3.865	0.053
Ca (mg/dL)	11,2-13,6	11.62±0.06	11.65±0.06	11.61±0.06	0.136	0.873	11.61±0.05	11.64±0.05	0.104	0.748	11.66±0.05	11.58±0.05	1.549	0.217
Mg (mg/dL)	2,2-2,8	2.33±0.06	2.40±0.09	2.27±0.06	0.711	0.494	2.30±0.07	2.36±0.05	0.368	0.546	2.40±0.06	2.25±0.05	3.469	0.066
Phos (mg/dL)	3,1-5,6	3.87±0.12	3.52±0.14	3.20±0.16	6.028	0.004*	3.33±0.14	3.75±0.10	6.323	0.014*	3.65±0.12	3.52±0.11	0.607	0.438

Abbrevetion: AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, GGT: Gamma Glutamyl Transferase, LDH: Lactate Dehydrogenase, CK: Creatinine Kinase, LA: Lactic Acide, TP: Total Protein, ALB: Albumin, CREA: Creatinine, BUN: Blood Urea Nitrogen, Ca: Calcium, Mg: Magnesium, Phos: Phosphate (Kaneko et al., 2008; Merck, 2022).

*: $p<0.05$, Differences between groups are statistically significant.

MCH from the hemogram parameters and the values of AST, ALP, LDH, TP, ALB, and P from the biochemical parameters were significant according to both age and gender factors (Table 1 and Table 2).

DISCUSSION

Blood parameters are frequently used for clinical examination, routine control, and to investigate the presence of disease. It is reported that hematological and biochemical tests, which are routinely used in human medicine, can also be widely used in veterinary medicine for the evaluation of diagnosis and treatment (Babaeski, 2023). In this context, hematological and biochemical parameters have an important place in the evaluation of racehorse health (Waller et al., 2009). There are numerous studies indicating that hematological and/or biochemical parameters are influenced by factors such as race, age, gender, nutritional level, type of activity, and physiological condition (Adamu et al., 2013; Arslan et al., 2002; Ayhan & Gürgöze, 2024; Benashour et al., 2024; Bonhomme et al., 2023; Bos et al., 2018; Çetelioğlu et al., 2001; Demirel et al., 2022; Fazio et al., 2011; Güzelbektaş et al., 2006; Harris et al., 1998; Kedzierski et al., 2009; Oktay & Eren, 2014; Tepeoglu, 2018).

In the present study, the mean values of WBC, NEU, MCV, MCH, and MPV were higher in Arabian Horses than in Thoroughbreds, but there was no statistically significant difference between the breeds ($p>0.05$). Similar to the results of this study, Bilal & Meral (2002) found no statistically significant difference in erythrocyte phase indices (MCV, MCH, MCHC) in Thoroughbred and Arabian horses in terms of breed in their study conducted at Veliefendi Hippodrome in Istanbul. Demirtaş (2018) conducted a study at the Istanbul Veliefendi Hippodrome and found that RBC, Hb, and HCT values were statistically significantly higher in 3-year-old Thoroughbreds than Arabian horses. This study evaluated that the lack of difference in terms of race may be due to the geographical location and altitude difference between the Adana and Istanbul regions.

In this study, WBC, LYM, NEU, MCV, MCH, and RDW parameters were found to be statistically significant in racehorses ($p<0.05$). MCV, MCH, and MCHC are routine erythrocyte indices commonly used in the clinic (Zhang et al., 2022). It was determined that at four years of age and above, WBC and LYM values were significantly lower compared to three years of age and below, and NEU, MCH, MCV, and RDW values were high (Table 1). The height determined in WBC and LYM values in foals compared to the older horses is in line with many research findings (Ayhan & Gürgöze, 2024; Cebulj-Kadunc et al., 2002; Lassen & Swardson, 1995; Mikniene et al., 2014; Satue et al., 2020). The decrease in the number of WBCs with advancing age found in the study was determined by Mcfarlane et al. (2001); as reported, it can be explained by a decrease in the level of immunity due to ageing. In the present study, NEU levels increased with ageing, and LYM levels decreased with ageing. Similar to the results of this study, it has been reported that NEU's are higher in older horses than in young horses (Czech et al., 2019; Jawor et al., 2007), while LYM counts decrease significantly with ageing, leading to a higher NEU/LYM ratio in older horses than in foals (Satué et al., 2010; Smith et al., 2002). This study showed a statistically significant increase in erythrocyte indices (MCV, MCH, and RDW values) with ageing. In this study, the erythrocyte indices MCV, MCH, and RDW values increased statistically significantly with aging. Among these findings, the increase observed in MCV and MCH values with ageing is consistent with the findings of many researchers (Benashour et al., 2024; Cebulj-Kadunc et al., 2020; Hodgson & Rose, 1994; Lassen & Swardson, 1995). In the current study, MCV, RDW, and MCH values were found to be higher during the ageing period. This is thought to result from an increased erythrocyte regeneration rate, which may be attributed to the rise in performance caused by the growing number of races the horses participated in and the higher oxygen demand required for transport.

Regarding gender, WBC, RBC, and MCHC values were higher, and MCV, MCH, and MPV values were lower in male horses than in female horses. In this study, the higher WBC and RBC values in male horses compared to females were found to be compatible with the findings of Cebulj-Kadunc et al. (2002), Kisadere et al. (2019) for WBC values, and Czech et al. (2019), Satue et al. (2020), Tomenendalova et al. (2014), and Weiss & Wardrop (2011) for RBC values. In terms of gender, it has been reported that androgens that stimulate erythropoiesis may be the reason for the high levels of hematological parameters in males compared to females (Kelani & Durotoye, 2002). The fact that the MCHC value was lower in female horses than in male horses is consistent with the literature (Mikniene et al., 2014; Udeh et al., 2021). It has been evaluated that the MCHC value may cause differences due to the physiological characteristics of male and female horses depending on gender. The MCV value was different from Ayhan & Gürgöze (2024), and Udeh et al. (2021), and MCH values were found to be lower in purebred male horses than female horses by Czech et al. (2019), and Weiss & Wardrop (2011), while compatible with Ayhan & Gürgöze (2024), and Udeh et al. (2021) with a difference. The fact that the MPV value was higher in females than in male horses is consistent with the literature (Isovic et al., 2023; Mesaric et al., 2023).

In the pathophysiology of the liver (ALP, GGT, AST, ALT, bilirubin, and albümin), globulin protein tests are important parameters (Comba et al., 2017). When biochemistry values were examined according to age, statistically significant differences were found in AST, LDH, ALP, TP, ALB, and P values ($p<0.05$). It was determined that AST, ALP, LDH, and ALB values decreased with advancing age ($p<0.05$), while TP values increased ($p<0.05$). Moreover, it was found that the values of AST, ALP, LDH, and ALB reached the highest level in those aged 3 and younger (Table 2). While the decrease observed in enzyme levels with advancing age was consistent with AL-Hadithy (2011) for AST and Munoz et al. (2012) for LDH, other researchers (Gürgöze et al., 2010; Halo et al., 2020; Oktay & Eren, 2014). The decrease observed in enzyme levels with advancing age is consistent with AL-Hadithy (2011) and Benashour et al. (2024) for AST and Munoz et al. (2012) for LDH, while it differs from the findings of other researchers (Gürgöze & İçen, 2010; Halo et al., 2020; Oktay & Eren, 2014). In this study, the high ALP levels determined in foals by many researchers (Czech et al., 2019; Gürgöze & İçen, 2010;

Halo et al., 2020; Miglio et al., 2019; Mikniene et al., 2014; Tomenendalova et al., 2014) were consistent with their findings. This situation can be explained by the fact that bone tissue growth is higher in foals, similar to Munoz et al. (2012). Gürgöze & İçen (2010) found that total protein levels were higher in older horses than in foals, and Mikniene et al. (2014) reported that a statistically significant increase in TP was detected with advancing age. Munoz et al. (2012) determined that ALB concentrations were lower in foals than adult horses. The TP and ALB levels obtained in this study are consistent with the researchers' findings. These high ALB concentrations may indicate liver function development (Munoz et al., 2012). The finding obtained at the P level was in line with the findings of other researchers (Gürgöze & İçen, 2010; Mikniene et al., 2014; Oktay & Eren, 2014; Tomenendalova et al., 2014). Braithwaite (1975) reported that young animals achieved much higher absorption rates in dietary phosphorus absorption than older animals. In light of Braithwaite's (1975) report, in this study, it is evaluated that the high P level obtained in foals or the P level decreases with advancing age, and the increased absorption in youth is due to the decreasing bone metabolism with advancing age.

In this study, AST, ALP, GGT, LDH, CK, ALB, CREA, and P values were higher in male horses than females, and TP values were lower in male horses than females. From these findings, AST and TP Mikniene et al. (2014), GGT Tomenendalova et al. (2014), LDH and CK Oktay & Eren (2014), ALB Paden et al. (2014), Mikniene et al. (2014) and CREA, on the other hand, many researchers (Kisadere et al., 2019; Paden et al., 2014; Souza et al., 2016) are consistent with the findings. However, from the study findings, GGT, CREA, P, and TP levels were determined by Oktay & Eren (2014), the ALP level was determined by Oktay & Eren (2014), and Paden et al. (2014); the CK level was determined by Paden et al. (2014); the ALB level was determined by Oktay & Eren (2014), and Mikniene et al. (2014). There was a difference in comparison in terms of gender. In this study, although the levels of AST, CK, and LDH, which are biomarkers of muscle damage, were statistically significantly different between females and males, these values were found to be within the normal reference ranges (Kaneko et al., 2008), and no clinical findings related to muscle damage were observed in horses. It was evaluated that the parameters related to muscle enzymes such as CK, Crea, AST, and LDH were higher in males, which may have been caused by the larger heart and muscle mass in male horses compared to female horses.

It is stated that the reference values of hematological and biochemical parameters of various horse breeds may vary depending on genetic factors, breed, geographical region (climate, altitude, etc.), living, and health conditions (nutritional quality, water availability, parasites, etc.), blood sampling time, analysis methodology, and equipment differences (Gürgöze & İçen, 2010; Paden et al., 2014).

CONCLUSION

Routine checks carried out by specialist veterinarians in hippodromes are important for racehorses' performance. Hemogram and antibiogram values are among the most important elements of these controls. Although Arabian and Thoroughbred are included in the same horse breed, there are differences in blood analysis results according to age, race, and gender.

Veterinarians and horse breeders consider these differences when assessing horses' health status. As a result, in horses that are seen as healthy in clinical examination, it has been concluded that there may be differences in hemogram and biochemistry values in terms of race, age, and gender.

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Conflict of interest

The authors declared that there is no conflict of interest.

Ethical statement or informed consent

The study was conducted with the approval of the Selçuk University Faculty of Veterinary Medicine, Experimental Animal Production, and Research Centre Ethics Committee (SÜVDAMEK, 27.12.2022/TS: 2022-15/KS: 2022/143).

Author contributions

The study was designed by NA and HY. YP collected data. MHS performed the statistical analyses. HY, NA, YP, and MHS writed article. Critical Reviews by NA. All authors read and approved the final version.

Availability of data and materials

All data and materials of the study are available in contact with the corresponsible author.

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An investigation on the potential role of Q fever and chlamydiosis of ovine abortion

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Abstract
Chlamydia abortus and *Coxiella burnetii* are among the significant pathogens that result in economic losses in small ruminants, particularly sheep, on a global scale. Both agents have been linked with disorders of the reproductive system in animals and are among the primary causes of abortion cases. This study aimed to evaluate the prevalence of Q fever and ovine enzootic abortion (OEA) in aborted sheep during lambing seasons. Samples of blood and vaginal swabs were obtained from sheep flocks in the Iğdır province. In total, both blood samples and vaginal samples from 100 aborted sheep were analyzed for *C. abortus* and *C. burnetii*. Both agents were investigated by ELISA in serum and by direct PCR in vaginal swabs. The results of the study indicate that 44% of the sheep sera exhibited positive antibody reactivity to *C. burnetii.* Five out of 100 sera samples from sheep tested were positive for ovine enzootic abortion. In addition, three samples were serologically positive for both agents. Molecular analyses of vaginal swabs were negative for both agents. The results of this study confirm the existence of exposure of sheep flocks in the Iğdır province to both agents. The detection of Q fever and OEA in abortive sheep indicates that these pathogens carry a risk of infection in humans due to their zoonotic properties.

Keywords: *Aborted sheep, chlamydiosis, Q fever, seroprevalence*

INTRODUCTION

Sheep breeding, which is an important part of the animal husbandry sector, is quite common in Eastern Anatolia, Türkiye (Ertaş et al., 2022). In our country, sheep are raised especially to meet the red meat deficit (Öztürkler, 2015), and many products such as milk, wool, and leather are also obtained (Ertaş et al., 2022). The issue of abortion represents a significant challenge for the livestock industry, particularly in the case of small ruminants. The loss of fetuses and subsequent reduction in milk production can result in considerable economic losses for the livestock industry. It is established that the occurrence of abortion in animals has an impact on human health and animal welfare (Karagül et al., 2019; Sebestiani et al., 2018). Several different factors, including both infectious and non-infectious agents may cause abortions. Infections are the main factor in abortions and can sometimes co-infection in the same cases (Santos et al., 2022; Sebastiani et al., 2018). Bacterial agents that are responsible for abortion in domestic mammals include *Brucella*, *Salmonella, Coxiella burnetii, Campylobacter,* and *Chlamydia* species (Abnaroodheleh et al., 2021; Sebastiani et al., 2018). In studies conducted in Türkiye, although abortion cases are generally caused by brucellosis, it has been demonstrated that *Chlamydia abortus* and *C. burnetii* are bacteria capable of causing abortions, with significant detection rates in ovine and caprine populations (Gülmez Sağlam and Şahin, 2016; Karaca et al., 2009; Karagül et al., 2019). The importance of identifying these diseases is related to their prevalence as causative agents and their potential for transmission between animals and humans.

Coxiella burnetii is a Gram-negative bacterium that can infect many animals, including livestock, domestic and wild mammals, and other bird and cold-blooded animal species. Q fever is a potential cause of infection in the reproductive system of ruminants (Agerholm, 2013; Alkahachi et al., 2020). It is postulated that the majority of human cases are contracted via domestic animals (Ramo et al., 2022; Wolf et al., 2020). In a study conducted by Kaplan et al. (2024) in Erzurum province, *C. burnetii* was detected in 14% of the milk samples offered for sale and revealed that animal products may be important in terms of public health.

Ovine enzootic abortion (OEA) is a disease of considerable economic importance to sheep and goat farming worldwide. It is caused by *C. abortus* and affects sheep and several other ruminants (Borel et al., 2018). Small ruminants infected with *C. abortus* may abort three weeks before parturition. However, some animals do not show any preliminary clinical signs of impending abortion. Nevertheless, in certain animals, the presence of vaginal discharge or changes in behavior may be observed 48 hours before the onset of abortion (Villagra et al., 2015). The contamination of the environment continues by vaginal discharge for up to two weeks (Rodolakis et al., 2015).

Chlamydia abortus and *C. burnetii* cause clinical findings such as miscarriage, stillbirth, weak offspring, in-

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fertility, and premature birth, which are considered reproductive disorders (Hamedi et al., 2020; Hireche et al., 2016). *C. abortus* and *C. burnetii* can cause co-infection in animals. The risk of transmission of these diseases is higher during birth when high amounts of individual bacteria are released. Infection may occur through inhalation, ingestion, or direct contact with birth fluids or the placenta. Bacteria have also the potential to be shed in milk, feces, and urine. Individuals who have direct contact with infected animals, including farmers and veterinarians, are considered to be at elevated risk of infection (Santos et al., 2022).

Chlamydia abortus and *C. burnetii* the obligate intracellular agents cannot be cultured in standard laboratory media due to their inability to survive and replicate outside of a host cell. Isolation is a prolonged and expensive process that carries inherent risks. A reliable and rapid laboratory diagnosis is essential for the accurate identification of infection within the herd. For laboratory diagnosis of both agents, Identification methods can be divided into two categories: direct and indirect. Direct diagnosis of the causative agent can be made using molecular techniques, isolation, and identification from clinical samples. Serological tests such as ELISA are defined as indirect methods and are used to detect the antibody response to the pathogen in the host (Gülmez and Şahin, 2016; Santos et al., 2022).

In this study, it was aimed to investigate the prevalence of Q fever and ovine enzootic abortion in sheep with an abortion history in the Igdır and to determine the role of these disease agents in abortion cases.

MATERIAL AND METHODS

Field study area

The city of Iğdır is situated within the Erzurum-Kars region of the Eastern Anatolia Region in Turkey. The northern and northeastern border is formed by the Aras River and the Armenian border along the bed of this river. The region is bordered to the east and southeast by Nakhichevan and Iran, to the south by Ağrı province, and the west and northwest by Kars Province.

Sampling

The present study was conducted on sheep with a known background of abortion in the Iğdır province, Türkiye. A total of 100 sheep from 5 different foci, all of which were aged between 2 and 4 years and belonging to the Morkaraman breed, were evaluated in 1 month after abortions. In the study, 5 ml blood samples were taken from sheep's *V. cephalica antebrachii* into one tube one of which contains a silica gel (for serum analysis).

Vaginal swabs were taken from sheep with a history of abortion within the first 30 days postpartum. After the vulva lips were thoroughly cleaned, the lips were separated, and samples were taken from the vaginal wall just anterior to the cervix. To prevent contamination with urine, the swab stick was directed inward from the upper part of the external urethral opening. The swab stick was rotated approximately 4-5 times before being slowly removed. It was then placed in a special medium inside the swab box and sent to the laboratory.

Serological methods

Detection of the Coxiella burnetii

The serum was obtained from the sheep blood samples through the centrifugation of tubes at 3000 rpm for 10 minutes. Thereafter, the ELISA, which is a serological test for the detection of host antibodies against *C. burnetii* and one of the most preferred tests for the detection of this disease, was used. To this end, a Q fever antibody test kit (IDscreen® Q fever indirect multi-species, France) was utilized, following the manufacturer's recommended protocol. The results were analyzed utilizing an ELISA reader at a wavelength of 450 nm. Results were calculated by the formula specified in the kit procedure, as shown below:

An S/P (%) value of more than 80% was considered as strong positive; an S/P value between 50% and 80% was considered positive; an S/P value between 40% and 50% was considered doubtful and an S/P value less than 40% was considered as negative.

Detection of the Chlamydia abortus

The ELISA test kit (CHLMS-MS, ID.vet, France, microwells coated with *C. abortus* specific antigen (Momp)) was used to detect the host response against *C. abortus* according to the instructions of the test kit. An ELISA reader was used to read the results. The results were expressed as a percentage of optical density readings for the test samples. Calculations were performed according to the following criteria: OD% < 50 was defined as negative, OD% > 50 and OD% > 60 were classified as doubtful, while OD% > 60 was considered positive about *C. abortus*.

Molecular Methods

To obtain a direct DNA extract, 100 vaginal swab samples were taken from the aborted animals and extracted using a DNA Mini Kit (PureLink™, K182002, USA). This was done by the instructions provided by the manufacturer.

Detection of the Coxiella burnetii

Coxiella burnetii was identified using Trans-PCR that targets IS1111A transposase gene. The Trans I and Trans II primers were obtained and used to target this region of the gene. The expected product is 687 bp (Berri et al., 2000). PCR was performed using 4 µL of each extracted DNA, with a total volume of 25 µL. The final mixture comprised 2 µM of each primer, 200 µM of each dNTP, 3 mM $MgCl_2$, and 0.5 U of Taq DNA polymerase.

DNA amplification was performed using a thermal cycler (Bio-Rad, MJ Mini Gradient Thermal Cycler, PTC-1148). The trans-PCR thermal program was modified by lowering the annealing temperature and 'touchdown' PCR was performed as suggested by Berri et al., (2000). Following the amplification, the products were analyzed with 1.5% agarose gel electrophoresis. Bands with 687 bp in length were considered as *C. burnetii*. *C. burnetii Nine-Mile I* strain obtained from Ankara University, Veterinary Faculty was used as a positive control. ddH_2O was used as a negative control.

Detection of the Chlamydia abortus

PCR analysis targeted the polymorphic membrane protein (pmp) gene of *C. abortus* (Greco et al., 2005). For this purpose, a total volume of 25 µL was used for the PCR reaction, consisting of 2.5 µL 10X PCR Buffer, 0.5 µL dNTP mix, 2 µL $MgCl_2$, 12.75 µL H_2O, 1 µL primers (20 pmol/µL), 0.25 µL Taq polymerase and 5 µL DNA. The reaction's thermal cycling was conducted by optimizing the methodology outlined by Greco. et al., (2005). The amplified products were examined using 1.5% gel electrophoresis, focusing on those with a size of 300 base pairs. *C. abortus* DNA previously obtained from sheep abortion and confirmed by Real-time PCR was used as a positive control (Büyük et al., 2020). ddH_2O was used as a negative control.

RESULTS

This study investigated of *C. burnetii* and *C. abortus* in the sera and vaginal swabs of sheep. Out of 100 sheep with aborted history for *C. burnetii*, 41 were negative, 15 were doubtful, 24 were positive, and 20 were strongly positive (Figure 1). 44 of 100 sheep's sera were determined to be positive for *C. burnetii* (Figure 2). *C. abortus* was investigated by indirect ELISA assay among the sheep's sera. Five out of 100 sera samples from sheep tested were positive for OEA. Three samples positive for *C. abortus* were also positive for Q fever (Figure 3). The molecular analyses of the vaginal swabs were found to be negative for both agents.

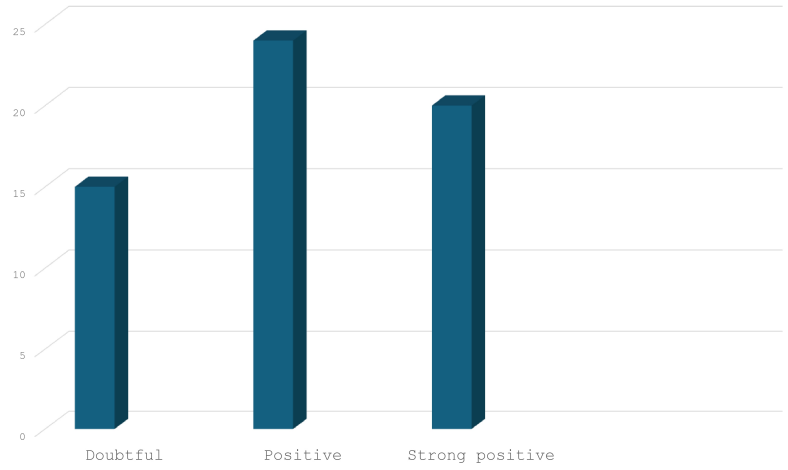

Figure 1. *Results of indirect ELISA for Q fever according to their ODs*

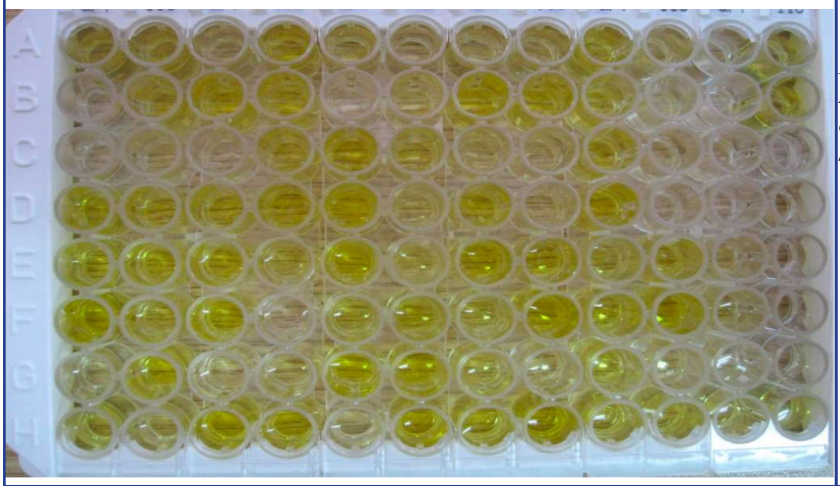

Figure 2. *View of the ELISA on the microplate. A-1: Positive control, B-1: Negative control, other wells show samples with positive and negative reactions*

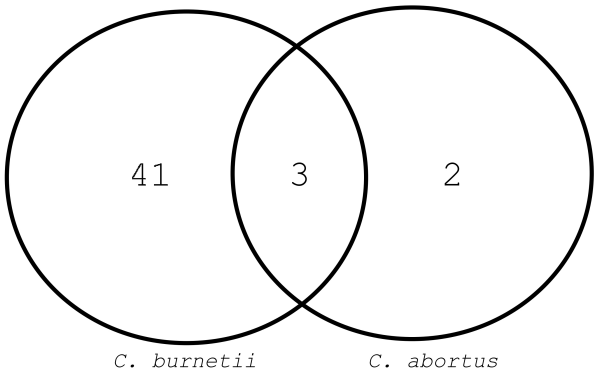

Figure 3. *Total prevalence of both pathogens in Iğdır province*

DISCUSSION

The etiology of abortion in ovine and caprine species is multifactorial, resulting from infection by a range of bacterial, viral, parasitic, and mycotic agents (Ali et al., 2022; Gülaydın et al., 2023). A major concern for breeders is the economic impact of abortion on sheep and goats. This study investigated the occurrence of *C. abortus* and *C. burnetii* infections, which are important abortigenic agents, in aborted sheep in the Iğdır province using serological and molecular methods. This was tested using an indirect ELISA. The observed antibody response indicates a natural immune response to microbial exposure, given that there is no vaccination program against both factors in Türkiye.

Coxiella burnetii is a zoonotic agent that has the potential to infect humans and many domestic animals (McQuiston and Childs, 2002), as well as healthy animals and the environment through aborted fetuses and fetal fluids, and secretions of infected animals (Kılbaş et al., 2023). As a result of reviewing worldwide studies on the prevalence of Q fever in livestock, it has been reported that the seroprevalence of Q fever levels is 15-27% in many countries, regardless of species. According to meta-analyses by Guatteo et al., (2011) and Nokhodian et al., (2017), the seroprevalence rates of *C. burnetii* in sheep and goats are closely compatible with findings from various global meta-analyses and systematic reviews. In Türkiye, Kılbaş et al., (2023) reported an overall prevalence of 13.49% in animals and an average prevalence of 19.1% in sheep for *C. burnetii*. In a study conducted on sheep by Kılıç and Kalander, (2016), the percentage of *C. burnetii* in the Eastern Anatolian region was determined by ELISA to be 16% in aborted sheep flocks and 7.60% in healthy sheep flocks. Karagül et al., (2019) stated that in their study conducted on sheep in Düzce province, the overall rate of seropositivity for Q fever was determined to be 26.38%. However, they determined the seropositive herd rate to be 50%, higher than the total seroprevalence. There are also studies aimed at investigating the status of *C. burnetii* in animals in the Northeast Anatolian region. In a study conducted on small ruminants in this region, *C. burnetii* was detected in 24.4% of sheep (Serifoğlu Bagatir et al., 2021). A study carried out in the province of Kars showed that 43.2% of the sheep were positive for *C. burnetii* using the ELISA (Gülmez Saglam and Şahin, 2016). In the current study, out of 100 sheep with aborted history for *C. burnetii*, 15 samples were found doubtful, 24 were positive, 20 were strong positive and, 41 were negative. 44 of the 100 sheep's sera were determined to be positive in terms of antibodies against phase I and phase II *C. burnetii*. Compared to other national and international studies, the overall positivity rate of *C. burnetii* in this research is higher than that reported previously. Following the study, the seroprevalence of *C. burnetii* was 44%, pointing to a considerable health risk for both animals and humans in these research areas. The importance of detecting *C. burnetii* increases, especially since small ruminants are considered reservoirs for humans.

Chlamydia abortus is an important bacterial pathogen because it causes abortions in sheep and can also cause infection in humans. Although it mostly causes infections in small ruminants, it can also infect other animal species (Sillis and Longbottom, 2011). In a study conducted by Hamedi et al., (2020) in Iraq, *C. abortus* was detected in 23.5% of aborted fetuses. In a study conducted in Germany by Runge, (2012), *C. abortus* was detected in 49% of sheep. In Iran, the seroprevalence value of *C. abortus* was reported to be 26.5% in small ruminants. (Esmaeili et al., 2015). Iraninezhad et al., (2020) found 44 (9.70%) of 452 sera positive for *C. abortus* in sheep and goats from Khorasan Razavi province in north-eastern Iran.

In Turkey, most cases of OEA-related abortion in domestic animals have been investigated using serological and molecular methods, and *Chlamydia* spp. have been detected at rates ranging from 1.56% to 32% (Gökçe et al., 2007; Karagül et al., 2019; Kaya and Öztürk, 2020; Küçükayan et al., 2007; Otlu et al., 2007, Öztürk et al., 2016; Türütoğlu et al., 2000). The seroprevalence of enzootic abortion in sheep was found to be 20.83% in a study conducted in Türkiye (Karagül et al., 2019). Çaya et al., (2006), in their study conducted in 9 provinces in the south-eastern and Mediterranean regions, detected *C. abortus* at rates varying between 2.5-30%, and *C. abortus* was detected at significant levels in most provinces. In Kars, the neighboring province of Iğdır, where the current study was conducted, Gökçe et al., (2007) found a seropositivity rate of 13.9% in aborted sheep and 8.33% in cattle. Otlu et al., (2007) found the seropositivity rate to be 5.4% in their study of aborted sheep in Kars. In a study conducted by Öztürk et al., in Burdur province in 2016, the disease prevalence in sheep was determined to be 32.0% by ELISA.

In the current study, serum samples from aborted sheep were tested by ELISA. *C. abortus* was detected in 5 of 100 aborted sheep. The average rate of enzootic abortion in the Iğdır region is low compared to other studies conducted in Türkiye. When results for both *C. burnetii* and *C. abortus* are compared with other studies, geographical location, test type and efficacy, race, sample size and type, grazing strategy and population density may play a role in differences in results (Abushahba et al., 2017; Radostits et al., 2007).

Given that *C. burnetii* and *C. abortus* are obligate intracellular pathogens, laboratory investigation was not feasible. Consequently, this study examined 100 vaginal swabs collected from aborted ewes via molecular analysis for these agents. Both agents were not identified using the molecular method. Among the reasons why molecular methods were negative is that after miscarriage, the shedding of pathogens is intense but decreases over time and is intermittent. Therefore, vaginal swabs do not have a sufficient bacterial load. Serologic tests used in epidemiologic studies show that the organism was previously exposed to the disease but do not show bacteremia or that it is still shedding the agent. Serologic tests and molecular methods may give different results.

CONCLUSION

As a result, it was observed that the seroprevalence of Q fever in small ruminants was high in the Iğdır province. It can be said that *C. burnetii* is one of the main causes of abortion, given the low abortion rate evaluated in the study. The diagnosis of both diseases is an important step in the identification of cases of abortion in flocks and the establishment of effective control measures. The results

of this study show a high incidence of Q fever in sheep in Iğdır province, followed by a lower seroprevalence for OEA. These results underlined the potential risk of the pathogens studied for animal and public health. In the Iğdır province, where the study was conducted, sheep farming is done as family-type enterprises. The animals are cared for, fed and milked using traditional methods. This situation poses a risk of transmission to humans of agents that may be excreted in milk. Based on these findings, prevention and control measures should be based on their potential impact on animals and humans. Additionally, it is recommended that further studies be conducted to improve comprehension of the transmission mechanisms of this pathogen and to develop strategies for the mitigation of the associated risks. Exploring potential reservoirs and intermediate hosts, improving surveillance systems, and enhancing biosecurity measures are crucial steps in controlling the spread of the pathogen.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical statement

The ethical approval of the study was obtained from the Local Ethics Committee for Animal Experiments of Kafkas University (Türkiye) (KAU-HADYEK/2023/168).

Availability of data and materials

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

Author contributions

Conceptualization was carried out by AGS, SK, and GK.; methodology, validation, and data curation, AGS, EÇ, SD, and MY; writing—original draft preparation, AGS, EÇ and SD; writing—review and editing, AGS, SK, and GK. All authors have read and agreed to the published version of the manuscript.

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Complications and therapeutic approaches in a sciatic nerve injury rat model

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Abstract
Sciatic nerve injury (SNI) is a common model for studying peripheral nerve damage and regeneration. This study investigates the complications associated with acute nerve injury (ANI) by laceration of sciatic nerve in rats including infection, edema, and cannibalism, and evaluates the effectiveness of therapeutic interventions to modulate the observed complications. For this purpose eighteen female wistar albino rats were divided into three groups: control, sham-operated, and ANI. The ANI model induced with dissection and repair of the right sciatic nerve. Post-surgical care included the administration of diclofenac sodium for pain management. Observations were made for signs of infection, edema, hematoma, and survival rates within 10 days. The ANI group showed significant complications, including a 41.6% incidence of symptoms of pain (paraesthesia, allodynia, hyperalgesia, decreased activity, piloerection, excessive licking, un-groomed appearance) within 3 days, which increased to 60% by day 5. Edema was observed in 8.3% of the ANI rats, and 33.3% developed hematomas. Cannibalism rates also increased, particularly within 10 days post-injury. Survival rates in the ANI group decreased to 16.6% by day 10, indicating severe post-operative complications. The current study highlights the critical complications associated with ANI in rats, particularly the high rates of pain related symptoms (i.e. paresthesia and cannibalism). These findings suggest the need for improved post-operative care and highlight the importance of therapeutic interventions like opioid analgesics to mitigate these complications and enhance recovery outcomes in peripheral nerve injury models.

Keywords: *Complications, laboratory animal models, sciatic nerve injury, survival*

INTRODUCTION

Sciatic nerve injury (SNI) often occurs due to trauma, disc herniations, or prolonged pressure, leading to pain, numbness, and muscle weakness in the lower extremities. Current medical treatment for sciatic nerve injury focuses on pain relief and functional restoration. Initial management often includes physical therapy and pain medications such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Minimally invasive procedures like nerve blocks can also provide relief. In severe cases, surgical intervention may be necessary to repair nerve damage (Ellis & Bennett, 2013). Emerging treatments, are being explored for their potential to reduce inflammation and promote nerve healing, offering hope for improved outcomes in the future. The ANI model of the sciatic nerve is widely used; however, there is limited information available regarding complications associated with ANI in rats.

Complications arising from sciatic nerve injury in rats encompass a range of physiological and behavioral issues, including infection, cannibalism, and altered recovery dynamics. The sciatic nerve injury model is widely utilized in research to explore the mechanisms of nerve regeneration and the associated complications. Infection is a significant concern following sciatic nerve injury, particularly in experimental settings where surgical procedures may introduce pathogens (Zhang et al., 2020) including peripheral nerve repair and regeneration. Symptoms of pain in rodent model of nerve injuries include paraesthesia, allodynia, hyperalgesia, decreased activity, piloerection, excessive licking, un-groomed appearance (Austin et al., 2012). Cannibalism, particularly in the context of maternal behavior, can also emerge as a complication in animal models. Research has shown that stressors, including those induced by environmental changes or injury, can lead to increased rates of cannibalism among rat mothers, particularly towards their offspring (Kusama-Eguchi et al., 2016). To sum up, complications following nerve injury in rats, including infection and cannibalism, are influenced by a myriad of factors such as inflammation, hormonal responses, and age-related changes in regenerative capacity. Understanding these complexities is essential for developing effective therapeutic strategies to enhance recovery and mitigate adverse outcomes in both experimental and clinical settings.

The aim of the present study is to investigate the observable complications and survival rates in rats subjected to acute nerve injury induced by sciatic nerve laceration.

MATERIALS AND METHODS

The experimental protocol of this study was approved by the Local Animal Studies Local Ethics Committee (No: 2024-027). Eighteen female rats (Wistar albino, 220-300 g) used in the study were individually housed during the experiment under standard controlled conditions (12 hrs dark/light cycle, at 22±2EC). Rats freely accessed food and water during housing. All procedures were performed according to the 'Principles of Laboratory Animal

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Experimental groups

Animals were randomly separated into 3 experimental groups, 1. Control (n = 3), 2. Sham-operated (n = 3), 3. Acute nerve injury (ANI, n=12) + Diclofenac (10 mg/kg/day, s.c.). Diclofenac sodium (Deva®), Ceftriaxone (Menarini®), Isoflurane (Adeka ®) were used for experiments. 0,9% NaCl were administered for controls, sham-operated and ANI groups (Bostan et al., 2018).

Experimental procedure

An experimental ANI model was performed. Rats in all experimental groups were anesthetized with 5% isoflurane and 1.5-2% oxygen concentration before surgery and maintained with 1.5-2% isoflurane and 1.5-2% oxygen concentration. Ceftriaxone (30 mg/kg, intraperitoneal,i.p.) was administered prophylactically to the groups before surgery. For the SNI group, the right sciatic nerve in each rat was dissected and cut to create a sciatic nerve injury model ((Bostan et al., 2018; Sonohata et al., 2023), and then repaired with sutures. All surgical procedures were performed by a single surgeon who is experienced on microsurgery (hand surgeon) using a standard surgical microscope. The right sciatic nerve was dissected and cut with a scalpel near the popliteal bifurcation through a skin incision. Subsequently, all injured nerves were repaired with primary end-to-end epineural sutures (Ethilon 8-0, Ethicon) (Figure 1). Rats received Diclofenac sodium (40 mg/kg) intraoperatively in operation area and the wounds were closed with 9-0 Vicryl (H. Kaya et al., 2021).

Post-experimental procedure and surgical care

All surgical procedures were performed under aseptic conditions. Rats received Diclofenac sodium (40 mg/kg/day) every 8 hours for three doses for postoperative pain. After surgery, all rats were given at least 3-4 hours of recovery and then placed in their individual cages (Bostan et al., 2018; Sonohata et al., 2023). Diclofenac sodium (40 mg/kg/day) administered once daily for 7 days after the protocol (Kaya et al., 2021; Kaya & Alizade, 2022).

Throughout the experimental process, changes in the animals were monitored and recorded by the facility's veterinarians and the researchers. Indicators of infection, such as hypothermia, reduced food and water intake, postural disturbances, blood in the stool, and decreased activity, were carefully documented. Signs of pain were evaluated through behavioral and physical indicators, including vocalization, guarding of the affected limb, reluctance to move or bear weight, postural disturbances, and decreased activity levels.

Edema was assessed through visual inspection for soft, swollen tissue at the injury site or along the hind limb, complemented by palpation. Paresthesia was evaluated based on behavioral signs, including excessive grooming or biting of the affected limb, as well as hypersensitivity or withdrawal reflexes in response to sensory stimuli, such as touch. Cannibalism was identified by the presence of self-inflicted wounds or missing tissue, particularly in the area surrounding the nerve injury.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism. Descriptive variables were presented as percentage distributions. Chi-square test is used for statistical analysis. A p-value of less than 0.05 was considered significant.

RESULTS

Paresthesia and cannibalism observed in the same ratio in the same animals in the operated legs. Similarly excessive licking, allodynia and need of stitches of operated legs also started within 3 days (Figure 2). Edema and hematoma only observed within 3-7 days. Signs of infection including hypothermia, decreased food and water intake, sign of disturbances in posture, blood in stool and decreased activity (as a sign of pain and infection) started in 3rd day of operation and observed in 10 days (Table 1). Cannibalism, a serious complication observed in this study, led to the sacrifice of animals during the 10-day experimental period in accordance with ethical guidelines. The inclusion of the 3-10 day range for edema and hematoma in the table was likely chosen based on the observed timeline of the onset of these complications and progression. In this study, edema and hematoma were not present within the initial 0-3 day period but were observed between 3-10 days post-injury. Therefore, this timeframe is critical to capture the development of these complications.

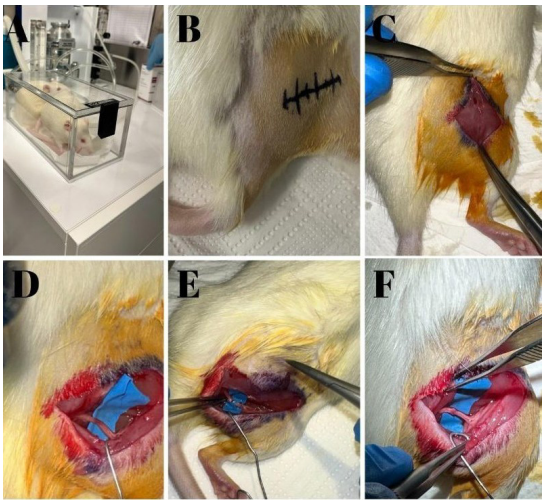

Figure 1. *SNI operation of rats. Shown are steps development of SNI model in rats; Anesthesia (A), Marking (B), Skin dissection(C), Isolation of sciatic nerve (D-F)*

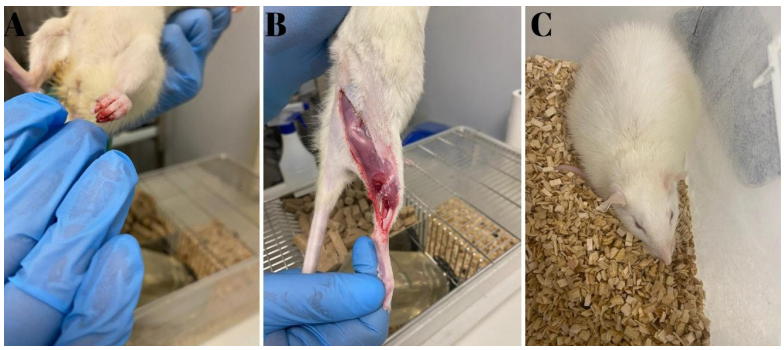

Figure 2. *Complications observed in rats with SNI. Shown are excessive licking (A), cannibalism (B) and piloerection with disturbance in posture (C)*

Table 1. *Observed complications of the experimental groups*

N (%)	Control (N=3)	Sham (N=3)	SNI (N=12)	p
Signs of infection within 3 days*	0/3 (0)	0/3 (0)	5/12 (41,6)	
Signs of infection within 5 days*	0/3 (0)	1/3 (33,3%)	6/10 (60%)	< 0.0001***
Signs of infection within 10 days*	0/3 (0)	2/3 (66,6%)	4/6 (33,3%)	
Edema within 3-5 days	0/3 (0)	0/3 (0)	1/10 (10%)	
Hematoma within 3-5 days	0/3 (0)	0/3 (0)	3/10 (30%)	
Paresthesia within 3 days	0/3 (0)	0/3 (0)	0/12 (0)	
Paresthesia within 5 days	0/3 (0)	0/3 (0)	1/10 (10%)	< 0.0001***
Paresthesia within 10 days	0/3 (0)	0/3 (0)	4/6 (66,6%)	
Allodynia in operated leg within 3 days	0/3 (0)	0/3 (0)	5/12 (41,6)	
Allodynia in operated leg within 5 days	0/3 (0)	0/3 (0)	6/10 (60%)	0.0002***
Allodynia in operated leg within 10 days	0/3 (0)	0/3 (0)	4/6 (66,6%)	
Decreased activity within 3 days	0/3 (0)	0/3 (0)	5 (41,6)	
Decreased activity within 5 days	0/3 (0)	1/3 (33,3%)	6 (60%)	0.0002***
Decreased activity within 10 days	0/3 (0)	2/3 (66,6%)	4/6 (66,6%)	
Piloerection within 3 days	0/3 (0)	0/3 (0)	5/12 (41,6)	
Piloerection within 5 days	0/3 (0)	0/3 (0)	6/10 (60%)	0.0700
Piloerection within 10 days	0/3 (0)	1/6 (33,3%)	4/6 (66,6%)	
Excessive licking within 3 days	0/3 (0)	0/3 (0)	1/12 (8,33%)	
Excessive licking within 5 days	0/3 (0)	0/3 (0)	1/10 (10%)	< 0.0001***
Excessive licking within 10 days	0/3 (0)	0/3 (0)	4/6 (66,6%)	
Un-groomed appearance within 3 days	0/3 (0)	0/3 (0)	5/12 (41,6)	
Un-groomed appearance within 5 days	0/3 (0)	1/3 (33,3%)	6/10 (60%)	0.0002***
Un-groomed appearance within 10 days	0/3 (0)	2 /3 (66,6%)	4/6 (66,6%)	
Cannibalism within 3 days	0/3 (0)	0/3 (0)	0/3 (0)	
Cannibalism within 5 days	0/3 (0)	0/3 (0)	1/10 (10%)	< 0.0001***
Cannibalism within 10 days	0/3 (0)	0/3 (0)	4/6 (66,6%)	
Survival (N,%) within 3 days	3/3 (100%)	3/3 (100%)	10/12 (83,3%)	
Survival (N,%) within 5 days	3/3 (100%)	3/3 (100%)	6/12 (50%)	< 0.0001***
Survival (N,%) within 10 days	3/3 (100%)	2/3 (66,6%)	2/12 (16,6%)	
Need of stitches within 3 days	0/3 (0)	0/3 (0)	2/12 (16,6%)	
Need of stitches within 5 days	0/3 (0)	0/3 (0)	4/10 (40%)	0.0017**
Need of stitches within 10 days	0/3 (0)	0/3 (0)	4/6 (66,6%)	

*Signs of infection: hypothermia, decreased food and water intake, sign of disturbances in posture, blood in stool

Chi-square test is used for statistical analysis. p<0.01: **; p<0.001: ***

DISCUSSION

ANI of sciatic nerve is a widely used model for studying peripheral nerve injuries due to its reproducibility and relevance to clinical conditions. However, the high mortality and morbidity rates observed in this study underscore the need for improved surgical techniques, postoperative care protocols, and preventive strategies. By understanding the complications and risks associated with ANI models, future studies can refine methodologies and contribute to the development of safer and more effective experimental designs. The complications observed during the study were meticulously documented and analyzed across experimental groups. These findings not only provide critical reference data for researchers conducting similar studies but also highlight the importance of addressing the challenges associated with experimental models like ANI.

Paresthesia and allodynia are common complications following sciatic nerve injury in rats, often resulting from neuropathic pain mechanisms that arise during the regeneration process. Paresthesia refers to abnormal sensations such as tingling or prickling, while allodynia is characterized by pain from stimuli that do not normally provoke pain. These phenomena are frequently observed in rodent models of peripheral nerve injury, including those involving the sciatic nerve, where nerve damage can lead to significant alterations in sensory processing and pain perception (Guo & Gu, 2014; Liu et al., 2019). Research indicates that following sciatic nerve injury, there is a marked increase in the expression of proteins associated with inflammation and nerve regeneration, which can contribute to the development of neuropathic. For instance, the upregulation of immune-related genes and inflammatory cytokines, such as interleukin-6 (IL-6) and interleukin-10 (IL-10), has been documented in the context of nerve injury, suggesting a robust immune response that may exacerbate pain conditions like allodynia (Huang et al., 2023; Xing et al., 2017). Moreover, cannibalism, or self-mutilation behavior, is another severe consequence observed in rodent models following sciatic nerve injury which may require sacrification during the experimental processes in accordance to ethical concerns, as in this study. This behavior is often linked to the intense pain and sensory abnormalities that arise from nerve damage, leading to self-inflicted injuries as the animals attempt to alleviate their discomfort (Heinzel et al., 2020). The phenomenon of autotomy, where animals remove or injure their own limbs, is particularly noted in cases of severe neuropathic pain, which can be induced by various types of nerve injuries, including complete transection or chronic constriction injuries (Andersson et al., 2018; Guo & Gu, 2014).

Observational studies of rats with sciatic nerve injury reveal significant complications such as edema and necrosis, which are critical to understanding the underlying mechanisms of nerve damage and recovery. The sciatic nerve, being the largest peripheral nerve, is particularly susceptible to injury due to its anatomical location and the nature of trauma it often endures. In experimental models, such as those involving sciatic nerve crush injuries, the immediate response includes inflammation characterized by edema, which is a common reaction to nerve trauma. This inflammatory response is mediated by various cytokines, including tumor necrosis factor-alpha (TNF-α), which plays a pivotal role in the pathophysiology of nerve injury and subsequent regeneration (Zhang et al., 2020). Furthermore, the upregulation of pro-inflammatory cytokines such as IL-1β and IL-6 has been documented in the context of peripheral nerve injuries, contributing to both edema and necrosis (Yu et al., 2023). Although inflammatory processes are considered one of the primary contributors to pain, NSAIDs may not be sufficient for managing pain and inflammation in the case of SNI. This insufficiency was also observed in the operated animals in this study, which showed signs of inflammation, pain, edema, and necrosis. Opioids may be more effective for SNI-related pain; however, the role of inflammation should not be overlooked. Adjuvant corticosteroid therapy could be considered part of the standard treatment protocol, although further studies are needed to establish solid evidence for its routine use in SNI models in rats.

Non-steroidal anti-inflammatory drugs (NSAIDs), particularly diclofenac, are commonly used to alleviate pain and inflammation in sciatic nerve injury in clinical practice and in vivo studies. Diclofenac's potent anti-inflammatory effects help reduce nerve compression and associated discomfort (Kaya & Alizade, 2022). Inflammation is also a significant concern following sciatic nerve injury, particularly in experimental settings where surgical procedures may introduce pathogens that can also be controlled with NSAIDs. The inflammatory response triggered by nerve injury can create an environment conducive to inflammation, complicating recovery. For instance, cytokines play a crucial role in mediating inflammation and immune responses post-injury, with studies indicating that their expression patterns significantly change following nerve damage (Zhang et al., 2020). The presence of pro-inflammatory cytokines can exacerbate tissue damage and delay recovery, highlighting the importance of managing inflammation to prevent secondary complications such as infection (Feng & Yuan, 2015). Along with the other research studies our study demonstrated that inflammation might be considered as a risk factor in rats with SNI along with the pain that diclofenac use failed to control in this experimental process which is also observed in increasing mortality within 10-day period. This study demonstrated that, although previous research reported fewer complications and higher survival rates in rats with SNI, future studies should consider its potential impact on mortality. Researchers should also take precautions, such as closely monitoring for signs of inflammation or loss of sensation, particularly within the first five days post-operation.

Moreover, therapeutic strategies aimed at mitigating these complications have been explored. For example, the administration of anti-inflammatory agents like dexamethasone has shown promise in enhancing functional recovery and reducing the extent of edema and necrosis following SNI (Feng & Yuan, 2015; Sun et al., 2012). These treatments can help modulate the inflammatory response, thereby promoting a more conducive environment for nerve repair and improve survival although in case of systemic inflammation there is conflicting data that corticosteroid use (Feng & Yuan, 2015; Sun et al., 2012). These treatments can help regulate the inflammatory response, creating a more favorable environment for nerve repair. However, NSAIDs may not be the most

effective option for managing pain and inflammation in sciatic nerve injury (SNI). While opioids offer stronger pain relief, they should be used with caution. Adjuvant corticosteroid therapy may be beneficial in controlling inflammation. Additionally, post-operative use of extended spectrum antibiotics might be necessary, as pre-operative administration alone may not sufficiently prevent infections. Strict adherence to surgical sterilization protocols is crucial, and close monitoring of the wound site, particularly within the first 5 days after surgery, is essential. Frequent checks, ideally every 6 hours, are recommended to detect early signs of infection and enable timely intervention, which could improve overall recovery outcomes in SNI.

Limitations

This study is observational, meaning no direct interventions were tested to address the complications. Therefore, while the findings are valuable for understanding the complications associated with sciatic nerve injury (SNI), the results may be limited in terms of establishing causality or offering definitive therapeutic solutions. Secondly, regular monitoring of biochemical parameters, such as inflammatory markers or cytokine levels, could provide additional insight into the physiological processes underlying complications like infection and inflammation. Lastly, the study only tracked complications over a 10-day period, which may not capture the long-term outcomes of sciatic nerve injury recovery. Extending the follow-up period could provide a more comprehensive understanding of both the progression of complications and the effectiveness of potential interventions over time for researchers.

CONCLUSION

This study highlights the significant challenges associated with the acute sciatic nerve injury (ANI) model in rats, particularly the high mortality and morbidity rates observed during the postoperative period. Complications such as inflammation, edema, hematoma, paresthesia, allodynia, and cannibalism were meticulously recorded, providing critical insights into the pathophysiology and behavioral consequences of ANI. The findings demonstrate that while NSAIDs like diclofenac can offer some relief, they are insufficient in managing the severe pain and inflammation associated with this model. Alternative therapeutic strategies, such as the incorporation of opioids, corticosteroids, and enhanced postoperative care protocols, should be explored in future studies to improve survival rates and reduce complications.

Furthermore, the detailed documentation of observed complications contributes valuable reference data for researchers utilizing SNI models, emphasizing the need for close monitoring and improved pain management strategies in experimental settings. These results not only underscore the importance of refining surgical and postoperative care techniques but also pave the way for the development of safer and more effective therapeutic approaches in peripheral nerve injury research.

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Conflict of Interest

NA

Ethical Statement

The experimental protocol of this study was approved by the Local Animal Studies Local Ethics Committee (No: 2024-027).

Author Contributions

VO and AED completed the experimental model. EB conducted the statistical analyses and figures. All authors write the main article and revised.

Availability of Data and Materials

The data and materials supporting the findings of this study are available from the corresponding author upon reasonable request.

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Investigation of gastrointestinal biomarkers in dogs with diarrhea

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Abstract

In this study, it was aimed to evaluate the zonulin and lactate levels, which are among the gastrointestinal biomarkers, in dogs with diarrhea due to various reasons. Thirty dogs with diarrhea and 15 healthy dogs, which were not classified as etiological, were included in the study. Blood samples were taken from Vena cephalica antebrachii of each dog in accordance with the technique. Serum zonulin levels were measured from the blood samples collected in tubes without anticoagulant, using the commercial test kit based on the ELISA principle. Plasma lactate levels were measured using a handheld analyzer from blood samples taken into heparinized tubes. While serum zonulin concentrations were found to be 9.80 ± 6.7 ng/mL in dogs with diarrhea, serum zonulin concentrations were found to be 1.94 ± 1.4 ng/mL in healthy dogs. It was determined that the plasma lactate levels of the dogs with diarrhea were 9.02 ± 4.7 mmol/L, whereas the plasma lactate concentrations of the healthy dogs were 1.21 ± 1.4 mmol/L. In the statistical evaluation, both zonulin and lactate concentrations were found to be highly significant ($p< 0.05$) for dogs with diarrhea. As a result, it was concluded that the gastrointestinal biomarkers zonulin and lactate levels increased in blood in dogs with diarrhea and that zonulin and lactate could be taken into account in detecting the damage that may occur in the intestines due to diarrhea, and these markers could be used in the follow-up of the prognosis and treatment of diarrhea.

Keywords: *Diarrhea, dog, lactate, zonulin*

INTRODUCTION

Gut health not only contributes to the prevention of various diseases but has also become a significant focus among nutritionists, veterinarians, and scientists (Kogut and Arsenault, 2016). Generally, gut health is discussed under six main areas: diet, digestion and absorption, normal and balanced microbiota, immune status, intestinal mucosa, and neuroendocrine-gut motor functions. Understanding these functions plays a crucial role in animal health, welfare, and performance (Celi et al., 2017). Diarrhea, the primary indication of impaired gut health in dogs, notably affects young dogs and is one of the leading causes of mortality in this age group (Mila et al., 2017; Münnich and Küchenmeister, 2014). Diarrhea in dogs is categorized as infectious or non-infectious based on etiology, and as acute (lasting less than 14 days) or chronic based on duration, making this classification highly relevant both clinically and for research (Nind, 2011; Schulz et al., 2008; Volkmann et al., 2017; Willard, 2013). The pathogenesis of diarrhea involves absorption disorders associated with damage to the villi and microvilli structures in the small intestine, while in the large intestine, it is linked to an increase in fecal water content due to inadequate water and electrolyte absorption by colonocytes. Moreover, the fermentation of indigestible nutrients by lactic acid bacteria can increase the osmolarity of intestinal contents, potentially exacerbating diarrhea (Burrows et al., 1995; Heyman, 2000; Volkmann et al., 2017). The primary pathology underlying intestinal diseases is leaky gut syndrome, caused by the disruption of the mucosal barrier and the resulting increase in intestinal permeability. Tight junction proteins between intestinal epithelial cells play a critical role in regulating the mucosal barrier, with the zonulin protein controlling the permeability of these connections (Sturgeon and Fasano, 2016). An increase in zonulin levels can lead to heightened intestinal permeability, contributing to the development of inflammatory, autoimmune, and neoplastic diseases. Disruption in the zonulin signaling pathway can alter immune responses and weaken mucosal tolerance, thereby playing a role in the pathogenesis of chronic inflammatory diseases (Fasano, 2012a). Both chronic and acute inflammatory conditions are marked by changes in intestinal permeability and dysbiosis, closely associated with compromised gut integrity. This disruption can allow microorganisms to enter the circulation through the gut, potentially triggering irregular immune responses that result in sepsis, septic shock, and irreversible organ damage (Koh et al., 2006; Vaishnavi, 2013). Hypovolemia and hypoperfusion, frequently observed in sepsis and septic shock, lead to impaired tissue oxygenation, triggering anaerobic metabolism and causing excessive lactate production (Shahrin et al., 2024). As a byproduct of bacterial fermentation, D-lactate levels increase in association with ischemic gut injury and enhanced gut permeability. This increase in D-lactate, released into the portal and systemic circulation, has been linked to D-lactic acidosis in conditions such as short bowel syndrome and exocrine pancreatic insufficiency. The aim of this study is to evaluate lactate and zonulin levels in dogs with acute diarrhea and to examine the relationship of these parameters with intestinal permeability and metabolic responses.

MATERIALS AND METHODS

Animal material

The animal material for this study consisted of 30 dogs of different ages and sexes presenting with diarrhea of unclassified etiology at the Small Animal Clinic of the Department of Internal Medicine, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, and 15 he-

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althy dogs determined to be clinically, hematologically, and biochemically normal. An additional criterion for including the diarrheic animals in the study was that they had not received antibiotics for at least one month, to ensure that zonulin levels would accurately reflect disrupted intestinal permeability.

Sampling procedure

In this study, blood samples were collected from each dog's *vena cephalica* antebrachii into heparinized tubes and serum tubes in accordance with proper techniques. Blood samples in serum tubes were centrifuged for 15 minutes at 3000 rpm to obtain serum samples. The serum samples were stored at -20°C until analysis.

Laboratory analysis

Zonulin and lactate analysis

Serum zonulin levels were determined using a Canine Zonulin ELISA Kit (Cat No. MBS2605074). The results from the test kit were evaluated using an ELX800 microplate reader. Plasma lactate levels were measured using a handheld analyzer (Lactate Pro 2, Arkray, Netherlands).

Statistical analyses

Descriptive statistics were conducted on the numerical data obtained in the study and presented in Table 1. The Shapiro-Wilk was used to check data distribution, revealing that distributions were not normal. Although all data underwent logarithmic (Logn) transformation, the distributions remained non-normal. Therefore, differences between groups were analyzed using the non-parametric Mann-Whitney U test. Data on lactate and zonulin levels were illustrated with box plots. Age and gender information for the healthy and diarrheic dogs was determined using cross-tabulation. All analyses were performed using SPSS® software (version 20.0, IBM, USA), and statistical significance was set at $p < 0.05$

RESULTS

Among the diarrheic dogs included in the study, 14 (46.6%) were male, and 16 (53.4%) were female. Based on age distribution, 18 dogs (60%) were younger than one year, and 12 dogs (40%) were between one and five years old. In the healthy group, gender distribution was balanced, with 7 males and 8 females. The age distribution of healthy dogs showed that 5 were under one year old, 9 were between one and five years, and 1 was older than five years. No statistically significant difference was found in age or gender distribution between healthy and diarrheic dogs. Serum zonulin levels in diarrheic dogs were significantly higher than in healthy dogs ($p < 0.05$). These differences were also depicted in Table 1. When examining lactate levels between diarrheic dogs and healthy dogs, a significant statistical difference was detected ($p < 0.05$). Plasma lactate levels were measured at 9.02 ± 4.7 mmol/L in diarrheic dogs and 1.21 ± 1.4 mmol/L in healthy dogs. Serum zonulin concentrations were determined to be 9.80 ± 6.7 ng/mL in diarrheic dogs and 1.94 ± 1.4 ng/mL in healthy dogs.

DISCUSSION

The identification of diet, digestion, absorption, microbiota balance, immune status, intestinal mucosa, and neuroendocrine-gut motor functions is crucial for monitoring animal health and welfare and assessing the impact of nutritional interventions on animal performance (Celi et al., 2017). Diarrhea is a clinical symptom characterized by an increase in both the fluid content and volume of feces, which can arise from infectious or non-infectious causes. In this study, no etiological classification was made for diarrheic dogs. However, it was noted that diarrhea, particularly in young dogs with immature immune systems, can lead to serious consequences (Mila et al., 2017; Münnich et al., 2014). The finding that 18 of the 30 diarrheic dogs in our study were under one year of age supports this observation. In recent years, the importance of gut health has grown among nutritionists, veterinarians, and scientists (Kogut and Arsenault, 2016). However, there remains a substantial gap in the identification of biomarkers related to gastrointestinal barrier function, permeability, and the gut endocrine system (Celi et al., 2017). Tight junctions between intestinal epithelial cells play a critical role in regulating the mucosal barrier, with zonulin acting as a key protein that reversibly regulates the permeability of these connections. Numerous clinical studies have examined zonulin as a biomarker of intestinal permeability (Fasano, 2012a; Sturgeon and Fasano, 2016). As another biomarker, lactate is present in the D-enantiomer form, typically undetectable in mammalian serum under normal physiological conditions. However, it may increase in serum due to bacterial fermentation in the gastrointestinal tract or as a result of metabolic disorders (Christopher et al., 1990). Increased gut permeability and bacterial overgrowth can lead to elevated circulating D-lactate levels, which, like zonulin, is thus regarded as a significant biomarker of gastrointestinal dysfunction (Peoc'h et al., 2018). In our study, we found statistically significant differences in zonulin and lactate levels between diarrheic dogs and the healthy control group ($p < 0.05$). Specifically, zonulin levels in diarrheic dogs were 9.80 ± 6.7 ng/mL, and serum lactate levels were 9.02 ± 4.7 mmol/L, both significantly higher than in the control group. The gut serves as a critical barrier against harmful substances in the body. Disruption of this barrier and increased permeability can allow pathogens to enter the circulatory system and lead to gastrointestinal symptoms like diarrhea (Fasano, 2012a). Zonulin plays a crucial role in the pathogenesis

Table 1. *Statistical comparison of serum lactate and serum zonulin level*

Group	Lactate (mmol/L)	Zonulin (ng/mL)
Diarrheal	9.02 ± 4.7	9.80 ± 6.7
Healthy	1.21 ± 1.4	1.94 ± 1.4
p-value	0.05	0.05

of many chronic inflammatory bowel diseases (Sturgeon and Fasano, 2016). In our study, high zonulin levels detected in diarrhea cases with various etiologies indicate a direct relationship between this biomolecule and gut health. Moreover, studies in the literature indicate that elevated zonulin levels are associated with systemic diseases such as autoimmune diseases, type 1 diabetes mellitus, and celiac disease (Fasano and Shea-Donohue, 2005; Fasano, 2012b). It has been shown that intestinal permeability begins to increase in individuals prone to type 1 diabetes before the onset of disease symptoms (De Magistris et al., 1996). Arrieta et al. (2009) demonstrated in a mouse study that treatment with the zonulin inhibitor AT-1001 reduced gut permeability, thereby preventing colitis. Zonulin has also been linked to asthma and brain tumors (Díaz-Coránguez et al., 2013; Fasano, 2011). Similar to findings in the aforementioned literature, we observed significant increases in zonulin levels in dogs with acute diarrhea in our study. These findings suggest that zonulin could serve as a biomarker for assessing intestinal permeability in dogs. Lactate, a byproduct of anaerobic glycolysis, is particularly elevated in serum in cases of shock, anemia, respiratory disorders, and gastrointestinal abnormalities (Fall and Szerlip, 2005). Elevated lactate levels are generally considered an indicator of hypoxia and tissue hypoperfusion (Kruse and Carlson, 1987). Besides hypoperfusion and hypoxia, lactate levels may also increase due to various drugs, toxins, mitochondrial defects, and conditions like sepsis (Kruse and Carlson, 1987; Luft, 2001). Chronically elevated lactate levels have been linked to an increased risk of mortality in clinical settings (Hayes et al., 2010, 2011). In this study, the significantly elevated total serum lactate levels in diarrheic dogs serve as an important indicator of increased intestinal permeability and anaerobic metabolism. Studies have shown that in cases with high plasma lactate levels, the mortality rate remains elevated despite treatment (Cortellini et al., 2015; Stevenson et al., 2007). While our study did not monitor mortality in dogs with high lactate levels, these findings underscore the need for future research examining the relationship between lactate levels and mortality.

CONCLUSION

Both biomarkers appear clinically valuable, especially for monitoring prognosis and treatment response in diarrhea cases. Furthermore, we conclude that treatment protocols should avoid medications that could negatively impact gut permeability and mitochondrial function. The routine use of zonulin and lactate levels in veterinary clinics may aid in identifying the underlying pathophysiological mechanisms of diarrhea.

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Conflict of interest

The authors declare no conflict of interest regarding this article.

Ethical stamement

This study was reviewed and approved by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (ADU-HADYEK) in accordance with ethical principles. The ethical approval was granted on 09/07/2020, with the approval number 64583101/2020/036.

Author contributions

Idea/Concept: SP; Design: SP, TŞ; Control/Supervision: SP, TŞ, TÖ; Data Collection and/or Processing: SP, TŞ; Analysis and/or Interpretation: SP, TŞ; Literature Review: SP, TŞ, TÖ; Writing the Article: SP, TŞ, TÖ; Critical Review: SP, TŞ, TÖ; References and Fundings: SP, TŞ; Materials: SP, TŞ.

Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request. All software and materials used in this study are commercially available or described in detail in the Materials and Methods section. Data supporting the findings of this study are included in the main text and supplementary materials.

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Factors affecting beef demand in Türkiye for 2010-2021 and the effects of restrictions imposed during the Coronavirus disease (COVID-19) period

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Abstract
In this study, the beef price, prices of substitute goods (lamb and chicken meat prices), change in national income and effects of COVID-19 on beef demand for the years 2010-2021 is examined using the ARDL Bounds Test Approach. The study finds that in the long run, an 1% increase in income leads to an approximately, on average, 0.54% increase in demand for beef whereas an 1% increase in beef prices results in, on average, an approximately 0.25% decrease in beef demand. Moreover it is determined that, in the long run, an increase of 1% in the price of lamb meat causes, on average, a 0.37% increase in beef demand, and an 1% increase in chicken meat prices results in on average, a 0.11% decrease in beef demand. This study also uses a dummy variable to account for COVID 19 pandemic effect on the demand of beef. According to the findings for this variable, the pandemic, reduces meat demand, on average, 11% with compared to non-pandemic period. As a result, the demand for beef is found to be affected by the income status of the consumer and the prices of the product itself and its substitutes.

Keywords: *Ardl model, beef demand, cattle fattening, covid-19, shri (Stay-at-Home Restriction Index)*

INTRODUCTION

In many countries around the world, a set of restrictions has been in the spotlight with the COVID-19 pandemic starting from China since January 2020. Public authorities have sought to limit the spread of this infectious disease by restricting social activities and face-to-face interactions to eliminate the direct negative effects of the COVID-19 virus on human health (Snuggs & Mcgregor, 2021).

COVID-19, which was responsible forthe death of approximately 6.7 million people as of January 01, 2023, caused radical changes in people's consumption and spending habits by keeping population mobility under control all over the world. Therefore, COVID-19 can be defined as a major disaster affecting the socio-economic structures and social behaviors of societies on a global scale (GCDL (Global Change Data Lab), 2022; Güney & Sangün 2021).

In particular, during the first half of 2020, with the effect of the epidemic, restrictions such as social distancing, limited occupancy and the ban on business activities have made most of the Hotel-Restaurant-Institutional (HRI) businesses struggling to survive (Kerr, 2021). At this point, the contraction in the sectors such as HRI, transportation (airlines), entertainment, etc., also affected the consumption of animal products negatively.

Studies in the meat industry after the pandemic, generally, focused on the changes in consumer's habits concerning about the meat consumption and problems in supply chain caused by the social distance, quarantine environment and similar existing restrictions. In this context, Bracale and Vaccaro (2020) in Italy, Martin-Neuninger and Ruby (2020) in New Zealand, Laguna et al. (2020) in Spain, Aydın and Demir Ayvazoğlu (2022) and Güney and Sangün (2021) investigate the effects of post-pandemic consumption habits in Türkiye and the problems of change in the supply chain.

The aim of this study is to determine the change in meat demand caused by restrictions during the pandemic period and the factors affecting beef demand. The Stay-at-Home Restriction (SHRI) ındex [BSG (Blavatnik School of Government), 2022] and the variables affecting the demand for beef (beef price, substitute pricesand income effect) at a quarterly frequency between 2010 and 2021 are examined by using the ARDL Bounds Test Approach. Thus, the factors affecting the demand for beef in Türkiye have been evaluated in a multidimensional way through the data of Turkish Statistical Institute (TSI).

The SHRI index used in the study is the index showing the level of restriction during the Covid 19 pandemic period. In this index, stay-at-home restrictions are calculated separately for each country with the help of three parameters: strictness, scope and duration (BSG, 2022).

MATERIALS and METHODS

In this study, the beef production in terms of kilogram, product price data for beef, lamb and white meat and national income statistics data in terms of national currency (TL) are included in the study. The source of dataset is Turkish Statistical Institute (TSI, 2022a; TSI, 2022b; TSI, 2022c; TSI, 2022d; TSI, 2022e; TSI, 2022f; TSI, 2022g). This study, as mentioned above, uses quaterly frequency data cover 2010-2021 in Türkiye. The definition of the dataset can be seen at Table 1.

The pre-analysis data of the variables are given in Figure 1. In this study, it is found that all of the variables show trends, and are seasonal variations. By using Holt-Winter exponenetial smoothing method, which is a statistical technique to detect if a time series has trend and/or seasonality, the study finds that two variables

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Table 1. *Definitions of data and variables used in the study*

LSET_SA = *f* (LSEF, LKEF, LTEF, LG_SA, Dummy 1, Dummy 2)

Variables	Definitions
LSET_SA	Seasonally Adjusted Natural logarithm of beef demand (Kg)
LSEF	Natural logarithm of beef price (TL)
LKEF	Natural logarithm of lamb meat price (TL)
LTEF	Natural logarithm of poultry meat price (TL)
LG	Natural logarithm of nominal national income (TL)
Dummy Variable 1 (COVİD pandemic)	Dummy variable takes 1 at and after 2020 Q1, 0 otherwise
Dummy Variable 2 (Decrease in imports)	Dummy variable takes 1 only at the third quarter of 2019, 0 ortherwise

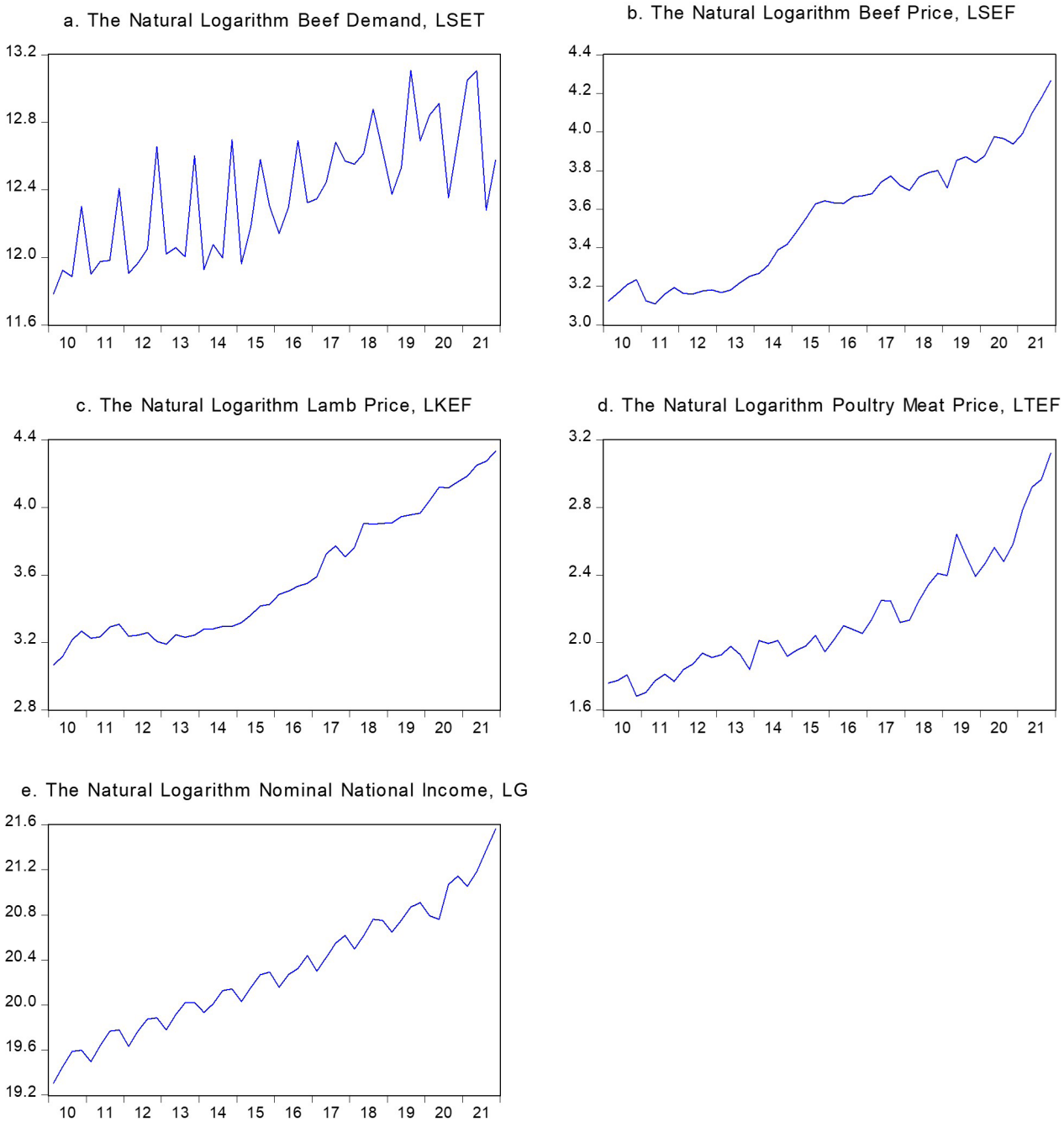

Figure 1. *Graphs of the data used in the study*

(the beef demand and income variables) have seasonal components. Therefore, these two series are adjusted for seasonality using the TRAMO-SEATS method which is very common approach for desesonalization. Thus, 'Beef demand' is called as 'Seasonally adjusted LSET (LSET_SA) and National income variable is called as 'Seasonally adjusted LG (LG_SA).

Afterthat, Augmented Dickey Fuller Unit Root Test (ADF) was applied to the variable under study to determine whether the series are stationary or not. If all series are stationary, time series literature suggests that Vector Autoregressive Model (VAR in short) is used to study relationship between variables. If all series become stationary after taking the first difference, Vector Error Correction Methods (VECM in short) is applied to dataset. On the other hand, if the variables are the mixed of stationary at level and after taking the first difference, ARDL is used for the further analysis of the series. Further, ARDL method does not require the unit root tests for the stationarity in advance, but all the studies in the literature run the unit root tests to make sure none of the variables integrated higher than the first degree. If any variable has a degree of integration higher than order 1, it is not appropriate to use ARDL. Instead, the preferred method is the Toda-Yamamoto method (Yamamoto, 1991). After investigation of time series properties of the variables with ADF tests, ARDL analysis method is used to investigate the cointegration relationship between the variables since as seen in the Table 2, which provides the unit root tests results, none of the variables under concern are integrated more than order 1.

Pesaran and Shin (1999) and Pesaran et al. (2001) suggest the Autoregressive Distribution Bound (ARDL) Test approach as a cointegration technique to investigate the existence of both short run and a long run relationship. The equation for the long-term and estimations of the associated short-term parameters in the study are given below:

RESULTS

In Figure 2, seasonally adjusted beef demand shown. When figure 2 is analysed, shows the deviation in the 3rd quarter of 2019 from the longrun trend. This is due to the increase in meat production due to the Eid al-Adha and the fall in real beef prices, which led to an increase of demand in the third quarter of 2019. Due to this sharp change, a dummy variable is included in the model for this point (Dummy 2, see Table 1 for its definition).

The results of the Augmented Dickey Fuller (ADF) test to determine the degree of integration of the relevant variables are given in Table 2. According to the results given in Table 2, the dependent variable LSET_SA is found as a stationary variable, whereas all other variables become stationary after taking the first differences. Therefore, ARDL approach can be applied to model estimation since there are a mixture of I(1) and I(0). Further the advantage of this method is that valid results are obtained regardless of whether the variables are I(1) or I(0) or a combination of the two.

With the ARDL bounds test, it is possible to determine both short-term relationship dynamics and long-term relationships between variables. Before interpreting the regression, results are obtained from ARDL estimations (ARDL (6, 5, 6, 0, 6), error diagnostics tests for any standard ARDL approach are conducted and the results from them along with cointegration results as short-term and long-term estimations are presented in Table 3.

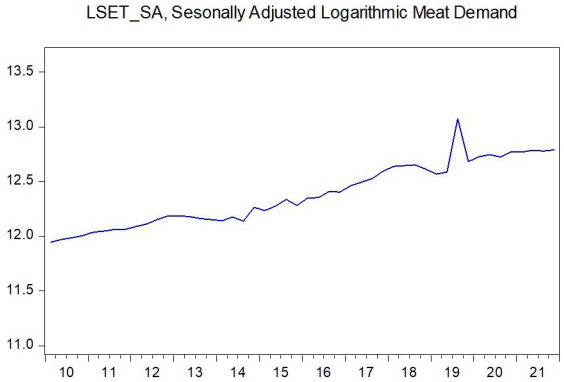

Figure 2. *Graph of seasonally adjusted beef demand variable*

Table 2. *ADF test results for variables*

Variable	Level		First Difference	
	Constant	Constant +Trend	Constant	Constant +Trend
LSET_SA	-0.5780	**-5.3560*****		
LSEF	1.0779	-1.7033	**-5.8949*****	**-6.1706*****
LKEF	2.5996	-0.9195	**-3.3463****	**-8.1308*****
LTEF	1.2227	-1.1360	**-6.8510*****	**-6.8153*****
LG_SA	2.3125	1.5139	**-7.2936*****	**-7.8495*****

***, ** and * denote statistical significance levels of 1%, 5% and 10%, respectively. Statistically significant results are in bold.

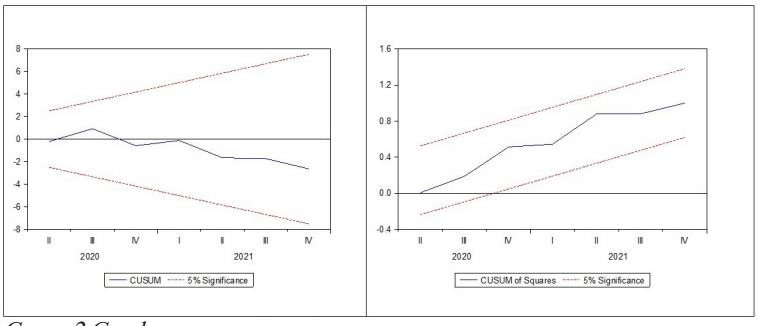

Figure 3. *Cusum and Cusum2 Graphs.*

Table 3. *ARDL (6, 5, 6, 0, 6) estimates and error diagnosis results*

Panel A: Short-Term Parameter Estimations						
Variables	**Lag Number**					
	0	1	2	3	4	5
		2.9086***	2.6929***	2.3028***	1.7670***	
	-0.5781	1.3169**	-0.4872	-0.3538	0.6726**	
	0.40850	0.3348	0.0611	0.1466	-1.1460**	-0.5440
	0.7556***	-1.0036**	-1.8677***	-2.3232***	1.5439***	-0.8985***
Dummy 1	-0.1100**					
Dummy 2	0.4979***					

Panel B: Long Term Estimations				
LSEF	**LKEF**	**LTEF**	**LG_SA**	**Constant**
-0.2475***	0.3730***	-0.1054*	0.5420**	1.1944**

Panel C: Cointegration Test Error Correction Term and Error Diagnosis Tests					
F	**$ECM_{(t-1)}$**	**LM**	**Reset**	**Cusum**	**$Cusum^2$**
5.4119***	-4.2821***	2.3032	0.537	S	S

***, ** and * denote statistical significance levels of 1%, 5% and 10%, respectively. Statistically significant results are in bold. S and SD expressions in CUSUM and $CUSUM^2$ mean Stable and Not Stable.

In table 3, for the parameter stability CUSUM and CUSUMSQ tests are conducted and found that they are stable. For the CUSUM and CUSUMSQ graphs can be found at the Figure 3. The model specification error is tested with Ramsey RESET test and LM test is used for whether the autoccorrelation is avaibable. The Panel C of the Table 3 shows the results of this tests and, according to them, there is no specification error and autocorrelation problem in the presented model. Further the scatterplots of the residuals and their autocorrelations and partial autocorrelations, which are not presented at the study, there is no information available and the residuals are randomly distributed around a certain mean.

According to the findings from the estimation, both dummy variables in the model are statistically significant. Accordingly, while Covid pandemic restrictions has a negative impact on meat demand, the Eid al-Adha increases the demand for meat.

In the case of short run results of model estimation, all the previous cattle demand variables (the beef price, prices of substitute goods, national income) are statistically significant effects on the current cattle demand. An 1% increase in the variables from the first to fifth lag cause, on average, respectively, 2,91%, 2.69%, 2.30%, 1.77% and 0.79% approximately on beef demand.

The effect of beef prices on beef demand are statistically significant at the 1st and 4th lags. Ceteris paribus an 1% increase in beef prices one period ago reduces today's beef demand by approximately 1.32% on average, whereas an 1% increase in the four periods ago has a positive effect on beef demand. While keeping all other variables constant, an 1% increase in beef prices in four periods ago increases today's beef demand by approximately 0.67%.

From the short run parameter estimates for lamb prices, only the 4th lag is statistically significant; When all other variables are kept constant, an 1% increase in lamb prices at four period ago reduces the current demand for beef by, on average, approximately 1.15%.

From the short run parameter estimates for chicken meat prices, the model selection procedure cannot obtain any short-term estimates. all the short-term parameter estimates of income are statistically significant; however, the effects are mixed. Parameter estimates for the current period is positive, while parameter estimates for other periods are negative.

When all other variables are held constant, an 1% increase in current income increases current beef demand by, on average, approximately 0.76%. On the other hand, 1% income increases occurring one, two, three, four and five periods ago cause a decrease in beef demand and these effects are, on average, approximately 1%, 1.87%, 2.32%, 1.54% and 0.9%, respectively.

The F statistic in the first column of Panel C is the F statistic for the cointegration test. The obtained value of 5.4119 is statistically significant at the 1% significance level and shows that there is long term equilibrium among the variables. Since there are long run relationship among variables, the error correction model can be estimated. According to the findings, the error correction term (ECMt-1) is -4.2821, which is negative and statistically significant. A value greater than 1 indicates that the short-term deviations from the equilibrium disappear and converge to the long run equilibrium value within a single period (namely, a quarter).

Considering the long run results, all our parameter estimates are statistically significant and generally in line with expectations. Here, as an exception, increases in chicken prices reduces demand for beef (Table 3, Panel B). While other variables are kept constant, in the long run, an 1% increase in beef prices leads to, on an average, a decrease in the beef demand approximately 0.25%. However, the same increase in lamb prices leads to, on an average, a 0.37% increase in beef demand. Lastly while other variables are kept constant, an 1% increase in income, in the long run, leads to, on an average, an increase of, approximately, 0.54% in beef demand.

DISCUSSION

In considering the TSI data for Türkiye, in general, between 2010 and 2021 at a quarterly frequency used in this study, the COVİD-19 restrictions negatively affects the beef demand, namely, it reduces the beef demand. The decrease in this demand was occurres due to the closure of food and beverage areas such as entertainment, cafes and restaurants, especially Hotel-Restaurant-Institutional in Türkiye due to the pandemic, which causes a significant contraction in the sector. As a matter of fact, in this study, it is found that the pandemic causes a 11% decrease in meat demand in the market. In parallel with the findings of this study, reported the slaughter of cattle decreased by 33% in April and May 2020 in the USA and 60% in Canada (LMIC (Livestock Marketing Information Center), 2022; USDA **(**United States Department Of Agriculture), 2022; Weersink, et. al. 2021). Other studies conducted in Canada reported that the possibility of not processing meat after slaughter reduces the demand for beef cattle in the market, and beef production decreased by 21% in April 2020 and 19% in May 2020 (Mallory, 2021; Rude, 2021).

However according to TSI data, the increase in beef demand in Türkiye, which was 8.46% on average every year between 2010 and 2019, increased by only 0.8% in 2020 due to the pandemic, reaching 1,341,446 tons. It can be said that beef demand in Türkiye has become stationary due to the pandemic. Tuncel (2023) found a positive relationship between Meat and Milk Board (MMB) meat demand data and the pandemic restriction level. Accordingly, while the Covid-19 results in household meat demand on the one hand, on the other hand, it has a negative effect as a result of the contraction in food and beverage areas such as Hotel-Restaurant-Institutional, entertainment, cafe restaruant etc. Tuncel (2023) reports that the increase in household consumption compensated for the decline in beef demand. On the other hand, the 0.8% increase in beef demand can be attributed to household consumption. As a matter of fact, Aydın and Demir Ayvazoğlu (2022) and Güney and Sangün (2021), in parallel with the findings of this study, report that household demand for beef increased during the pandemic.

The increase in household consumption expenditures is also directly related to beef prices. As a matter of fact, during the 2020 pandemic period, the rate of increase in beef prices in Türkiye (6.9%) remained below the inflation rate in 2020 (14.6%) (TSI, 2022g). Accordingly, in real terms, the decline in beef prices in 2019 continued to a certain extent in 2020. The 'Dummy 2' used for a sudden upward shift in the third quarter of 2019 indicates that the effect of real beef prices on the demand for meat coincides with the Eid al-Adha period also.

It has been determined that the imbalances in beef supply in the USA, Canada and many European countries during the pandemic intensified at the beginning of the pandemic; after the initial shock was over, the prices in many countries followed a balanced course from the 6th month of the pandemic (BLS (Bureau of Labor Statistics**)**, 2022; STATCAN(Statistics Canada), 2022; Weersink, et. al. 2021). In particular, the closure of meat processing facilities during the pandemic and indirectly the possibility of livestock enterprises not being able to find a channel to offer their livestock for final consumption at the end of the fattening period can be considered the main reason for the supply imbalance (LMIC, 2022; USDA, 2022; Weersink, et. al., 2021). When COVID- 19 is evaluated in terms of the food sector, Nordhagen et al. (2021) conduct a study on 367 small- and medium-scale enterprises in 17 countries and they find that 84% of food companies made changes in their production volumes as a result of the COVID-19 epidemic, and they reports a complete cassation in the production in 13 % and a decrease in 82% of these enterprises.

Within the scope of this study, the price fluctuations of beef in Türkiye during the pandemic period were more stable. In Akter's (2020) research using the Stay-at-Home Restriction Index, milk, cheese and eggs, as well as oils and fats, were not affected by the stay-at-home restrictions, and he attributed the change in meat price indices to problems in the fish and seafood supply chain. Coluccia et al. (2021) report in their study that agricultural food demand problems can be solved by taking into account many parameters such as export, consumer behavior, supply chain reaction and epidemic restrictions.

A more flexible supply chain structure in Türkiye than in many western countries and less dependence on meat

packaging as well as packaged and processed food can be shown among the reasons for this situation (Akter, 2020). Indeed, hoarding behavior can lead to higher price changes in societies with a high dependence on processed food. Tuncel (2023) finds a 60.7% correlation between the level of restriction and beef demand during the Covid 19 pandemic period. He reports that this correlation is influenced by the favorable meat prices during the pandemic period, as well as the hoarding behavior that develops with herd psychology to a certain extent. Indeed, Long and Khoi (2020), in their study conducts in Vietnam during the COVID-19 pandemic, finds that consumers' perception of risk and the expectation make the products more expensive in the future due to increasing the tendency to stockpile food.

The regression results obtained within the scope of the study provide information on the elasticity of demand for beef, cross elasticities of demand for beef with substitute goods and income elasticity of beef. ARDL bounds test is used to test whether there is a long-run relationship between variables, and long and short run elasticities are calculated under the condition of the existence of a cointegration relationship (Narayan & Smyth, 2006). Accordingly, in this study, the elasticity of demand for beef is found to be 0.25% in the long run. In parallel with the finding of this study, Hatırlı et al. (2008) determines the elasticity of demand for beef as 0.20% in Türkiye. Accordingly, the elasticity of demand for beef is characterized as a necessary good.

In study determination, in terms of cross elasticity of demand, in the long run, a 1% increase in lamb prices increases the current beef demand by approximately 0.37%, whereas a 1% increase in chicken meat prices causes an average decrease of 0.11% in beef demand. Accordingly, while the substitution relationship between beef and lamb was found, the substitution relationship between chicken and beef was found to be weak. Hatırlı et al. (2008), unlike this research finding, reported that beef and chicken are substitutive and lamb meat is complementary. The difference between these two studies can be explained by the fact that chicken meat has been at the top of consumer preferences over the years and its substitution with red meat is low due to the lower price of chicken meat.

In addition, it was determined in the study that a 1% increase in current income increased the demand for beef by an average of 0.54% in the long term Saygın et al. (2018) found in their study that the income elasticity of beef was 0.32%. Accordingly, the relationship between beef demand and income elasticity coincides with the expected income elasticity level.

CONCLUSION

As a result, a negative relationship is found between the level of restriction and the demand for beef during the COVID-19 period in Türkiye. This situation has shown that the contraction in sectors such as transportation (airlines), entertainment and similar sectors, especially HRI, and the decrease in beef demand, which emerged as an obstacle to the consumption of animal products through these channels, were compensated, to a certain extent, by encouraging an increase in household consumption with the effect of low carcass prices. However, the livestock sector has entered into a major bottleneck since 2021 due to the contraction problem in beef consumption points during the pandemic periods and production with low carcass prices despite the increasing fattening costs of cattle breeding enterprises. At this point, in order to increase the demand for animal products, it is necessary to ensure that the demand for beef is met in a cheap way through measures to reduce production costs and to establish an appropriate economic model for the sustainability of production.

In Türkiye, instead of suppressing carcass meat prices in the market by importing meat, structural problems should be solved first. Accordingly, ensuring stability in milk prices by supporting dairy farming plays a fundamental role in stabilising meat prices. In addition, practices such as pasture improvement and development of pasture animal husbandry, reduction of calf mortality rates, support to encourage production in the domestic market instead of balancing the supply deficit with meat imports, encouragement of ovine consumption in accordance with Türkiye's realities and pasture structure should be implemented.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

ST planned, designed and contributed to the literature review, data collection and writing. TTD designed the study material and conducted the analyses. PAD contributed to data collection, literature review and editing of the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

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

Ethical Statement

This study does not present any ethical concerns.

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Effects of body condition score on milk yield and calf birth weight in dairy cattle

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Abstract
This study investigated the effects of pre-calving body condition scores (BCS) on milk yield and calf birth weights in pregnant Holstein heifers. For this purpose, data from 66 primiparous heifers, their first lactation milk yields, and the birth weights of 66 calves born from these heifers were analyzed. Two different rations consisting of concentrate feed and roughage with 36% HP and 1718 kcal/kg ME were used in the feeding of the animals pre- and post-calving. Approximately three weeks pre- calving, the heifers were classified into three groups based on their BCS as low (BCS ≤ 3.00), moderate (BCS 3.25 ≤ 3.50), and high (BCS ≥3.75). To determine changes in body condition, a second scoring was performed immediately post-calving. The differences between pre- and post-calving BCS values were calculated, and the BCS changes for each animal were identified. The effects of these changes on milk yield and calf birth weight were statistically analyzed using one-way analysis of variance (ANOVA). According to the results, BCS at calving had no significant effect on 305-day adjusted lactation milk yield ($p>0.05$). Additionally, pre-calving BCS and post-calving BCS changes did not affect calf birth weights ($p>0.05$). However, the findings indicated that a post-calving BCS loss of 0.50–0.75 points significantly increased milk yield ($p<0.05$). This finding suggests that controlled energy mobilization in early lactation may support milk production. Therefore, properly planned transition period rations are thought to be essential for ensuring herd health and increasing milk yield.

Keywords: *Birth weight, body condition score, holstein, milk yield, pregnant heifer*

INTRODUCTION

Productivity in dairy cattle is dependent on economically significant traits such as milk yield and reproductive performance. These traits are largely influenced by the physiological status of the animals. Body condition score is a crucial indicator, particularly in the pre-calving period, as it reflects cows' energy reserves and metabolic status. BCS is assessed visually and through palpation, evaluating the body fat reserves of dry or lactating cows without considering live weight or body measurements (Gallo et al., 1996; Hady et al., 1994). Thanks to BCS, the efficiency performance of cows can be increased by ensuring optimum nutrition in periods when energy needs vary, such as insemination, dry period, birth and lactation (Daşkın, 2011). By evaluating the current metabolic profiles and determining the energy balances of cows exhibiting different physiological characteristics, it is possible to prevent certain metabolic and production-related problems (Ural & Erdoğan, 2018)

In dairy cattle, milk yield is particularly high during the early stages of lactation. During this period, if essential nutrients such as energy, protein, and minerals are not adequately supplied, cows utilize their body reserves to sustain milk production. Consequently, a decline in body weight and condition occurs (Aeberhard et al., 2001). When nutrient requirements are not met and feeding is inadequate, a negative energy balance develops. BCS is widely used in dairy cattle to assess negative energy balance and to implement feeding programs that meet nutritional requirements (Edmonson et al., 1989; Lassen et al., 2003; Samarütel et al., 2006). The negative energy balance resulting from inadequate and unbalanced nutrition adversely affects reproductive performance and overall health in cows (Dechow et al., 2002; Fallah, 2022; Gillund et al., 2001; Lassen et al., 2003; Maršálek et al., 2008; Roche et al., 2007).

Numerous studies have examined the effects of pre-calving body condition scores on post-calving health disorders, fertility, and milk yield, yielding varying results (Butler & Smith, 1989; Kara et al., 2013; Meikle et al., 2004; Pedron et al., 1993; Roche et al., 2009; Ruegg & Milton, 1995; Tapkı et al., 2005ab; Waltner et al., 1993; Wathes et al., 2007). The optimal lower limit for BCS at calving is reported to be between 3.00 and 3.50 (Roche et al., 2009; Samarütel et al., 2006). Butler & Smith, (1989) suggested that low pre-calving BCS could negatively impact post-calving fertility and milk yield. Additionally, cows with an optimal BCS range have been shown to return to reproductive cyclicity more rapidly and achieve higher first-service conception rates (Roche et al., 2009; Walsh et al., 2011).

One of the economically important traits in cattle breeding is calf birth weight. Birth weight is a critical factor that directly influences calf viability, growth performance, and future milk yield. In addition to genetic factors, calf birth weight is affected by environmental factors such as maternal age, nutritional status, gestation length, and season of birth. Calves born with normal birth weights grow more rapidly under proper feeding and management conditions, contributing to early reproductive and milk production potential. In contrast, calves with low birth weights may have weaker immune systems, making them more susceptible to diseases. On the other hand, excessively high birth weights can lead to dystocia, posing significant risks to both the dam and the calf (Mee, 2008). Studies by Berry et al., (2007) and Lom-

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bard et al.,(2007) have reported that high pre-calving BCS can increase calf birth weight but may also elevate the risk of calving difficulties.

This study aimed to evaluate the effects of pre- and post-calving BCS on milk yield characteristics and calf birth weights in primiparous pregnant heifers. By doing so, the study sought to highlight the importance of BCS management during both pre- and post-calving periods.

MATERIALS AND METHODS

This study was conducted at a private dairy farm located in Aksaray (38°19'11.7"N, 33°54'13.7"E) and was approved by the Ethics Committee of the SÜVDAMEK (Protocol No. 2025/02-17).

The study utilized data obtained from Holstein heifers and cows in a private dairy farming operation in Aksaray. The research material consisted of 66 primiparous heifers and the birth weights of their calves. All animals were subjected to the same feeding and management protocol, and milk yield records were collected using an automated robotic milking system. The animals were housed in modern free-stall barns and fed ad libitum with two different rations during the dry period and early lactation. Feeding was conducted twice daily, in the morning and evening.

The ration composition included wheat straw, dry alfalfa hay, barley silage, corn silage, corn flakes, a vitamin-mineral premix, bypass fat, a toxin binder, and a concentrate dairy feed containing 36% CP and 1718 kcal/kg ME. The ration was formulated according to NRC (2001) guidelines, and the composition and quantities are provided in Table 1. The chemical composition of the concentrate feed used in the ration was analyzed at a specialized feed manufacturing facility.

Body condition scores of heifers were determined three weeks before and at the time of calving according to the methodology of Ferguson et al., (1994) based on a 5-point scale with 0.25 points interval. The groups were formed as Low Body Condition Score (LBCS ≤3.00; n=25), Moderate Body Condition Score (MBCS 3.25 ≤3.50; n=25) and High Body Condition Score (HBCS ≥3.75; n=16) according to their fitness scores. The BCS of the animals was reassessed immediately post- calving.

***Table 1**. Ingredients and chemical composition of the rations used in the study.*

Ingredients	Dry period ration (kg/day/head)	Post-calving ration (kg/day/head)
Wheat straw	3.00	0.40
Alfalfa hay	1.25	2.70
Concentrate dairy feed	1.80	7.60
Barley silage	5.00	5.50
Corn silage	6.00	19.50
Corn flake	2.00	5.50
Vitamin-mineral premix*	0.15	0.55
Bypass fat	0.00	0.40
Toxin binder	0.03	0.03
Ration dry matter	10.50	22.20
Ration dry matter ratio (%)	49.00	50.00
Ration crude protein (%)	12.50	17.30
Chemical analysis values of concentrated milk feed		
Crude protein (%)	36	
Crude fiber (%)	8	
Crude fat (%)	2.5	
Ash (%)	7	
Sodium (%)	0.4	
Metabolizable energy (kcal/kg)	1718	

*: Per kilogram of contains: 246.000 IU Vit. A, 61.500 IU Vit. D_3, 1538 mg Vit. E, 923 mg Mn, 923 mg Zn, 923 mg Fe, 369 mg Cu, 6 mg Co, 25 mg I, 9 mg Se.

The differences between the pre- and post-calving BCSs were calculated, and the body condition changes for each animal were determined. Based on the changes in BCS, the animals were classified into two groups: a 0.00-0.25 score change group and a 0.50-0.75 score change group.

Birth weights and genders of calves were recorded by weighing and observing the calves immediately after birth. Milk yield records were kept daily through the robotic milking system. Additionally, the 305-day milk yield predictions were calculated. The robotic milking system accurately measured the daily milk yields and maintained records on an individual animal basis. The system also provided data on milking frequency, milk quantity, and milk quality. These data were transferred to the farm management software at regular intervals for analysis.

Statistical analyses

The collected data were analyzed using IBM Corp. (2012) SPSS Statistics software (v.21). The normality of the data was assessed using the Kolmogorov-Smirnov

test. For data that followed a normal distribution, one-way analysis of variance (ANOVA) was applied, while the Kruskal-Wallis test was used for data that did not follow a normal distribution. A significance level of $p<0.05$ was considered statistically significant.

RESULTS

The average body condition scores of all animals used in the study were determined to be 3.67 pre- calving and 3.30 post- calving, with an average BCS change of 0.36. The average corrected 305-day milk yield of the animals was found to be 7987 kg. Based on the birth weight measurements of the calves, the average birth weight was determined to be 38.07 kg (Table 2).

The effects of pre-calving BCS and post-calving BCS changes on the 305-day corrected milk yields and calf birth weights are shown in Table 3.

Regarding pre-calving BCS, no statistically significant differences were found among all groups in terms of 305-day milk yields ($p>0.05$). However, the highest milk yield was observed in the group with the moderate BCS score (BCS = 3.25 ≤ 3.50), which was 8263 ± 316 kg. The group with the heaviest calf birth weight was found to be the group with a BCS ≥ 3.75, with an average weight of 40.160 ± 1.190 kg. However, no significant differences were found for calf birth weights across the groups ($p>0.05$).

Post-calving, a BCS change of 0.00-0.25 was observed in 40 animals, while a BCS loss of 0.50-0.75 points was recorded in 26 animals. When the effects of these BCS changes on 305-day milk yields and calf birth weights were examined, no differences were found in calf birth weights ($p>0.05$). However, in the group with greater post-calving BCS loss, the 305-day milk yield was approximately 900 kg higher, and this difference was found to be statistically significant ($p<0.05$).

DISCUSSION

In this study, the effects of pre-calving and post-calving BCSs on 305-day milk yield and calf birth weight were examined. The general averages for BCS and post-calving BCS changes observed in the heifers were consistent with some findings in the literature, while differing from others. Kertz et al., (1997) reported similar average BCS at calving, with a value of 3.36. In a comparable study, Bayram et al., (2012) reported average BCS at calving and during the first month of lactation as 3.14 and 3.01, respectively, which aligns with the results of this study. However, Domecq et al., (1997), in their study examining the relationship between BCS and milk yield, reported lower pre-calving BCS in heifers, with a value of 2.66, which is lower than the pre-calving BCS found in this study.

In studies conducted by Yaylak & Kumlu, (2005) and Ural, (2012), it was reported that BCS significantly influenced 305-day milk yield, with animals having higher BCS exhibiting higher milk yields. In contrast, Bouska

Table 2. *Means and ranges of variables of the study herd (n=66).*

Variables	Mean	SEM	Range
Pre-calving BCS	3.67	0.50	4.50 to 2.75
Post-calving BCS	3.30	0.46	4.00 to 2.50
Post-calving BCS loss	0.36	0.02	0.75 to 0.00
305-day adjusted milk yield (kg)	7987	173	12215 to 5462
Birth weight (kg)	38.07	0.48	48.00 to 30.00

Note. BCS: Body condition score

Table 3*. Effect of body condition score on milk yields and calf birth weights.*

Groups	n	305-day adjusted milk yield X±Sx	p	Birth weight X±Sx	p
Pre-calving BCS					
≤3.00	25	7882 ± 308		37.300 ± 0.694	
3.25 ≤3.50	25	8263 ± 316	0.440	37.500 ± 0.647	0.237
≥3.75	16	7721 ± 188		40.160 ± 1.190	
Post-calving BCS loss					
0.00 ve 0.25	40	7620 ± 163	0.017	38.263 ± 0.650	0.820
0.50 ve 0.75	26	8551 ± 336		37.769 ± 0.691	

Note. Statistically significant when p values <0.05.

et al., (2008) found that cows with low BCS (≤ 3.5, 4, ≥4.5) during the dry period achieved the highest milk yields (7,345, 6,980, 6,868 kg, respectively). Similarly, Bayram et al., (2012) reported that cows with low BCS (BCS <3.00) at calving had significantly higher actual milk yield and 305-day milk yield compared to those with moderate BCS (BCS ≥ 3.00). However, they found no significant effect of BCS during early lactation on 305-day milk yield.

In contrast to these findings, in the present study, the highest milk yield was observed in the moderate BCS group (3.25–3.50 range) with a value of 8,263 ± 316 kg, while cows with BCS ≤3.00 had a lower milk yield (7,882 ± 308 kg). However, the differences between the groups were not statistically significant (p=0.440). Similarly, Poczynek et al., (2023) reported a milk yield of 8,288.0 ± 560 kg for the BCS group of 3.00-3.25, concluding that differences in milk yield based on BCS were not significant. In another study, Metin et al., (2023) found that milk yield was higher in the BCS 3.75 group (moderate score) compared to both BCS 3.50 and BCS 4.00 groups. Tapkı et al., (2005b) reported that cows with a moderate BCS (≤4) during the dry period had significantly higher milk yields compared to those with a high BCS (BCS >4). Contreras et al., (2004) similarly found a positive correlation between milk yield and moderate BCS values, with the correlation turning negative as BCS values increased. Butler & Smith, (1989), in line with the findings of this study, reported that low pre-calving BCS could negatively affect fertility and milk yield post-calving due to insufficient energy reserves.

In several previous studies, post-calving BCS changes were reported to range between 0.29 and 1.20 units (Dechow et al., 2002; Domecq et al., 1997; Koenen et al., 2001; Maršálek et al., 2008; Samarütel et al., 2006; Waltner et al., 1993). However, Bayram et al., (2012) reported a lower post-calving BCS loss (0.11) compared to the aforementioned studies. In the present study, the average post-calving BCS loss was determined to be 0.36. When the effects of these losses on milk yield were examined, cows that experienced a 0.50-0.75 BCS loss had significantly higher 305-day milk yields (8,551 ± 336 kg) compared to those with lesser BCS loss (p=0.017). This finding supports the idea that post-calving BCS loss may contribute to milk production through the mobilization of body reserves during lactation.

Consistent with the present study, other research has reported higher milk yields in cows with higher BCS loss (Berry et al., 2007; Dechow et al., 2002; Roche et al., 2007). Additionally, Dechow et al., (2002) found a genetic correlation between BCS loss and milk yield characteristics ranging from 0.17 to 0.55. In contrast, Bayram et al., (2012) reported no significant effects of BCS changes on reproductive and milk yield traits. This discrepancy may be explained by the very low level of BCS loss in their study. There are also studies reporting no effect of BCS at calving on milk yield (Domecq et al., 1997; Jilek et al., 2008; Markusfeld et al., 1997; Pedron et al., 1993; Ruegg and Milton, 1995; Samarütel et al., 2006; Waltner et al., 1993). The varying results in studies examining the effect of BCS on milk yield suggest that the relationship between pre-calving BCS and milk yield is not always linear. Moreover, discrepancies in findings may be attributed to differences in breed, age, management practices, and scoring methods used in the studies.

In this study, the average birth weight of the calves was found to be 38.07 kg. When BCS values were evaluated in conjunction with calf birth weights, an increase in birth weight was observed in parallel with higher pre-calving BCS. Specifically, calves born from cows in the ≥3.75 BCS group had a higher average birth weight (40.160 ± 1.190 kg) compared to the other groups. However, the difference between the groups was not statistically significant (p=0.237). Similarly, the post-calving BCS changes had no significant effect on calf birth weight (p=0.820). This finding is consistent with those reported by Karslıoğlu & Galiç, (2021). Bayram et al., (2012) also found that calves born to cows with lower BCS had an average birth weight 0.5 kg lower than those born to cows with higher BCS, though the difference was not significant.

The average birth weights in this study were lower than those reported in some other studies (Bayram et al., 2012; Karslıoğlu & Galiç, 2021; Kertz et al., 1997). Similar average calf birth weights were reported by Tapkı et al., (2005a), Metin et al., (2023), and Poczynek et al., (2023) (37.5 and 37.9, 40.1 kg), but in these studies, the effect of BCS on calf birth weight and growth performance was found to be significant. Many studies have reported that cows with higher pre-calving BCS give birth to heavier calves. These studies also indicate that high BCS positively affects placental development and fetal nutrient intake, although the risk of dystocia increases in cows with excessively high BCS (Berry et al., 2007; Roche et al., 2007). The inconsistencies in these results suggest that the energy balance during the post-calving period may not have a direct effect on calf birth weight, and pre-calving nutrition could be a more determining factor. Additionally, when evaluating calf birth weight, environmental factors such as maternal age, gestation length, and calving season, in addition to genetic factors, should be considered.

CONCLUSION

In this study, the effects of pre-calving BCS and post-calving BCS loss on 305-day milk yield and calf birth weight were evaluated. The results showed that while numerical differences in calf birth weights were observed due to pre-calving BCS and post-calving BCS loss, these differences were not statistically significant. However, post-calving BCS losses had significant effects on milk yield. This finding suggests that controlled energy mobilization in the early stages of lactation may support milk production.

Nevertheless, there are differing opinions in the literature regarding the impact of BCS loss on milk yield. Some studies report that slight BCS loss contributes to increased milk production, while other studies emphasize that excessive BCS loss poses risks to reproductive performance, metabolic health, and long-term milk yield. Therefore, optimal BCS loss should be achieved at the beginning of lactation, and excessive energy mobilization should be prevented.

In this regard, it is recommended that feeding programs be adjusted according to energy balance, BCS changes be monitored regularly, and balancing nutritional strate-

gies be implemented during the post-calving period. Future studies investigating the relationships between BCS loss and milk composition, reproductive performance, and metabolic health in more detail would be beneficial. Furthermore, long-term follow-up studies assessing the effects of genetic differences and management conditions could provide more definitive conclusions on the subject.

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Conflict of interest

The authors declared that there is no conflict of interest.

Ethical statement or informed consent

This study was approved by the Ethics Committee of the SÜVDAMEK (Approval No. 2025/02-17).

Author contributions

TK and OE conceptualized and designed the study. They also contributed to data collection. Statistical analyses were performed by TK and OE. TK drafted the manuscript, and all authors reviewed and approved the final version.

Availability of data and materials

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Estimating the production losses related fasciolosis in water buffaloes in Türkiye (Journal of Advances in VetBio Science and Techniques, 2024, 9(3), 242-246.)

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Erratum

In the article with "Sarıözkan, S., Küçükoflaz, M. Estimating the production losses related to fasciolosis in water buffaloes in Türkiye. Journal of Advances in VetBio Science and Techniques, 9(3), 242-246." citation information which was published in the three issue of nine volume of Journal of Advances in VetBio Science and Techniques, authors inadvertent error in Table 2 and Table 3. DOI number of the Original Article: https://doi.org/10.31797/vetbio.1553089

Erratum

***Table 2**. Calculation method for estimating the total production losses due to fasciolosis in water buffaloes in Türkiye*

Loss Items	Calculation Method
1. Meat losses	No. of slaughtered water buffaloes × prevalence of disease × reduction in carcass weight × price of meat
2. Milk losses	No. of milked water buffaloes × prevalence of disease × reduction in milk yield × price of milk
3. Liver losses	No. of slaughtered water buffaloes × prevalence of disease × price of liver
4. Extended calving interval	No. of slaughtered water buffaloes × prevalence of disease × extended day for calving interval × cost of extended calving
TOTAL LOSSES	**(1+2+3+4)**

***Table 3**. Total production losses due to fasciolosis in water buffaloes in Türkiye*

Loss Items	Quantity of Losses (US$)	%
1. Meat losses	69,597× 0.069 × 5.2 × 9 = 224,742	23.7
2. Milk losses	79,333 × 0.069 × 44.3 × 1 = 242,497	25.6
3. Liver losses	69,597 × 0.069 × 10 = 48,021	5.1
4. Extended calving interval	69,597 × 0.069 × 20 × 4.5 = 432,197	45.6
TOTAL LOSSES	**947,457**	**100.0**

During the printing of the article, the above versions of Table 2 and Table 3 were inadvertently printed instead of Table 2 and Table 3 presented below. Table 2 and Table 3, which were intended to be presented in the article, are given below.

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Table 2. *Calculation method for estimating the total production losses due to fasciolosis in water buffaloes in Türkiye*

Loss Items	Calculation Method
1. Meat losses	No. of slaughtered water buffaloes × prevalence of disease × reduction in carcass weight × price of meat (US$/kg)
2. Milk losses	No. of milked water buffaloes × prevalence of disease × reduction in milk yield × price of milk (US$/kg)
3. Liver losses	No. of slaughtered water buffaloes × prevalence of disease × price of liver (US$/whole liver)
4. Extended calving interval	No. of milked water buffaloes × prevalence of disease × extended day for calving interval × cost of extended calving (US$/day)
TOTAL LOSSES	**(1+2+3+4)**

Table 3. *Total production losses due to fasciolosis in water buffaloes in Türkiye*

Loss Items	Quantity of Losses (US$)	%
1. Meat losses	69,597× 0.069 × 5.2 × 9 = 224,742	22.3
2. Milk losses	79,333 × 0.069 × 44.3 × 1 = 242,497	24.1
3. Liver losses	69,597 × 0.069 × 10 = 48,021	4.8
4. Extended calving interval	79,333 × 0.069 × 20 × 4.5 = 492,658	48.8
TOTAL LOSSES	**1,007,918**	**100.0**