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Paraoxonase, haptoglobin, serum amyloid A, tumor necrosis factor and acetylcholinesterase levels in ewes with pregnancy toxemia

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ABSTRACT

Objective: In this study were investigated serum paraoxonase, haptoglobin, tumor necrosis factor-α, acetylcholinesterase, serum amyloid A, nonesterifie fatty acids, glucose, beta hydroxybutyric acid, total protein, aspartat aminotransferase, gama glutamil transferase, cholesterol and triglyceride in ewes with pregnancy toxemia

Materials and Methods: This study material consisted 10 control and 10 group with pregnancy toxemia, total 20 merino hybrid ewes at aged between 2-6 years.

Results: The analysis of blood serum samples revealed that serum amyloid A (SAA), haptoglobin (HPT), tumor necrosis factor-α (TNF), paraoxonase (PON1), acetylcholinesterase (ACHE), aspartat aminotransferase (AST), gama glutamil transferase (GGT) and cholesterol did not differ statistically significant between two groups although SAA, HPT, TNF, PON1, ACHE, AST, GGT levels were higher in ewes with pregnancy toxemia when compared to healthy ewes. Beta hydroxybutyric acid (BHBA) and nonesterifie fatty acids (NEFA) ($p<0.001$), triglyceride and total protein ($p<0.01$) increased that glucose ($p<0.001$) levels decreased in sheep with pregnancy toxemia compared with healthy ewes.

Conclusion: Acetylcholinesterase, paraoxonase, haptoglobin, tumor necrosis factor-α, serum amyloid A concentration researched may prove beneficial laboratory findings disease in sheep with pregnancy toxemia.

Keywords: *Acetylcholinesterase, Paraoxonase, Haptoglobin, Serum amyloid A, Tumor necrosis factor-alfa, Sheep*

INTRODUCTION

Pregnancy toxemia is a disease observed in sheep and goats during the final stages of pregnancy due to the disruption of carbohydrate, glucose, and fat metabolisms. The disease is observed during the last stage of pregnancy and milking period in goats (Bastan et al., 2013; Salar et al., 2018).

There are two forms of cholinesterase in mammals: acetylcholinesterase and pseudocholinesterase. Acetylcholinesterase and pseudocholinesterase are mainly produced in the liver (Kaplay, 1976; Stojevic et al., 2005). Cholinesterase has been measured in cattle, camel, goat, and sheep plasma and liver, and the highest cholinesterase levels were determined in goats (Qarawi et al., 2003).

SAA and haptoglobin levels were higher in Saanen goats with pregnancy toxemia when compared to healthy goats (Albay et al., 2014). Higher SAA levels were found in sheep with pregnancy toxemia when compared to healthy non-pregnant (Mahmoud et al., 2016).

Serum paraoxonase enzyme is associated with HDL cholesterol in mammals and protects the body against several harmful chemicals (Mackness et al., 2017). Paraoxonase levels are low in sheep with fatty liver when compared to healthy sheep (Cao et al., 2017). Paraoxonase-1 levels decrease on the first day of the last month of pregnancy in Turkish Saanen goats and increase on the prenatal 15th and 30th days (Salar et al., 2018).

Tumor necrosis factor-alpha (TNFα) is induced by various stimuli such as tumor cells, bacteria, viruses, parasites, cytokines, and inflammation. TNFα is responsible for neoplastic tissue destruction, and certain local and systemic effects induced by tumors (Çömez, 2006). TNF levels are higher in sheep with pregnancy toxemia when compared to healthy sheep. Higher TNF levels were reported in Saanen goats with pregnancy toxemia when compared to healthy pregnant goats (Albay et al., 2014).

The present study investigated the acetylcholinesterase, paraoxonase, tumor necrosis factor-α, serum amyloid A, haptoglobin, glucose, BHBA, NEFA, cholesterol, triglyceride and total protein levels in ewes with pregnancy toxemia.

MATERIALS and METHODS

Animal material

The animal material included 2-5 years old merino hybrid ewes on a farm in Balikesir province in Turkey with two or more lactation counts, 40-60 kg live body weight and were in the last 4 weeks of pregnancy and exhibited loss of appetite, depression, lethargy, muscle dysfunction, lack of coordination, ataxia and were bedridden. The ewes were clinically examined. Ten pregnancy toxemia and 10 healthy, a total of 20 merino hybrid ewes were used in the study. After the clinical examination of the merino hybrid ewes, 10 ewes with blood BHBA levels ofover 1.5 mmol/L were assigned to the pregnancy toxemia group, and 10 healthy non-pregnant ewes with BHBA levels of below 1 mmol/L were assigned to the control group. All ewes with pregnancy toxemia were in the last 4 weeks of pregnancy and were kept under the same feeding, care, and environmental conditions. All healthy non-pregnant ewes were keptunder the same feeding, care, and environmental conditions.

Blood analysis

10 ml blood samples were collected from V. jugularis of the ewes diagnosed with pregnancy toxemia into tubes without anticoagulant after the clinical examination. The blood samples were immediately centrifuged at 5000 rpm for 5 min to obtain the serum. Two samples were transferred into eppendorf tubes and stored at -80ºC until the analysis. After the blood serum samples were collected, they were sent to a private laboratory for analysis.

Biochemical analysis

Paraoxonase, acetylcholinesterase, tumor necrosis factor-α, haptoglobin, serum amyloid A levels were measured with the ELISA (Enzyme-Linked Immunosorbent Assay) method (SunRed ELISA Kit cat. No: E90440, Eastbiopharm, China). Biochemical analyses were conducted with a Randox brand Daytona model device (United Kingdom). Randox brand kits were used to study the biochemical parameters. BHBA (Cat. No: RB1007) was determined with the enzymatic kinetic method, while the principle of total cholesterol (Cat. No: CH3810) analyse based on the enzymatic endpoint method, glucose (Cat. No: GL 3815), NEFA (Cat. No: FA115) were determined with the colorimetric method, total protein (Cat. No: TP38669) was determined with the Biuret Reagent endpoint method, triglyceride (Cat. No: TR3823) was determined with the lipase/GPO-PAP method.

Ethical statement

This study was conformed according to Balikesir University Animal Experiments Local Ethics Committee Presidency instructions and approved with consensus at the meeting (2019/12-7).

RESULTS

Clinical findings

Anorexia, lagging the herd, lethargy, bad breath, shaky gait, teeth grinding, ataxia, difficulty in standing, blindness, confinement to the ground, head resting, muscle tremors, loss of consciousness, and mortality were observed in the ewes with pregnancy toxemia included in the study.

Biochemical findings

The results of the biochemical analyzes regarding to ewes with pregnancy toxemia and healthy ewes are presented in (Tables 1 and 2). The analysis of blood serum samples revealed that serum amyloid A (SAA), haptoglobin (HPT), tumor necrosis factor-α (TNF), paraoxonase (PON1), acetylcholinesterase (ACHE), aspartat aminotransferase (AST), gama glutamil transferase (GGT) and cholesterol did not differ statistically significant between two groups although SAA, HPT, TNF, PON1, ACHE, AST, GGT

levels were higher in ewes with pregnancy toxemia when compared to healthy ewes. Beta hydroxybutyric acid (BHBA), nonesterifie fatty acids (NEFA), triglyceride and total protein increased that glucose levels decreased in sheep with pregnancy toxemia compared with healthy ewes (Tables 1 and 2).

Table 1. SAA, HPT, TNF, PON1, ACHE levels in merino hybrid ewes with pregnancy toxemia and healthy merino hybrid ewes

Parameters	Healthy merino hybrid ewes (n=10)	Merino hybrid ewes with pregnancy toxemia (n=10)	P value
SAA μg/ml	5.10±3.73	8.70±6.48	NS
HPT μg/ml	112.32±63.73	172.81±101.30	NS
TNF μg/ml	45.28±26.02	73.09±50.04	NS
PON 1 μg/ml	67.20±48.74	110.42±75.77	NS
ACHE ng/ml	10.69±7.18	15.03±9.57	NS

* p<0.05, ** p<0.01, *** p<0.001, NS: Not Significant

Table 2. Biochemical parameters in merino hybrid ewes with pregnancy toxemia and healthy merino hybrid ewes

Parameters	Healthy merino hybrid ewes (n=10)	Merino hybrid ewes with pregnancy toxemia (n=10)	p value
BHBA mmol/L	0.47±0.12	2.83±1.34	p<0.001
Glucose mg/dL	43.80±6.72	24±6.46	p<0.001
NEFA mmol/L	0.14±0.16	1.30±0.63	p<0.001
Total Cholesterol mg/dL	44.5±10.90	47.2±13.25	NS
Triglyceride mg/dL	15.80±4.02	22.10±6.53	p<0.01
Total Protein g/dL	4.75±0.99	5.98±1.25	p<0.01
GGT U/L	33.90±8.06	40.30±9.35	NS
AST U/L	78.1±19.93	129.4±109.06	NS

* p<0.05, ** p<0.01, *** p<0.001, NS: Not Significant

DISCUSSION

Pregnancy toxemia is a disease observed in sheep and ewes during the last stages of pregnancy due to the disruption of carbohydrate, glucose, and fat metabolisms (Bastan et al., 2013; Albay, 2014; Salar et al., 2018).

It was reported that lagging behind the herd, loss of appetite, lethargy, shaky gait, bad breath, difficulty in standing, teeth grinding, blindness, head resting, confinement to the ground, loss of consciousness, muscle tremors, and mortality are observed in ewes with pregnancy toxemia (Mahmoud et al., 2016; Salar et al., 2018; Asmaa et al., 2019; Gaadee et al., 2021). In the present study, loss of appetite, lagging behind the herd, teeth grinding, bad breath, shaky gait, ataxia, head resting, blindness, muscle tremors, incoordination, the difficulty of standing, confinement to the ground, loss of consciousness, and mortality were observed in ewes with pregnancy toxemia.

Glucose levels were lower in sheep with pregnancy toxemia when compared to healthy sheep (Kabakçı et al., 2003; Mahmoud et al., 2016; Prasannkumar et al., 2016; Gaadee et al., 2021). It was reported that glucose levels were lower in Awassi sheep with pregnancy toxemia when compared to both healthy non-pregnant and healthy pregnant sheep (Khaled, 2011). The glucose level in Barki sheep with pregnancy toxemia was also decreased when compared to both healthy non-pregnant and healthy pregnant sheep (Mahmoud et al., 2016). In the present study, it was determined that the glucose levels in ewes with pregnancy toxemia were lower when compared to the glucose levels of healthy ewes.

It was reported that total protein levels were lower in sheep with pregnancy toxemia when compared

to healthy non-pregnant and healthy pregnant sheep (Gaadee et al., 2021). Total protein levels were lower in sheep with pregnancy toxemia when compared to healthy pregnant sheep (Asmaa et al., 2019). In the present study, although total protein levels were increased in ewes with pregnancy toxemia when compared to healthy sheeps.

It was found that BHBA levels were higher in sheep with pregnancy toxemia when compared to healthy sheep (Khaled, 2011; Gurdoğan et al., 2014; Mahmoud et al., 2016). Higher BHBA levels were reported in sheep with pregnancy toxemia when compared to healthy non-pregnant and healthy pregnant sheep in the last month of pregnancy (Mahmoud et al., 2016). BHBA levels were higher in ivesi sheep with pregnancy toxemia when compared to both healthy and pregnant sheep with subclinical toxemia (Gurdoğan et al., 2014). It was reported that BHBA levels were higher in Awassi sheep with pregnancy toxemia when compared to healthy non-pregnant and healthy pregnant sheep (Khaled, 2011). In the present study, it was established that BHBA levels in ewes with pregnancy toxemia were higher when compared to healthy ewes.

The NEFA levels increased more in sheep with clinical pregnancy toxemia when compared to both healthy non-pregnant and healthy pregnant sheep in the last period of pregnancy (Asmaa et al., 2019). The NEFA levels were found to be higher in sheep with pregnancy toxemia when compared to healthy sheep (Sauza et al., 2019). It was found that NEFA levels were higher in sheep with pregnancy toxemia when compared to non-pregnant and healthy sheep in the last month of pregnancy (Mahmoud et al., 2016). In the present study, NEFA levels were higher in ewes with pregnancy toxemia when compared to healthy ewes.

A study conducted on Barki sheep indicated that the cholesterol levels were lower in healthy sheep in the last period of pregnancy when compared to the healthy non-pregnant sheep (Asmaa et al., 2019). It was reported that cholesterol levels decreased in sheep with pregnancy toxemia when compared to healthy non-pregnant and healthy pregnant sheep in the last month of pregnancy (Gaadee et al., 2021). It was found that the cholesterol levels were lower in sheep with pregnancy toxemia compared to healthy sheep (Prasannkumar et al., 2016). In the present study, no statistically significant difference was found between the cholesterol levels of the ewes with pregnancy toxemia and healthy ewes.

Triglyceride levels were higher in sheep with pregnancy toxemia when compared to healthy non-pregnant and healthy pregnant sheep in the last month of pregnancy (Mahmoud et al., 2016). Higher triglyceride levels were determined in sheep with pregnancy toxemia when compared to healthy sheep (Kabakçı et al., 2003). It was found that the triglyceride levels were higher in Saanen goats with pregnancy toxemia when compared to healthy pregnant goats (Albay et al., 2014). In the present study, triglyceride levels were higher in ewes with pregnancy toxemia when compared to healthy ewes.

Pseudocholinesterase levels were determined in forty Nubian goats, and the topical fenthion administration decreased the pseudocholinesterase levels (Fuentes et al., 2006). Acetylcholinesterase levels were lower in clinical ketosis cows when compared to the control group (Simonov et al., 2015). In a study conducted on the liver parameters of cows with ketosis, it was found that the cholinesterase values were lower when compared to the control group (Sun et al., 2015). Three distinct types of cholinesterase levels were measured in the plasma and liver tissue of the cattle, camels, goats, and sheep, and the highest cholinesterase levels were determined in goats (Qarawi et al., 2003). In the present study, it was found that the acetylcholinesterase levels in ewes with pregnancy toxemia were not statistically significantly different when compared to healthy ewes.

SAA levels were higher in Saanen goats with pregnancy toxemia when compared to healthy pregnant goats (Albay et al., 2014). Higher SAA levels were found in sheep with pregnancy toxemia when compared to healthy non-pregnant (Mahmoud et al., 2016). In a study conducted on Barki sheep, SAA levels were found higher in healthy pregnant sheep in the last period of the pregnancy when compared to the healthy non-pregnant sheep (Asmaa et al., 2019). Another study conducted on Ivesi sheep also found that SAA levels increased in sheep with pregnancy toxemia when compared to healthy sheep (Gurdoğan et al., 2014). In the present study, it was found that the SAA levels in ewes with pregnancy toxemia were not statistically significantly different when compared to healthy ewes.

It was also determined that the haptoglobin levels increased more in sheep with pregnancy toxemia when compared to healthy non-pregnant sheep (Asmaa et al., 2019). Haptoglobin levels were found to be higher in sheep with pregnancy toxemia when

compared to healthy non-pregnant (Mahmoud et al., 2016). In Ivesi sheep with pregnancy toxemia haptoglobin levels were increased when compared to healthy and pregnant sheep with subclinical pregnancy toxemia (Gurdoğan et al., 2014). In the present study, it was found that the haptoglobin levels in ewes with pregnancy toxemia were not statistically significantly different when compared to healthy ewes.

It was reported that the paraoxonase levels in sheep with fatty liver decreased on the 8th and 16th days when compared to healthy sheep (Cao et al., 2017). It was found that the serum paraoxonase levels were lower in postpartum Holstein cows with fatty liver when compared to healthy non-pregnant cows (Farid et al., 2013), however, the authors also argued that there was no standardized method for paraoxonase measurement, commonly used method was paraoxon hydrolysis and different results could be obtained due to methods that measured paraoxonase, thus, concluded that the physiological interpretations of these levels were challenging (Farid et al., 2013; Camps et al., 2009). In a study conducted on Saanen goats in Turkey, it was reported that the serum paraoxonase-1 levels in the last month of pregnancy were lowest on the 0th day, high on the -15th day, and highest on the -30th day (Salar et al., 2018). In the present study, it was found that the paraoxonase levels in ewes with pregnancy toxemia were not statistically significantly different when compared to healthy ewes.

It was found that the TNF values were higher in animals with ketosis when compared to healthy animals (El-Deep et al., 2017; Zhanga et al., 2018). The TNF levels in cows with subclinical ketosis were higher when compared to healthy cows (Brodzki et al., 2021). A study conducted on Barki sheep revealed that TNF levels increased in sheep with pregnancy toxemia when compared to healthy non-pregnant and healthy pregnant sheep in the last period of pregnancy (Asmaa et al., 2019). TNF levels increased in sheep with mild and severe pregnancy toxemia when compared to healthy sheep (Yarım et al., 2007). In the present study, it was found that the TNF levels in ewes with pregnancy toxemia were not statistically significantly different when compared to healthy ewes.

CONCLUSION

In conclusion, acetylcholinesterase, paraoxonase, haptoglobin, tumor necrosis factor-α, serum amyloid A concentration researched may prove beneficial laboratory findings disease in sheep with pregnancy toxemia.

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The effect of potassium levels on electrocardiographic data in calves with neonatal diarrhea

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ABSTRACT

Objective: The objective of the current study was to assess the potassium levels and electrocardiographic (ECG) data in calves that diagnosed with newborn diarrhea.

Materials and Methods: This study was conducted on a dairy farm located in Ankara, involving a population of 500 dairy animals. The calves were divided into two groups: one group with normal potassium levels (NKg, n=24) and another group with hyperkalemia (HKg, n=16) due to neonatal diarrhea. After the initial clinical examination was conducted on the farm, electrocardiographic investigations were carried out utilizing an ECG equipment. A biochemical analysis was conducted on electrolytes Na^+, K^+, and Cl^- extracted from blood samples collected in red plain tubes, utilizing an automated analysis system

Results: The findings indicate that there were no statistically significant differences observed across all ECG variables between the two groups.

Conclusion: Acidemia and metabolic acidosis are commonly observed in calves suffering from hyperkalemic diarrhea, as documented in previous investigations, often accompanied by heart rhythm problems. In contrast, the current investigation revealed that neonatal calves with diarrhea and hyperkalemia had just a deepening of the T wave, without any additional abnormalities. Nevertheless, it is important to note that acidemia and metabolic acidosis were not of a severe nature. The implementation of Holter monitoring is indicated for calves experiencing hyperkalemia in conjunction with diarrhea.

Keywords: *Calf, Diarrhea, Electrocardiography*

INTRODUCTION

Hyperkalemia, characterized by an increased concentration of potassium in the bloodstream, has the potential to induce significant cardiac rhythm disturbances, particularly in neonatal calves affected by diarrhea (Constable et al., 2005; Trefz et al., 2015). Potassium is an essential electrolyte that serves a crucial purpose in preserving optimal electrical conductivity in the cardiac system and facilitating regular muscle operation. Nevertheless, in the event that the quantities of this substance exceed a certain threshold, it has the potential to interfere with the heart's regular rhythm and overall operation (Elliott and Braun, 2017).

The presence of hyperkalemia in individuals can be identified through various electrocardiographic (ECG) manifestations. These include the observation of tall and symmetric T waves, widening of the QRS complex, progressive flattening and eventual disappearance of P waves, as well as the occurrence of life-threatening dysrhythmias and bradycardia. These distinct patterns can be utilized to diagnose hyperkalemia and assess its severity (Mattu et al., 2000; Diercks et al., 2004). Comparable results are observed in calves

who have been subjected to experimental hyperkalemia, as well as those that have had either experimentally generated or naturally occurring diarrhea (Bergman and Sellers, 1954; Lewis and Phillips, 1973; Weldon et al., 1992; Constable, 1999; Özkan et al., 2011; Başoğlu and Aydoğdu, 2013). However, it has been demonstrated through retrospective studies that, ECG may exhibit a relatively low sensitivity in the diagnosis of hyperkalemia (Wrenn et al., 1991; Montague et al., 2008).

The objective of the current study was to assess the potassium levels and electrocardiographic (ECG) data in a cohort of 40 calves (age<30 days) diagnosed with newborn diarrhea, originating from dairy farms located in Ankara, Türkiye.

MATERIALS and METHODS

This study was conducted on a dairy farm located in Ankara involving a population of 500 dairy animals. A total of 40 Holstein calves were selected as participants for the study. The calves were divided into two groups: one group with normal potassium levels (NKg, n=24) and another group with hyperkalemia (HKg, n=16) due to neonatal diarrhea. Calves were excluded from the study if diarrhea wasn't considered to be the primary problem. The investigation carried out during this study received approval from the Ankara University Animal Experiments Local Ethics Committee, under permit number 2023-4-29.

After the initial clinical examination was conducted on the farm, electrocardiographic investigations were carried out utilizing an ECG equipment (Carewell 1103 L Vet, China) The electrocardiograms were acquired when the calves were in a standing position. The application of electrocardiography electrodes involved the utilization of alligator clips. These clips were affixed to the skin in specific locations: the olecranon on the caudal aspect of the forelimbs, the cranial aspect of the hindlimbs over the patellar ligament, and the 8th intercostal space of the left thorax near the costochondral junction. Additionally, a neutral electrode was positioned over the right patellar ligament. All recordings were standardized at 10 mm/mV and 50 mm/s. Calves that underwent monitoring following the recording of an ECG that lasted for at least five minutes, with the aim of identifying any arrhythmias that may have been present. There was no application of digital filters.

A biochemical analysis was conducted on electrolytes Na^+, K^+, and Cl^- extracted from blood samples collected in red plain tubes, utilizing an automated analysis system (Fujifilm Dri-Chem 4000i, Fujifilm, Japan).

A commercially accessible software package (GraphPad Prism, version 7.01, GraphPad Software, La Jolla) was used to perform the statistical analysis of the obtained data. Statistical significance was determined by considering p values of <0.05. The normal distribution of data was assessed using the D'Agostino & Pearson, Shapiro-Wilk, and Kolmogorov-Smirnov tests. The ROUT (Q=1%) method was utilized to identify and flag outliers. A series of t-tests were performed to compare 15 variables between the two groups.

RESULTS

The age of the cases was represented by the median value of 14 days, with an interquartile range of 10.25–15 days. Given that the study was carried out within the facilities of a dairy farm, it was observed that a majority of 95% (n=38) of the calves belonged to the Holstein breed.

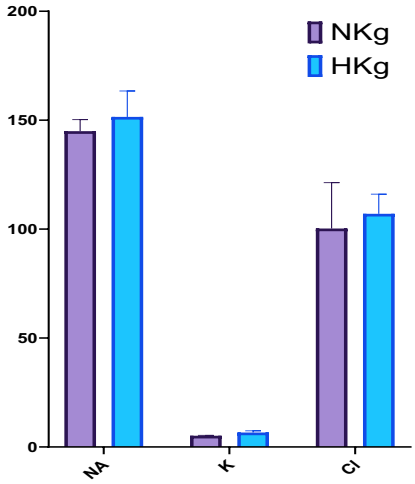

Figure 1. Electrolyte status in 40 neonatal diarrheic calves.

Table 1. Serum biochemistry variables between groups.

	NKg (n:24)		HKg (n:16)		p value
	Mean	SD	Mean	SD	
Na^+ mmol/L	145	5.29	151.50	11.91	0.0225
K^+ mmol/L	5.14	0.19	6.61	0.79	<0.0001
Cl^- mmol/L	100.30	21.04	107.00	9.04	0.2343
Na^+/K^+-ratio	0.035	0.001	0.044	0.007	<0.0001

Table 2. The ECG findings of Lead II of 40 neonatal calves with diarrhea

	NKg (n:24)		HKg (n:16)		*p* value
	Mean	SD	Mean	SD	
Heart Rate (beats/min)	174.30	49.45	173.90	38.95	0.9809
PR ınt (ms)	89.42	27.73	98.00	31.97	0.3726
P wave duration (ms)	44.25	15.20	42.63	21.92	0.783
QRS duration (ms)	126.30	89.48	118.60	58.09	0.7641
T wave duration (ms)	36.58	10.27	42.06	17.51	0.2193
QT interval(ms)	242.60	45.36	250.30	46.72	0.6078
QTc interval(ms)	403.40	56.53	418.60	62.29	0.4285
P Axis (°)	76.83	90.89	59.00	98.24	0.5595
QRS Axis (°)	116.90	106.91	90.38	108.30	0.4489
T Axis (°)	62.29	88.33	106.60	125.58	0.1971
II/ST amplitude (mV)	0.05	0.18	-0.01	0.11	0.2795
P amplitude (mV)	-0.02	0.26	-0.04	0.37	0.617
Q amplitude (mV)	-0.32	0.43	-0.25	0.37	0.5795
R amplitude (mV)	0.35	0.37	0.35	0.27	0.4573
S amplitude (mV)	-0.22	0.43	-0.18	0.36	0.7364
T amplitude (mV)	0.08	0.46	0.02	0.38	0.0569

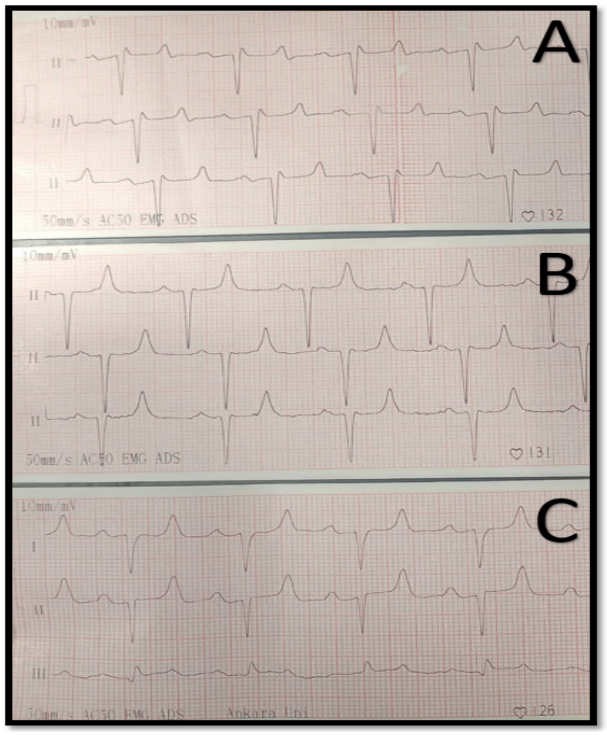

Figure 2. A: ECG findings of a calf with neonatal diarrhea in the hyperkalemia group with a serum potassium level of 8,12 mmol/L. B: ECG findings of a calf with neonatal diarrhea in the hyperkalemia group with a serum potassium level of 8,33 mmol/L. C: ECG findings of a calf with neonatal diarrhea in the normokalemia group with a serum potassium level of 4.77 mmol/L.

Figure 1 and Table 1 present data pertaining to the levels of electrolytes in venous blood samples collected from calves. A total of 16 calves (40%) exhibited hyperkalemia (cK+:5.84-8.33), while 24 calves (60%) had normokalemia. Figure 2 and Table 2 present a comparative analysis of lead II ECG measurements conducted on two distinct groups of calves. The findings indicate that there were no statistically significant differences observed across all ECG variables between the two groups.

DISCUSSION

According to the results of our research, the ECG variables of calves suffering from diarrhea and having high potassium levels did not significantly differ from one another. While it is acknowledged that previous research has been conducted in this field, we believe that the data we have collected during the decision-making process for diagnosing and treating calf diarrhea on a specific local farm holds significance.

Trefz et al. (2018) performed a study. In their investigation conducted on calves exhibiting

diarrhea and aged less than 21 days, the researchers observed certain outcomes, including elevated voltages of S and T waves, as well as changes in the amplitudes of P and Ta waves, which serve as early indicators of hyperkalemia. This study involved the examination of 130 calves that were admitted to a university animal hospital in Germany over a span of approximately 2 years. The calves were divided into four groups based on their blood pH and potassium levels, taking into account the treatment procedures. There could be multiple factors contributing to the observed disparities between the outcomes of our study and the one just mentioned. The following items can be listed as:

- Calves originating from various locations within the city.
- Calves of the Simental breed selected based on the breeder's preference.
- Admission of the calves to the hospital for medical care and subsequent monitoring of their treatment procedures.
- A total of 130 calves can be systematically divided into four distinct groups, allowing for a more comprehensive examination of each group with a greater level of detailed data analysis.

There are two enantiomeric forms of lactic acid. L-lactic acid is a frequently seen molecule in human metabolism, whereas D-lactic acid is generated by certain strains of microorganisms or by less significant metabolic routes. Although L-lactic acid is a naturally occurring molecule within the body, D-lactic acid is considered a detrimental enantiomer (Pohanka, 2020). Research findings have indicated that D-lactate plays a significant role in the metabolic acidosis commonly observed in calves suffering from diarrhea. The significance of bacterial colonization in the intestinal tract of calves experiencing diarrhea has garnered increased attention following the identification of the involvement of D-lactate in the onset of metabolic acidosis (Ewaschuk et al., 2003; Lorenz, 2004; Ewaschuk et al., 2005; Constable, 2009; Lorenz, 2009; Trefz et al., 2012). In their investigation, Naseri et al. (2019) performed an examination of L-lactate levels and observed a statistically significant elevation in the group of patients with septic shock. Upon assessing the individual blood lactate concentrations of septic calves, it was observed that there was no discernible disparity in mortality rate between hyperlactatemic calves and those with blood lactate concentrations falling within the reference range. In the present study, a comparative analysis of lactate levels between different groups was not carried out. The evaluation of lactate is a crucial criterion that demands consideration in future research endeavors.

In a study conducted by Kızıl et al. (2016) in our country, the potassium values and ECG data of healthy and diarrheic calves were compared. The findings of the ECG indicated the absence of P-waves and the presence of elevated T-wave peaks in hyperkalemic calves exhibiting moderate dehydration. Additionally, a reduction in the amplitude of P-waves, prolonged QRS complex, and, negative T-wave peaks were observed in moderately hyperkalemic calves with mild dehydration. Consequently, calves experiencing diarrhea may exhibit varying degrees of hyperkalemia, a condition that can be clinically diagnosed and potentially detected through electrocardiogram (ECG) analysis. Implementing preventive measures to address hyperkalemia may prove beneficial in devising treatment strategies for diarrheic and dehydrated calves. In the study, the difference between the two groups was not investigated and the findings were evaluated separately for each group. In contrast to the methodology employed in this particular study, the groups in our investigation were formed based on varying quantities of potassium. Therefore, our results reflect the effects of potassium.

CONCLUSION

Consequently, acidemia and metabolic acidosis are commonly observed in calves suffering from hyperkalemic diarrhea, as documented in previous investigations, often accompanied by heart rhythm problems. In contrast, the current investigation revealed that neonatal calves with diarrhea and hyperkalemia had just a deepening of the T wave, without any additional abnormalities. Nevertheless, it is important to note that acidemia and metabolic acidosis were not of a severe nature. The implementation of Holter monitoring is indicated for calves experiencing hyperkalemia in conjunction with diarrhea.

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Three-dimensional pelvimetric evaluation of the pelvic cavity in different dog breeds

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ABSTRACT

Objective: The mean of pelvis diameters and development of pelvis: It is known that it varies depending on many factors such as race, body size, gender, nutrition, movement rate and hormones. In this context, the study was carried out to examine the cavum pelvis of different breeds of dogs using multidetector computed tomography (MDCT).

Materials and Methods: A total of 43 dogs, 19 different breeds, were used in the study. The pelvis region was scanned with a 64-detector MDCT device with a section thickness of 0.625 mm. The obtained images were transferred to the RadiAnt DICOM Viewer program to create a three-dimensional model of the cavum pelvis, and then pelvic measurements were taken.

Results: In the pelvimetric examination, it was determined that conjugated diameter was highest in Kangal Shepherd dogs and vertical diameter was highest in Kangal Shepherd dogs. The lowest average of both diameters was found in Pomeranian dogs. Pelvis slope formed between these two ages was observed to be highest in Alabai breed dogs with 38° and lowest in Pekingese, Pomeranian and Setter breed dogs with 18°.

Conclusion: It is thought that it will be beneficial for many disciplines, especially gynecology, to reveal the differences between the breeds by comparing the results of the dog breeds whose metric characteristics of the cavum pelvis were examined.

Keywords: *3D, Computed tomography, Dog, Pelvis*

INTRODUCTION

The pelvis is the part that provides the transition between the body and the lower limbs, it is the cavity formed by the ossa coxae from the sides and lower part, the os sacrum from the back and the first caudal vertebrae. it contains important structures such as urinary, digestive and reproductive organs as well as the birth canal ((Moore, 2006; Liebich et al., 2007; Demiraslan et al. Hang on, 2022).. The size and shape of the bony framework of the pelvis are important factors in the duration of labor (Sporri, 1994).

Gender determination using the skeletal system can be determined using the morphological or the morphometric features of the bone (Steyn, 2009; Gundemir et al., 2020a; Duro et al., 2021; Jashari et al., 2022; Pazvant et al., 2022; Şenol et al., 2022; Szara et al., 2022; Dayan et al., 2023; Güzel et al., 2023; İşbilir et al., 2023). The pelvic bone is an important structure used in gender determination (Gundemir

et al., 2020b; Manuta et al., 2023). While there is a 95% accuracy rate in gender prediction using the pelvic skeleton and 90% in gender prediction using the cranium, this rate can reach a very strong prediction level of 98% when both the pelvis and the cranium are used together (Şahiner, 2007; Garvin, 2012). In terms of archeology and forensic sciences, the pelvis is a very important part of the skeletal system in determining gender. The reason why the pelvis provides such a large amount of estimation in gender prediction is the effect of sex hormones on the pelvis (Komar and Buikstra, 2008; Dawson and Ross, 2011; Charles, 2013).

Pelvis diameters were first determined by manual pelvimetry, and with the developing technology, it was replaced by imaging systems (Radiography, Computed Tomography, Magnetic Resonance Imaging, Ultrasonography) that provide clearer results (Stark, 1985; Ohlerth and Scharf, 2007). Among these methods, the most reliable and accurate method is the measurements taken using computed tomography (Lenhard, 2009).

The presence of pelvic diameters and the development of the pelvis are assumed to depend on breed, body size, sex, nutrition, movement speed, hormones, environment and climatic conditions (Karakaş, 1988). In this context, in our study, it was aimed to determine the relations between the breeds and the genders of the same breed by performing the pelvic evaluation of different breed dogs. It is purposed that the obtained data will provide basic data for different disciplines, especially gynecology, and contribute to studies in the fields of zooarchaeology, evolutionary biology and taxonomy by determining the gender dimorphism data of the breeds.

MATERIALS and METHODS

Ethical approval

Istanbul University-Veterinary Faculty Unit Ethics Committee 15.11.2022/22-38 Local Ethics Committee Approval was obtained for this study.

Samples

19 different dog breeds (Alabai, German Shepherd, American Staffordshire, Beagle, Chihuahua, Cocker Spaniel, Golden Retriever, Siberian Husky, Jack Russell terrier, Kangal Shepherd, Cavalier King, Pekingese, Pompeian, Poodle, Rottweiler, Russian Tsvetnaya) were used in our study. A total of 43 dogs were used: Bolonka, Samoyed, Setter, Terrier. There were no health problems in the research. The samples were made at Istanbul University, Faculty of Veterinary Medicine, Department of Radiology.

Retrieval of three-dimensional pelvimetry data

MDCT images were obtained by scanning the pelvis of the dogs used in the study with a 64-slice multidetector computed tomography (MDCT) (General Electronic Revolution) device at 80 kV, 200 mA, 639 mGY and 0.625 mm section thickness, and saved in DICOM (Digital Imaging and Communications in Medicine) format. Images were transferred to the RadiAnt DICOM Viewer program to take the pelvis measurements of the dogs whose MDCT images were taken, and pelvic measurements were taken from the points mentioned below. The study terminology was based on Nomina Anatomica Veterinaria (2017).

Pelvimetric measurements and reference points (Özkadif et al., 2014; Yılmaz et al., 2019; Demircioglu et al., 2020; Özkadif et al., 2022):

A. Diameter conjugata: Diameter between the cranial tip of the symphysis pelvina and the promontorium (Figure 1).

B. Diameter verticalis: Diameter between the cranial tip of the symphysis pelvina and the facies pelvina of the sacrum (Figure 1).

C. Inclinatio pelvina: Angle between diameter verticalis and diameter conjugata (Figure 1).

D. Diameter transvera

D.1. Apertura pelvis cranialis in transvers diameters (Figure 2)

D.1.1. Dorsal transversal diameter: Diameter between the ends of two ala osis sacri.

D.1.2. Intermedial transversal diameter: Diameter between two tuberculum muscle psoas minoris.

D.1.3. Ventral transversal diameter: Diameter between two eminentia ilio-pubica.

D.2. Transverse diameters of the pelvic cavity (Figure 3)

D.2.1. Cranial transversal diameter: two inc. Diameter between anterior ends of ischiadica majors.

D.2.2. Medial (bispinous) transversal diameter: Diameter between two ischial spine (spina ischiadica).

D.2.3. Caudal (bituberous) transversal diameter: The distance between the inner faces of two ischial tuberosity (tuber ischiadicum).

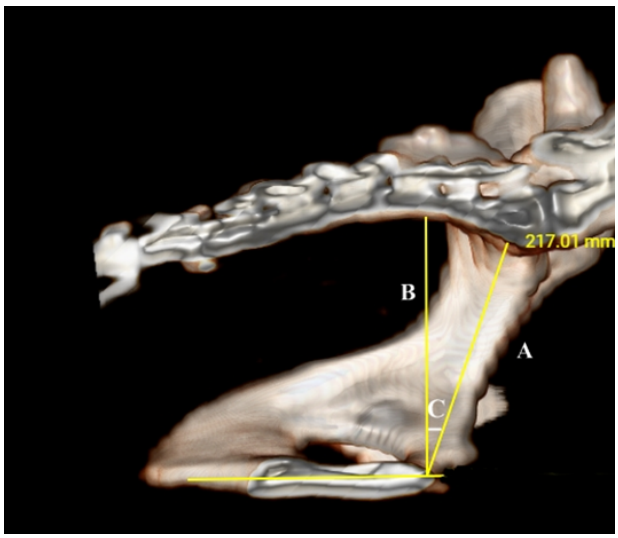

Figure 1. Measurement on lateral view of pelvis. A: Conjugate diameter, B: Vertical diameter, C: Pelvic inclination

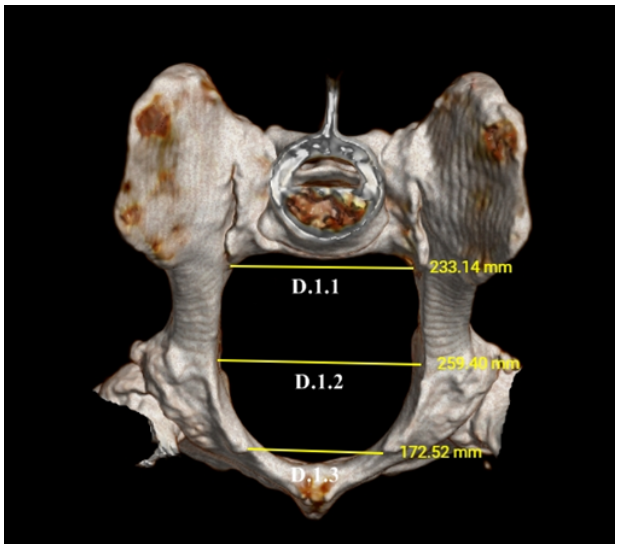

Figure 2. Transverse diameters of apertura pelvis cranialis (cranial view); D.1.1. Dorsal transverse diameter, D.1.2. Intermediate transverse diameter, D.1.3. Ventral transverse diameter

Statistical analysis

In the study, the minimum and maximum values of the pelvimetric measurements were taken separately for each group of dogs. Inclinatio pelvis values between females and males were obtained. These values were compared with ANOVA and the statistical difference between males and females was tried to be revealed. SPSS 22 package program was used for statistical analysis.

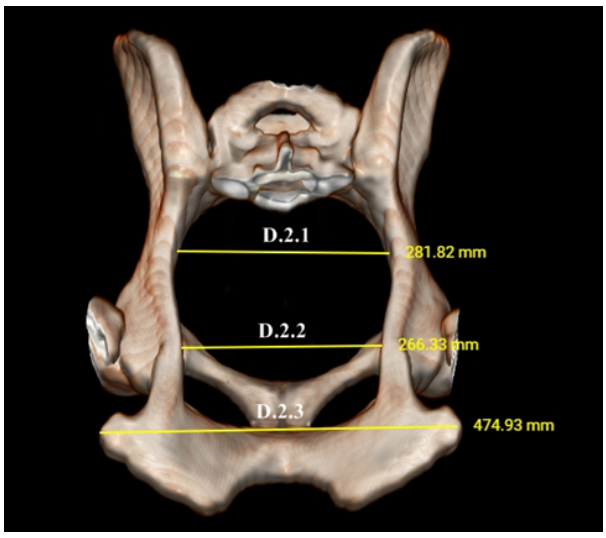

Figure 3. Transverse diameters of the pelvic cavity (caudal view); D.2.1. Cranial transverse diameter, D.2.2. Medial (bispinous) transverse diameter, D.2.3. Caudal (bituberous) transverse diameter

RESULTS

Radiographic pelvimetry data of the dog breeds that reveal the study material are given in Table 1. Diameter conjugata (A) was determined to be highest in Kangal Shepherd dogs with an average of 75.62 mm, and the lowest average in Pomeranian breed dogs. Diameter verticalis (B) was found to be 73.32 mm average in Kangal Shepherd dogs, and the lowest average was 29.50 mm in Pomeranian breed dogs. Inclinatio pelvis (C) was found to be highest in Alabai breed dogs with 38° and lowest in Pekingese, Pomeranian and Setter breed dogs with 18°. When the transversal diameters of the aperture pelvis cranialis were examined, it was determined that D.1.1 diameter was the largest in Alabai breed dogs, and D.1.2 and D.1.3 diameters were found in Rottweiler dogs. It was determined that D.2.1 and D.2.3 diameters in the width diameters of the pelvic cavity were higher in Alabai breeds and D.2.2 diameter in German Shepherd breeds. When the statistical comparison of the inclinatio pelvis in all dog breeds was made between the genders, no significant difference was observed between them (Table 2). In the dog breeds, we examined in this study, it was determined that the diameter verticalis fell to the more caudal of the sacrum in breeds with large body structures (Kangal Shepherd, German Shepherd, Alabai, Rottweiler).

Table 1. Pelvimetric data of dog breeds

Dogs		A (mm)	B (mm)	C (°)	D1.1 (mm)	D1.2 (mm)	D1.3 (mm)	D2.1 (mm)	D2.2 (mm)	D2.3 (mm)
Alabai (n:1)	Min.	79.30	71.20	38.00	56.00	62.70	41.40	63.80	63.80	139.60
	Max.	79.30	71.20	38.00	56.00	62.70	41.40	63.80	63.80	139.60
	Mean	79.30	71.20	38.00	56.00	62.70	41.40	63.80	63.80	139.60
German Shepherd (n:3)	Min.	72.90	70.10	22.00	49.90	57.20	40.80	56.60	55.70	128.10
	Max.	81.50	77.10	33.00	57.70	62.50	47.90	61.70	72.80	135.80
	Mean	77.93	72.77	28.33	54.40	60.07	44.03	58.77	65.67	132.03
American Staffordshire (n:1)	Min.	54.30	59.50	25.00	41.10	49.00	42.30	48.20	43.20	93.90
	Max.	54.30	59.50	25.00	41.10	49.00	42.30	48.20	43.20	93.90
	Mean	54.30	59.50	25.00	41.10	49.00	42.30	48.20	43.20	93.90
Beagle (n:2)	Min.	42.50	43.90	24.00	34.60	43.60	40.70	39.40	40.80	78.70
	Max.	46.60	50.50	38.00	38.70	50.30	41.40	44.00	41.40	79.80
	Mean	44.55	47.20	31.00	36.65	46.95	41.05	41.70	41.10	79.25
Chihuahua (n:2)	Min.	30.90	32.60	23.00	17.70	24.40	21.60	24.60	24.70	45.40
	Max.	31.30	33.50	35.00	26.90	36.50	23.00	31.90	31.60	55.80
	Mean	31.10	33.05	29.00	22.30	30.45	22.30	28.25	28.15	50.60
Cocker Spaniel (n:1)	Min.	48.90	53.60	29.00	41.6	48.40	36.10	43.10	42.60	89.10
	Max.	48.90	53.60	29.00	41.6	48.40	36.10	43.10	42.60	89.10
	Mean	48.90	53.60	29.00	41.6	48.40	36.10	43.10	42.60	89.10
Golden Retriever (n:10)	Min.	50.60	57.50	17.00	45.5	51.50	34.40	45.60	48.00	102.20
	Max.	71.80	75.30	31.00	51.80	58.80	44.10	54.10	60.80	130.40
	Mean	64.85	67.39	24.20	44.04	54.34	38.03	51.21	53.67	114.52
Siberian Husky (n:3)	Min.	48.10	64.80	25.00	47.50	53.30	32.00	53.40	43.90	110.40
	Max.	69.30	68.70	29.00	58.30	55.30	45.30	54.60	56.10	114.90
	Mean	60.13	67.23	27.33	54.27	54.10	38.33	53.83	51.47	111.93
Jack Russell terrier (n:1)	Min.	34.50	38.90	29.00	33.60	39.30	30.20	37.50	35.10	68.50
	Max.	34.50	38.90	29.00	33.60	39.30	30.20	37.50	35.10	68.50
	Mean	34.50	38.90	29.00	33.60	39.30	30.20	37.50	35.10	68.50
Kangal Shepherd (n:5)	Min.	69.70	66.80	21.00	50.60	53.70	35.90	54.20	52.50	121.50
	Max.	82.80	77.10	38.00	59.50	61.90	44.90	59.70	60.60	141.60
	Mean	75.62	73.32	32.00	54.32	58.04	40.62	56.42	57.66	128.86
Cavalier King Charles (n:1)	Min.	44.30	45.90	19.00	38.00	46.10	38.00	42.90	38.00	71.10
	Max.	44.30	45.90	19.00	38.00	46.10	38.00	42.90	38.00	71.10
	Mean	44.30	45.90	19.00	38.00	46.10	38.00	42.90	38.00	71.10
Pekingese (n:1)	Min.	36.40	38.40	18.00	27.20	39.50	30.10	38.00	33.20	62.00
	Max.	36.40	38.40	18.00	27.20	39.50	30.10	38.00	33.20	62.00
	Mean	36.40	38.40	18.00	27.20	39.50	30.10	38.00	33.20	62.00
Pompeian (n:1)	Min.	27.00	29.50	18.00	22.20	26.90	18.80	27.70	24.10	48.10
	Max.	27.00	29.50	18.00	22.20	26.90	18.80	27.70	24.10	48.10
	Mean	27.00	29.50	18.00	22.20	26.90	18.80	27.70	24.10	48.10
Poodle (n:2)	Min.	45.50	42.70	35.00	30.50	35.10	16.00	35.00	34.00	67.10
	Max.	52.40	45.30	37.00	35.80	42.90	31.70	38.90	40.90	77.10
	Mean	48.95	44.00	36.00	33.15	39.00	23.85	36.95	37.45	72.10
Rottweiler (n:1)	Min.	76.60	73.00	24.00	58.6	64.00	46.40	58.30	60.30	125.60
	Max.	76.60	73.00	24.00	58.6	64.00	46.40	58.30	60.30	125.60
	Mean	76.60	73.00	24.00	58.6	64.00	46.40	58.30	60.30	125.60
Russian Tsvetnaya Bolonka (n:1)	Min.	55.60	52.00	32.00	39.80	50.00	36.00	41.60	43.30	88.20
	Max.	55.60	52.00	32.00	39.80	50.00	36.00	41.60	43.30	88.20
	Mean	55.60	52.00	32.00	39.80	50.00	36.00	41.60	43.30	88.20
Samoyed (n:1)	Min.	58.80	55.70	32.00	43.70	49.70	33.80	46.90	46.30	99.00
	Max.	58.80	55.70	32.00	43.70	49.70	33.80	46.90	46.30	99.00
	Mean	58.80	55.70	32.00	43.70	49.70	33.80	46.90	46.30	99.00
Setter (n:1)	Min.	62.30	67.80	18.00	50.50	53.60	35.10	56.20	62.50	115.40
	Max.	62.30	67.80	18.00	50.50	53.60	35.10	56.20	62.50	115.40
	Mean	62.30	67.80	18.00	50.50	53.60	35.10	56.20	62.50	115.40
Terrier (n:5)	Min.	32.90	33.60	18.00	22.50	30.10	21.40	27.70	25.10	48.00
	Max.	47.70	46.20	32.00	37.70	40.80	31.70	41.30	36.90	76.80
	Mean	39.02	39.62	25.60	30.30	37.96	27.16	34.38	32.84	62.50

Table 2. Statistical analysis of inclinatio pelvis (C) between genders

Sex	N	Mean	SD	Min	Max	F	P Value
Female	14	25.79	6.42	17.00	37.00	1.057	0.310
Male	29	27.93	6.41	18.00	38.00		

DISCUSSION

In carnivores, the pelvis is the most suitable for birth among domestic animals. This is because the base of the pelvis canal is flat and backward. Moreover, due to the backward extension of the canal, the diameter verticalis falls back, causing the diameter to be longer and therefore easier delivery (Bahadır and Yıldız, 2016).

In studies on different carnivorous species and breeds, Özkadif et al. (2022) reported the diameter verticalis in males 49.75±0.90 mm, in females 37.46±5.18 mm, the diameter conjugata in males 52.54±1.22 mm, in females 48.54±3.71 mm, Inclinatio pelvis in males 49.60°, in females 49.50±0.46° degrees in red foxes. Yilmaz et al. (2020) determined the same diameters and angles in female Van cats, respectively, as 38.9 mm, 32.2 mm, and 37.3°. Atalar et al. (2017), in the pelvimetric evaluation of kangal dogs reveal that the diameter vetricalis in males is 92.21±0.98 mm, in females, 83.55±0.68 mm, the diameter conjugata in males is 93.12 ± 1.53 mm, in females 82.61±2.32 mm, inclinatio pelvis in males 39.01°, in females, they reported it 44.67° degrees. Dobak et al. (2018) reported diameter conjugata as 59±5 mm in English bulldogs, and Nganvongpanit et al. (2017) reported diameter conjugata as 64.19 mm in males and 60.31 mm in females in Retriever dogs. In our study, diameter conjugata belonging to 19 different dog breeds was the highest in Kangal Shepherd dogs and the lowest average was in Pomeranian breed dogs, diameter verticalis was highest in Kangal Shepherd dogs and lowest also in Pomeranian breed dogs, and for inclinatio pelvis, it was determined highest in Alabai breed dogs and lowest in Pekingese, Pomeranian and Setter breed dogs.

In this study, when the transverse diameters of the aperture pelvis cranialis were examined, it was observed that the intermediary transversal diameter had the highest value in all dog breeds, while the ordering of the dorsal transversal diameter and ventral transversal diameter values between breeds was determined. König and Liebich (2015) reported that the intermediary transversal diameter has the highest value among the transverse diameters of the aperture pelvis cranialis in dogs. Among the transverse diameters of the aperture pelvis cranialis in red foxes (Özkadif et al., 2022), the intermediary transversal diameter is the highest and the ventral transversal diameter is the narrowest, while in Kangal dogs (Atalar et al. 2017), it was reported that the dorsal transversal diameter is the highest and the ventral transversal diameter was the narrowest.

CONCLUSION

In conclusion, three-dimensional pelvimetry results of 19 different dog breeds were obtained and pelvimetric measurements were revealed for different species. Although pelvis morphology and morphometry can be affected by many reasons, it is thought that the data revealed will be beneficial for many disciplines, especially for gynecology. Also, it is thought that determining the probability of difficult or easy birth by revealing the pelvimetric characteristics of these breeds is very important for the enterprises engaged in animal production and breeding, and it is thought that it will contribute in an industrial way.

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The effect of gender on growth performance, live weight gain, growth pattern modeling and, survival rate in Turkish native geese of the Kars region

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ABSTRACT

Objective: This study presents a comprehensive investigation into the impact of gender on various aspects of growth performance in Turkish native geese, specifically in the Kars region.

Materials and Methods: A total of 62 goslings, comprising 25 males and 37 females, were monitored over 12 weeks. The study focused on growth performance, live weight gain, survival rates, and growth pattern modeling.

Results: In the course of our investigation, the acquired findings have brought to light a discernible gender effect during specific measurement periods, wherein a notable influence of sex on growth performance was observed. Males consistently manifested markedly higher live weights in comparison to females during weigh-ins. This gender-related disparity was statistically significant on both the hatching day ($p<0.01$) and throughout the period spanning weeks 6 to 12 ($p<0.05$), underscoring substantial differences in growth performance between the two genders. Remarkably, male geese displayed significantly higher daily live weight gains during weeks 5 to 6 ($p<0.01$). Linear regression analyses emphasized gender's influence on live weight gain, with female geese demonstrating an R^2 value of 0.9044 ($p<0.001$) and male geese showing an R^2 value of 0.8912 ($p<0.001$). Gompertz growth models unveiled distinctive growth patterns. In females, an R^2 value of 0.9300 highlighted their growth trajectory, while males exhibited an R^2 value of 0.9194. Survival rates after a 12-week period were 81.7% for females and 80.0% for males.

Conclusion: In conclusion, this meticulous exploration of the impact of gender on growth trajectories underscores the significant role it plays.

Keywords: *Geese, Gender, Growth, Survival rate, Weight gain*

INTRODUCTION

Over millennia, humans have harnessed aquatic birds for diverse purposes, consuming their meat, utilizing fatty tissue for lighting and heating, and employing feathers for insulation. The goose, exemplifying adaptability, plays a dual role—providing delicious meat and valuable fat reserves for cooking. Goose eggs offer impressive nutritional benefits, and their ability to produce fatty livers, down, and feathers showcases their multifaceted utility. Thriving globally in diverse environments, geese have seamlessly integrated into various sectors, heightening their significance. While the consumption of goose byproducts may not be paramount, its popularity has notably risen in recent decades, reflecting a noteworthy shift in consumer preferences (Buckland and Guy, 2002;

Johnsgard, 2010; Scanes and Christensen, 2020; Kozák, 2021; Ni et al., 2022).

In Türkiye's poultry farming, a hierarchy prevails with broiler chickens, layer hens, turkeys, geese, and ducks. The limited demand for goose products nationwide, mostly in specific regions, is a key factor. Challenges like reduced egg yields, difficulties in obtaining fertilized eggs, and complexities in incubation contribute to the intricate landscape. The lack of dedicated scientific studies on geese further complicates the issue. Despite not being driven primarily by market demand, goose husbandry is practiced in various regions, notably in Northeastern Anatolia, Southern Anatolia, Western Black Sea, and Central Anatolia, including provinces like Kars, Muş, Ardahan, and Kütahya. Small-scale goose farming is a prevalent phenomenon across Turkish provinces (Saatcı et al., 2021).

When juxtaposed against other poultry farming practices in Türkiye, the domain of goose husbandry emerges as conspicuously marginalized. Geese, regarded comprehensively from their anatomical constituents such as feet and head, feathers to intestines, are reared exclusively within extensive husbandry systems across the national landscape. Nonetheless, recent years have borne witness to an augmented interest in goose meat consumption, subsequently galvanizing the establishment of enterprises in Kars and Ardahan, each boasting capacities ranging from 3,000 to 5,000 head. These ventures are dedicated to harnessing pasture-based feeding modalities fortified by supplemental grain provisions, thus strategically addressing the escalating demand for goose-derived culinary offerings (Kırmızıbayrak, 2018; Saatcı et al., 2021).

Research on post-hatch growth performance and live weight gain in Turkish native geese from the Kars region up to the 12th month remains limited (Saatcı et al., 2011; Tilki et al., 2011; Arslan, 2012; Önk and Kırmızıbayrak, 2022). While gender's influence on growth performance has been a general focus, studies with weekly weigh-ins are notably sparse (Saatcı et al., 2011; Tilki et al., 2011; Önk and Kırmızıbayrak, 2022). Furthermore, the survival rate and growth pattern modeling of Turkish native geese have not received comprehensive attention. To address these gaps, our study aims to comprehensively investigate the impact of gender on various aspects: growth performance, live weight gain, survival rate, and growth pattern modeling in Turkish native geese raised in the Kars region.

MATERIALS and METHODS

Ethical statement

The geese included in this research were managed in accordance with the legal provisions and regulations of Türkiye. Furthermore, the study was initiated after securing authorization from the Kafkas University Local Ethics Committee for Animal Experiments (KAÜ-HADYEK/2020-179).

Location

The study was conducted in the province of Kars, which is located at coordinates 40°36'18''N and 43°5'48''E, at an altitude of 1760 meters above sea level. Kars province is situated in the easternmost region of Türkiye and shares a border with Armenia. Our study was conducted at the Goose Unit within the Faculty of Veterinary Medicine at Kafkas University, Kars, Türkiye.

Animal and feeds

A total of 62 goslings, consisting of 25 males and 37 females, were included in this study. Goslings hatched simultaneously were integrated into the research. These goslings were reared under standard conditions in the Goose Unit, without additional interventions or modifications in feeding practices.

During the initial four weeks (starter phase), all goslings were provided ad libitum access to diets containing 22% crude protein and 3000 kcal/kg of metabolizable energy. Subsequently, up to 12 weeks of age (finisher phase), they continued ad libitum feeding with diets containing 18% crude protein and 3100 kcal/kg of metabolizable energy (Table 1). The geese were also allowed pasture grazing during daylight hours, with unrestricted access to water throughout the study.

Upon hatching, artificially incubated goslings were carefully dried, weighed, and wing-tagged for easy identification. The weighing of geese began in May and weekly weighing were conducted up to 12 weeks of age. Additionally, any mortalities during the specified periods were recorded to determine the survival rate. Data from deceased geese were excluded from the analysis.

Statistical analyses

The Independent Samples T-Test was employed to assess the impact of gender on daily or weekly increases in growth performance and live weight. The evaluation of growth performance, whether

involving daily or weekly live weight augmentation, encompassed data derived from the surviving population of geese throughout the entirety of the study. Furthermore, linear regression models predicated on gender were constructed to elucidate the influence on live weights, complemented by Gompertz growth curve models. An analysis of Kaplan-Meier survival curves was conducted, grounded in records documenting the longevity of both deceased and surviving individuals, aligned with the progression of sampling weeks. These analyses were executed utilizing GraphPad Prism® version 9.5.1 (GraphPad Software Inc., San Diego, CA, USA). The presentation of data was structured as mean values ± standard deviation (SD), and statistical significance was established at a threshold of $p<0.05$.

Table 1. Composition of basal diets for the starter and finisher phases

Ingredients	Starter	Finisher
	%	%
Barley	6.10	6.00
Vegetable oil	4.40	5.50
Wheat bran	4.60	5.70
Wheat	6.40	5.50
Corn	40.00	48.20
Corn gluten. 62% HP	3.50	-
Soy meal. 44%	32.00	26.10
Dicalcium phosphate	1.50	1.50
L-threonine	0.09	0.09
Marble dust	0.81	0.81
Salt	0.35	0.35
Vitamin - Mineral mixture[1]	0.25	0.25
Nutrient analysis		
Dry matter (%)	89.7	89.8
Crude protein (%)	22.0	18
Metabolized energy (Kcal/kg)	3000	3100
Calcium (%)	0.78	0.76
Phosphorus (%)	0.42	0.40

[1]: The provided supplementation consisted of 1,000,000 IU of Vitamin A and 400,000 IU of Vitamin D3. In terms of minerals, the mixture included 30 mg of iron (iron sulfate monohydrate), 1.5 mg of iodine (calcium iodide anhydride), 0.5 mg of cobalt (cobalt carbonate monohydrate), 5 mg of copper (copper sulfate pentahydrate), 80 mg of manganese (manganese oxide), 80 mg of zinc (zinc oxide), and 0.3 mg of selenium (sodium selenite). This vitamin-mineral combination was provided per kilogram of diet.

RESULTS

Notably, during the weighing weeks, males consistently demonstrated significantly higher live weights compared to females. In the context of Turkish native geese, a statistically significant gender difference in growth performance was observed both on the hatching day ($p<0.01$) and during weeks 6 to 12 ($p<0.05$). On the hatching day, female and male geese displayed initial average live weights of 79.4 g and 90.4 g, respectively. Over the 12-week period, their live weights experienced substantial increases, reaching 3140.1 g for females and 3414.3 g for males. These findings underscore the influence of gender on the growth performance of Turkish native geese. A comprehensive overview of the growth performance based on gender and the total dataset for the entire 12-week period since hatching in Turkish native geese is provided in Table 2. The study demonstrates that gender significantly affects the growth performance of Turkish native geese, particularly during specific weeks of their development.

According to the obtained results, numerical variations have been discerned in the daily live weight gain attributed to gender in Turkish native geese. Particularly, a statistically significant higher daily live weight gain was observed in males during the period spanning weeks 5 to 6, in comparison to their female geese ($p<0.01$). However, for the remaining time intervals, the gender-associated impact on daily live weight gain did not attain statistical significance. Remarkably, the zenith of daily live weight gain in Turkish native geese was attained during the time frame of weeks 3 to 4, with females and males registering figures of 68.9 g and 73.3 g, respectively. Furthermore, an evident trend of gradual attenuation in daily live weight gain was observed following the 7th week (Table 3).

Notably, male geese consistently exhibited higher live weight increments compared to their female counterparts throughout consecutive weighing weeks. This observation underscores a noteworthy difference in growth performance between male and female geese. Nevertheless, it is interesting to highlight that from the 7th week onwards until the 12th week, a distinct and statistically significant disparity emerged between the live weight gain of male and female geese ($p<0.05$). These findings underscore the significance of gender in shaping the patterns of live weight gain among Turkish native geese and are further detailed in Table 4.

During the 6th week, male geese displayed a notably superior rate of live weight gain compared to their female geese, and this distinction held statistical significance ($p<0.05$). The comprehensive statistical disparities pertaining to weekly live weight gain and its association with gender within the context of Turkish native geese are comprehensively detailed in Table 5.

Table 2. Gender-based changes in growth performance of Turkish native geese over a 12-week period

Weeks	Female		Male		P value	Total	
	Mean ± SD	n	Mean ± SD	n		Mean ± SD	n
0#	79.4 ± 13.2	30	90.4 ± 12.8	20	**	83.8 ± 14.0	50
1	194.4 ± 43.6	30	212.2 ± 60.2	20	NS	201.5 ± 51.0	50
2	414.2 ± 109.8	30	449.9 ± 143.0	20	NS	428.5 ± 124.1	50
3	702.5 ± 163.8	30	778.6 ± 169.5	20	NS	733.0 ± 168.6	50
4	1185.3 ± 228.2	30	1291.6 ± 274.7	20	NS	1227.8 ± 250.7	50
5	1646.7 ± 307.7	30	1758.8 ± 323.4	20	NS	1691.5 ± 315.5	50
6	1876.9 ± 337.5	30	2068.8 ± 318.1	20	*	1953.6 ± 340.1	50
7	2276.9 ± 379.6	30	2546.8 ± 412.3	20	*	2384.8 ± 411.1	50
8	2609.1 ± 371.6	30	2885.9 ± 460.1	20	*	2719.8 ± 427.3	50
9	2795.3 ± 382.3	30	3068.1 ± 468.5	20	*	2904.4 ± 435.7	50
10	2908.8 ± 368.3	30	3201.0 ± 471.9	20	*	3025.7 ± 433.1	50
11	3026.5 ± 401.2	30	3302.4 ± 529.2	20	*	3136.8 ± 471.7	50
12	3140.1 ± 392.6	30	3414.3 ± 504.7	20	*	3251.0 ± 456.9	50

#: Hatching weight, *: $p<0.05$, **: $p<0.01$, NS: Not significant, SD: Standard deviation

Table 3. Daily live weight gain based on gender in Turkish native geese over a 12-week period

Weeks	Female		Male		P value	Total	
	Mean ± SD	n	Mean ± SD	n		Mean ± SD	n
0-1	16.4 ± 4.9	30	17.4 ± 7.6	20	NS	16.8 ± 6.1	50
1-2	31.4 ± 10.1	30	34.0 ± 13.2	20	NS	32.4 ± 11.3	50
2-3	41.2 ± 11.2	30	47.0 ± 10.7	20	NS	43.5 ± 11.2	50
3-4	68.9 ± 12.1	30	73.3 ± 27.8	20	NS	70.7 ± 19.8	50
4-5	65.9 ± 21.7	30	66.7 ± 31.1	20	NS	66.2 ± 25.6	50
5-6	32.9 ± 11.7	30	44.3 ± 15.4	20	**	37.4 ± 14.3	50
6-7	57.1 ± 13.9	30	68.3 ± 37.9	20	NS	61.6 ± 26.5	50
7-8	47.5 ± 14.2	30	48.4 ± 17.9	20	NS	47.9 ± 15.7	50
8-9	26.6 ± 9.6	30	26.0 ± 14.4	20	NS	26.4 ± 11.6	50
9-10	16.2 ± 14.7	30	19.0 ± 16.9	20	NS	17.3 ± 15.5	50
10-11	16.8 ± 14.4	30	14.5 ± 17.5	20	NS	15.9 ± 15.6	50
11-12	16.2 ± 7.7	30	16.4 ± 10.6	20	NS	16.3 ± 8.9	50

**: $p<0.01$. NS: Not significant, SEM: Standard deviation

Table 4: Daily live weight gain in Turkish native geese up to the week of weighing

Weeks	Female		Male		P value	Total	
	Mean ± SD	n	Mean ± SD	n		Mean ± SD	n
0-1	16.4 ± 4.9	30	17.4 ± 7.6	20	NS	16.8 ± 6.1	50
0-2	23.9 ± 7.2	30	25.7 ± 9.8	20	NS	24.6 ± 8.3	50
0-3	29.7 ± 7.9	30	32.8 ± 7.9	20	NS	30.9 ± 7.7	50
0-4	39.5 ± 8.6	30	42.9 ± 9.7	20	NS	40.9 ± 8.7	50
0-5	44.8 ± 7.9	30	47.7 ± 9.1	20	NS	45.9 ± 8.8	50
0-6	42.8 ± 7.6	30	47.1 ± 7.5	20	NS	44.5 ± 7.9	50
0-7	44.8 ± 6.5	30	50.1 ± 8.3	20	*	47.0 ± 8.3	50
0-8	45.2 ± 5.9	30	49.9 ± 8.2	20	*	47.1 ± 7.5	50
0-9	43.1 ± 5.2	30	47.3 ± 7.4	20	*	44.8 ± 6.8	50
0-10	40.4 ± 5.1	30	44.4 ± 6.7	20	*	42.0 ± 6.1	50
0-11	38.3 ± 5.1	30	41.7 ± 6.9	20	*	39.6 ± 6.1	50
0-12	36.4 ± 4.6	30	39.6 ± 6.0	20	*	37.7 ± 5.4	50

*: p<0.05, NS: Not significant, SEM: Standard deviation

Table 5: Gender-dependent variation in weekly live weight gain in Turkish native geese

Weeks	Female		Male		P value	Total	
	Mean ± SD	n	Mean ± SD	n		Mean ± SD	n
0-1	115.0 ± 34.9	30	121.8 ± 53.3	20	NS	117.7 ± 42.8	50
0-2	219.8 ± 70.0	30	237.7 ± 92.3	20	NS	227.0 ± 79.3	50
0-3	288.4 ± 78.1	30	328.7 ± 75.4	20	NS	304.5 ± 78.8	50
0-4	482.8 ± 84.9	30	513.0 ± 194.5	20	NS	494.9 ± 138.4	50
0-5	461.4 ± 152.2	30	467.1 ± 217.5	20	NS	463.7 ± 179.1	50
0-6	230.1 ± 81.9	30	310.0 ± 107.6	20	**	262.1 ± 100.1	50
0-7	400.0 ± 97.5	30	478.0 ± 265.3	20	NS	431.2 ± 185.5	50
0-8	332.3 ± 99.7	30	339.1 ± 125.6	20	NS	335.0 ± 109.6	50
0-9	186.2 ± 67.5	30	182.2 ± 100.8	20	NS	184.6 ± 81.5	50
0-10	113.6 ± 103.4	30	132.9 ± 118.5	20	NS	121.3 ± 108.9	50
0-11	117.7 ± 101.1	30	101.5 ± 122.4	20	NS	111.2 ± 109.2	50
0-12	113.6 ± 53.9	30	114.9 ± 74.9	20	NS	114.1 ± 62.2	50

**: p<0.01, NS: Not significant, SEM: Standard deviation

In this study, a simple linear regression analysis was conducted to assess the weekly growth performance data of Turkish native geese up to a 12-week period. For female geese, the simple linear regression analysis revealed an R^2 value of 0.9044, indicating a substantial explanatory power of approximately 90.44% for the weekly growth performance variations (p<0.001). The regression equation was computed as Y=287.1*X+35.33, where Y represents the weekly growth performance and X denotes the weeks. Similarly, for male geese, the simple linear regression analysis yielded an R^2 value of 0.8912, signifying an explanatory capability of around 89.12% for the weekly growth performance patterns (p<0.001).

The derived regression equation was Y=314.6*X+40.84. These findings underscore the significant impact of gender on the weekly growth performance of Turkish native geese. The growth rate for female geese increased by an average of

287.1 g over the 12-week period, while male geese exhibited a growth rate of 314.6 g. This analysis contributes to a better understanding of the gender-related disparities in growth performance (Figure 1A).

Investigating the trajectory of weekly growth performance among female geese, the application of the Gompertz growth model yielded noteworthy parameters: An asymptotic maximum weight of 3361 g, an initial weight of 53.52 g, a rate constant of 0.3401, and a coefficient of determination (R^2) amounting to 0.9300. These parameters unveil valuable insights into the developmental trajectory and distinctive attributes characterizing the growth pattern of female geese throughout the observed weeks. Similarly, in the context of male geese, the weekly growth performance was examined using the Gompertz growth model, leading to the identification of the following parameters: an asymptotic maximum weight of 3657 g, an initial weight of 52.01 g, a rate constant of 0.3486, and a coefficient of determination (R^2) of 0.9194. These elucidated parameters provide a comprehensive understanding of the dynamic growth patterns and inherent characteristics exhibited by male geese during the observed weeks (Figure 1B). The equations of the obtained simple linear regression model and Gompertz growth model in our study are presented in Figure 1A and Figure 1B, respectively.

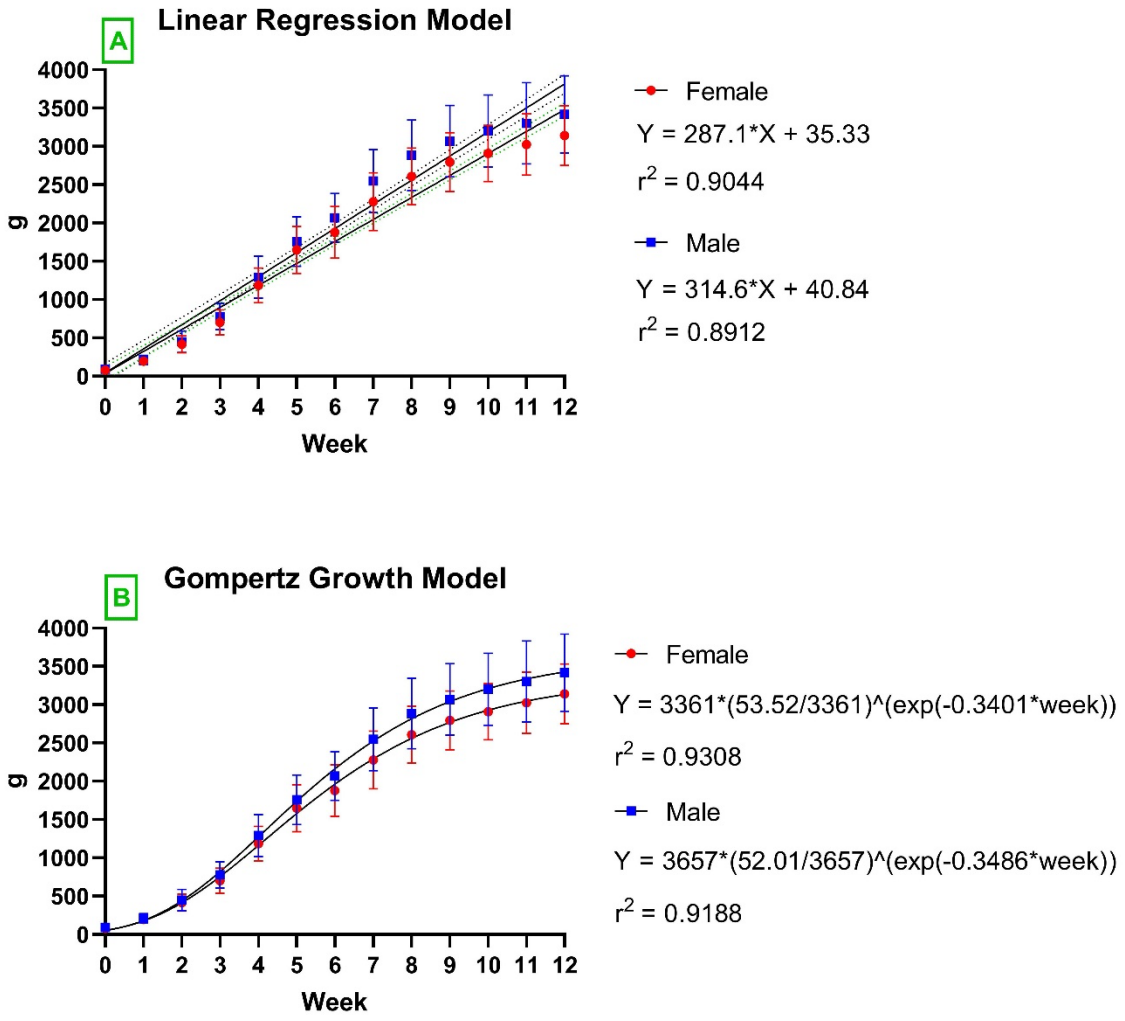

Figure 1. Equations of the simple linear regression model (A) and the Gompertz growth model (B) obtained based on the live weights determined over the 12-week period in Turkish native geese.

Following a comprehensive 12-week monitoring period of Turkish native geese, it was determined that the survival rates stood at 81.7% for females and 80.0% for males, revealing no statistically significant distinction between the genders ($p>0.05$). Noteworthy is the observation that the mortality rate exhibited a prominent increase during the initial 4-week interval. The application of the Kaplan-Meier survival curves to the Turkish native geese dataset spanning 12 weeks yielded

significant insights into their survival dynamics (Figure 2).

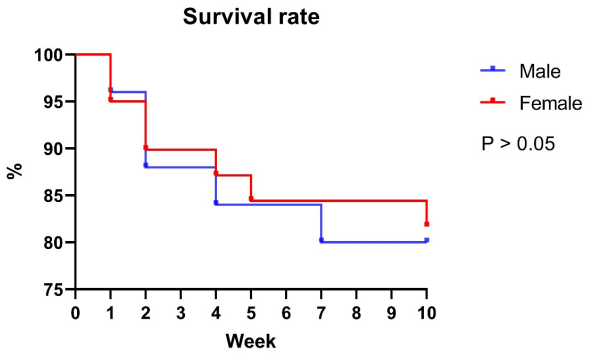

Figure 2. Kaplan-Meier survival curves on the 12-week dataset of Turkish native geese. The survival rates of Turkish native geese were 81.7% for females and 80.0% for males during the 12-week period, with no significant difference between genders ($p>0.05$). The noticeable increase in mortality rates during the first 4 weeks was noteworthy.

The statistical examination, exemplified by the Log-rank (Mantel-Cox) test, resulted in a Chi-square value of 0.02819, accompanied by a corresponding p-value of 0.87. Additionally, the Hazard Ratio (Mantel-Haenszel) was computed as 0.9043, along with a 95% Confidence Interval (CI) that ranged from 0.2794 to 2.927. This interval quantifies the degree of uncertainty around the Hazard Ratio estimate.

DISCUSSION

Goose farming is vital for Kars province, Türkiye, given its favorable conditions. The region's climate and terrain make it ideal for native goose breeding, offering economic and cultural benefits. Goose meat is integral to local cuisine, and goose feathers contribute to crafts and textiles, supporting trade. Sustainable goose farming is essential for Kars' agricultural and animal husbandry sectors (Kırmızıbayrak, 2001; Saatcı et al., 2021; Kırmızıbayrak, 2002; Demir et al., 2013; Kırmızıbayrak, 2018). Studies regarding the growth and fattening performance of Turkish native geese raised in Kars and its surrounding regions are considerably limited (Sahin et al., 2008; Tilki et al., 2009; Arslan and Tufan, 2009; Tilki et al., 2011; Saatcı et al., 2011; Arslan, 2012; Önk and Kırmızıbayrak, 2022). The prevalence of family-owned goose farms has limited comprehensive studies in the past. However, recent years have seen increased importance and popularity of goose farming in Kars and its vicinity. To address this, we conducted a study evaluating growth performance (Table 2), live weight gain (Table 3-5), and survival rates in Turkish native geese. Weekly growth data were recorded from hatching to 12 weeks, and live weight gains were analyzed using a linear regression equation (Figure 1). A Gompertz growth model was constructed (Figure 1), and Kaplan-Meier survival curves (Figure 2) were determined. This comprehensive approach enriches our understanding of the growth and survival dynamics of Turkish native geese.

In the province of Yozgat, the termination of a 12-week period yielded recorded live weights for male and female native geese at 3663.2 g and 3373.8 g, respectively, while for white male and female geese, the corresponding figures stood at 3331.5 g and 3215.4 g. Notably, no statistically significant gender-based impact was discernible. Furthermore, the aspect of gender evinced an absence of statistical significance concerning live weight gain up to the culmination of the 12-week duration (Boz and Sarıca, 2021). Within the context of Konya province, the native geese manifested weight variations spanning from 3522.7 g to 3984.7 g post a 12-week interval, thereby suggesting a modulating influence of gender upon growth performance. In respect to the trajectory of daily live weight gain, a statistically significant gender-based influence became apparent during the 8th and 9th weeks (Tilki and İnal, 2004). In a study undertaken on Turkish native geese native to the Kars region, it was revealed that subsequent to a 12-week period, male and female individuals achieved live weights of 3800.4 g and 3337.9 g, correspondingly. Furthermore, a statistically noteworthy gender-based influence on growth performance emerged during the 8th to 12th week interval. Additionally, the influence of gender exhibited statistical significance concerning daily live weight gain throughout the 6th to 8th, as well as the 10th to 12th weeks (Tilki et al., 2011). In alignment with this context, alternative studies conducted within the Kars region reported distinct live weights at 12 weeks of age. These weights were documented as 3569.0 g and 3256.1 g (Önk and Kırmızıbayrak, 2022), along with 4112.1 g and 3856.2 g (Saatcı et al., 2011) for males and females, respectively. However, it is noteworthy that the gender effect displayed variations when juxtaposed with prior investigations (Saatcı et al., 2011; Önk and Kırmızıbayrak, 2022). Reports on the impact of gender on live weight in native geese from the Kars region have yielded conflicting results (Tilki et al., 2004; Kırmızıbayrak and Boğa Kuru, 2018). Within

the realm of studies conducted on native geese in Kars, the average live weight at the 12-week mark has been reported as 3425.8 g (Arslan, 2012) and 3572.3 g (Kırmızıbayrak et al., 2011). In our conducted study, the live weights of Turkish native geese at the culmination of the 12-week period were determined to be 3140.1 g for females, 3414.3 g for males, and a total mean of 3251.0 g (Table 2). This gender effect reveals distinct growth trajectories, particularly diverging between weeks 5 and 6 (Table 3). Male geese, especially from the 7th week onwards, demonstrated a notable advantage in cumulative live weight gain (Table 4). The statistical significance of this gender-based difference reinforces its relevance. The dataset differs from previous studies on native geese, likely due to factors such as rearing conditions, nutrition, genetics, and the environment. To ensure the accuracy of similar investigations, meticulous elimination or consideration of these factors is essential for a more precise understanding of growth performance. By mitigating their impact, a substantial improvement in live weight gain can be anticipated.

Growth assumes paramount significance within the realm of animal husbandry, playing a pivotal role in bolstering economic returns. From the moment of hatching to the attainment of maturity, both physiological and morphological transformations occur, influencing weight and volume dynamics (Kaplan and Gürcan, 2018; Tirink et al., 2022; Boğa Kuru and Kırmızıbayrak, 2023). Vigilant growth tracking is crucial for effective herd management, directly impacting economic gains. The use of growth curves, depicting changes in body weight or length over time, is pervasive and pivotal. Growth curve modeling serves to understand the trajectory of biological systems over time, revealing the intricate interplay between genetic potential and environmental conditions. These models offer valuable insights for breeders, aiding in predicting growth patterns, determining optimal feed quantities, calibrating medication dosages, and identifying opportune moments for market entry. Nonlinear models like exponential, logistic, von Bertalanffy, Brody, and Gompertz models are effective due to their sigmoidal structures, accurately characterizing growth dynamics (Bahreini Behzadi et al., 2014; Do and Miar, 2020; Tirink et al., 2022). Moreover, growth curve modeling has found utility in goose research, facilitating comparisons among diverse breeds such as the Linda goose, Turkish native goose, Jilin White goose, Landes goose, Pomeranian goose, and Steinbacher goose (Önder et al., 2017; Ibtisham et al., 2017; Hrncar et al., 2021; Kaya and Yurtseven, 2021; Tirink et al., 2022; Wang et al., 2023). In our study, both simple linear regression and the Gompertz growth model assessed the weekly growth performance data of Turkish native geese over 12 weeks (Figure 1). For female geese, simple linear regression revealed a significant gender effect (R^2=0.9044, $p<0.001$), with a weekly growth rate increase of 287.1 g, while male geese showed 314.6 g. Further analysis using the Gompertz model for female geese yielded parameters such as an asymptotic maximum weight of 3361 g, an initial weight of 53.52 g, a rate constant of 0.3401, and an R^2 value of 0.9300, providing insights into their growth trajectory. Similarly, for male geese, the Gompertz model revealed parameters like an asymptotic maximum weight of 3657 g, an initial weight of 52.01 g, a rate constant of 0.3486, and an R^2 value of 0.9194, offering a comprehensive understanding of their dynamic growth patterns. Growth curve modeling, especially in livestock like geese, proves valuable in elucidating growth dynamics, providing insights for breeders and researchers.

In the context of a study focused on geese, it becomes evident that various factors play a role in influencing the first-year survival rates. Specifically, the survival rates among Greylag geese exhibit a notable range, spanning from 65% to as high as 92%. Furthermore, a distinct survival rate of 74% has been documented during the first year (Nilsson and Persson, 1993). Parallel investigations into Barnacle geese have showcased survival rates varying between 54% and 83% (Owen and Black, 1989). Analogously, a comprehensive evaluation conducted within a goose farming context has divulged a survival rate of 65%, which notably falls below the expected benchmark (Shen and Saeheaw, 2023). In our research on Turkish native geese in the Kars region, a detailed 12-week examination revealed a survival rate of 81.7% for female geese and 80.0% for male geese (Figure 2). Statistical analysis showed no significant difference between these groups. Notably, higher mortality was observed in the initial 4 weeks, emphasizing the need for intensive monitoring and strict control measures during this crucial period. This strategic approach has the potential to significantly reduce overall mortality, ensuring the well-being and sustained survival of the geese population.

CONCLUSION

As a result, this study, which investigated the gender effect on the growth performance of geese, provides significant findings. The analyses conducted reveal that gender has a statistically significant impact on growth performance during certain periods. Disparities in live weight at hatching signify that gender influences growth potential in early stages. Analysis of weekly live weight gain indicates gender's influence on growth rates during specific periods, particularly observing an increase in males around the 6th week. Simple linear regression analysis reveals that a substantial portion of weekly growth performance variation can be attributed to gender. Moreover, while survival rates do not significantly differ by gender, a notable mortality peak in the first 4 weeks is evident. Thus, this research meticulously delves into the growth performance of Turkish native geese in the context of gender, underscoring gender's role in shaping their growth trajectories. These findings underscore the necessity for further research in geese farming, genetic studies, and growth management.

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Prevalence, molecular identification and determination of antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* in raw meat

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ABSTRACT

Objective: The aim of this study was to determine the prevalence, molecular identification, and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) in raw meats of retail sale in Balikesir, Türkiye.

Materials and Methods: A total of 250 raw meat samples (beef n=100, chicken n=100, and turkey n=50) were collected from various supermarkets. Mueller-Hinton Broth medium containing 6.5% NaCl was used for pre-enrichment and Baird Parker Agar (BPA) was used as a selective medium. Polymerase Chain Reaction technique was used to confirm the suspected colonies with the *nuc* gene for *S. aureus* and the *mec*A gene for MRSA. Kirby-Bauer standard disc diffusion method was applied for antibiotic susceptibility of MRSA.

Results: Of the 250 investigated raw meat samples, 21.2% were positive for *S. aureus*, which comprised 31% beef, 14% chicken, and 16 %turkey samples. Of the 250 investigated raw meat samples, 4% were positive for MRSA, which comprised 6% beef, 3% chicken, and 2% turkey samples. All MRSA isolates were found to be resistant to penicillin, sulfamethoxazole, trimethoprim, cefoxitin, and oxacillin, but they were susceptible to vancomycin.

Conclusion: In recent years, MRSA has been called a zoonotic pathogen that poses a serious risk for food safety and public health. Therefore, we believe that this study will shed light on new studies on the prevalence of MRSA in various animal-originated foods.

Keywords: *Antibiotic susceptibility, MRSA, Prevalence, Raw meat*

INTRODUCTION

Staphylococcus aureus, which is in the *Staphylococcaceae* family, is a bacterium that causes foodborne intoxications with hospital-acquired bacteremia (Tiemersma et al., 2004). Methicillin has been put into use with the resistance of penicillin to *S. aureus* species, most of which have beta-lactamase activity. However, it was reported that it was isolated from MRSA strains after a short time (Robinson and Enright, 2003). The *mec*A gene-encoded with penicillin-binding protein 2a (PBP2a) is mediated against methicillin resistance in staphylococci, penicillin-like methicillin, oxacillin, and all other beta-lactam antibiotics (Yasuda et al., 2000) and cause serious infections.

MRSA infections are grouped mainly as hospital-acquired MRSA (HA-MRSA), community-acquired MRSA (CA-MRSA), and livestock-acquired (LA-MRSA). The first report from livestock was published in 1975 with MRSA isolation from mastitis cows (Devriese and Hommez, 1975). However, reports on this subject have been more frequently started to be published since 2000. It was reported in 2007 that animals are MRSA reservoirs (Smith and Pearson, 2011) and there is mutual MRSA (spa-type T127 ST1) contamination between

cattle and humans (Juhasz-Kaszanyitzky et al., 2007). In two different cases in Denmark, MRSA isolates, isolated from cows and sheep, and from whole-genome sequences similar to humans on two different farms, revealed that this may be related to animal husbandry (Harrison et al., 2013; Petersen et al., 2013). In the USA, there are approximately 94.000 invasive infections and an estimated 18,650 deaths from MRSA annually (Klevens et al., 2007). On the other hand, in the European Union in 2010, it has been reported to cause illness more than 150.000 people (Köck et al., 2010).

In recent years, with the definition of MRSA in animals used in food production, the presence of MRSA in foods of animal origin has come to the agenda (Febler et al., 2011). Although direct contact with animals appears to be the most likely route of infection, it is reported that the role of MRSA as a food pathogen needs further investigation (Verkade and Kluytmans, 2014). The aim of this study was to investigate MRSA prevalence, molecular characterization, and antibiotic sensitivity in beef, chicken, and turkey meat.

MATERIALS and METHODS

Collection of samples

In this study, a total of 250 raw meat samples (100 beef, 100 chicken meat, and 50 turkey meat) were collected from the markets in Balikesir, Türkiye. The samples were transported to the laboratory under the cold chain (+4°C) and analyzed on the same day.

S. aureus and MRSA isolation and identification

Twenty-five g of each meat sample was weighed, and 225 mL of Mueller-Hinton Broth (Oxoid CM0405) containing 6.5% NaCl was added and homogenized in the stomacher (IUL) for 2 min. The homogenate was incubated at 35±2°C for 16-20 h (EFSA, 2009). At the end of the incubation, 0.5 ml was taken from the pre-enrichment medium and spread to Baird Parker Agar (Merck, Germany) medium and incubated at 35±2°C for 48h (TS 6582-1 EN ISO 6888-1: 2001). Then, Gram staining and coagulase test (Staphytect Plus; Oxoid-DR0850) was performed according to the manufacturer's instructions to the black suspect colonies that grow in petri dishes. As phenotypically Gram (+) and coagulase-positive isolates were incubated in Brain Heart Infusion Broth (BHI; Merck, Germany) medium at 35±2°C for 24 h. After, all isolates were stored at -80°C in a BHI broth medium containing 20% glycerol (20 w/v) until genotypic was identified.

Molecular characterization

DNA extraction was performed using a commercial kit (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche, Germany) according to the manufacturer's instructions. The process is initial denaturation (94°C for 10 min) and 23 cycles in total; denaturation (94°C for 1 min), binding (51°C for 1 min), extension (72°C for 2 min) and final extension (72°C for 5 min). Primarily for the *nuc* gene; *nuc* 1 (5'-GCGATTGATGGTGATACGGTT-3'); *nuc* 2 (5'-AGCCAAGCCTTGACGAACTAAA GC-3') and for the mecA gene; *mec*A1 (5'-AAAATCGATGGTAAAGGTTGGC-3'); *mec*A2 (5'-AGTTCTGCAGTACCGGATTTGC-3') sequence was used (Maes et al., 2002). Electrophoresis was performed for 75 minutes at 90 V in agarose prepared in 1.5%. Samples of 279 bp lengths were considered positive for *S. aureus*, while 533 bp samples were considered MRSA positive.

Antibiotic susceptibility test

According to Kirby-Bauer's standard disc diffusion method (Baur et al., 1961), all isolates identified as MRSA were incubated in Trypticase Soy Agar (TSA; Biomerieux 43011) medium for 35±1°C 24 h. The antibiotic discs (Thermo Scientific Oxoid) used in the test were placed on the medium at 24 mm intervals. Antibiotics contained in discs; penicillin G 10 µg, gentamicin 10 µg, erythromycin 5 µg, ampicillin 10 µg, sulfamethoxazole-trimethoprim 25 µg, ciprofloxacin 5 µg, tetracycline 30 µg, chloramphenicol 30 µg, cefoxitin 30 µg, and oxacillin. Results were interpreted according to CLSI instructions (2012; 2014).

Reference Strains

In this study, reference strains of *S. aureus* (ATCC 25923) and MRSA (ATCC 43300 *mec*A positive and ATCC 33592 *mecA* negative standard strains) were obtained from Microbiologics Inc. (Saint Cloud, USA).

RESULTS

In this study, *S. aureus* was detected in 53 (21.2%) of 250 raw meat samples. These isolates were isolated from 31 beef, 14 chickens, and 8 turkey meat, respectively (Table 1). On the other hand, 10 isolates from 53 *S. aureus* isolates were evaluated as MRSA. Of these, 6 isolate beef, 3 isolate chickens and 1 isolate turkey meat were detected in raw meat samples (Table 1, Figure 1). In this study, 10 isolates

detected as MRSA were tested for sensitivity to 11 different antibiotics according to Kirby-Bauer's standard disc diffusion method. Antibiotic resistance levels of the isolates were evaluated according to CLSI (2012; 2014). As a result of antibiotic resistance tests, 1 isolate was found to be resistant to gentamicin, 2 isolates of erythromycin, 2 isolates of tetracycline, 1 isolates of ciprofloxacin and 2 isolates of chloramphenicol, all of them were resistant to ampicillin, penicillin, sulfamethoxazole-trimethoprim, cefoxitin and oxacillin. One of the isolates was found to be moderately sensitive to erythromycin and 1 isolate of found to be moderately sensitive to ciprofloxacin.

Table 1. Distribution of *S. aureus* and MRSA isolates in beef, chicken, and turkey meat

Samples	(n)	Positive samples of *S. aureus* (%)	Positive samples of MRSA (%)
Beef	100	31 (31)	6 (6)
Chicken	100	14 (14)	3 (3)
Turkey meat	50	8 (16)	1 (2)
Total	250	53 (21.2)	10 (4)

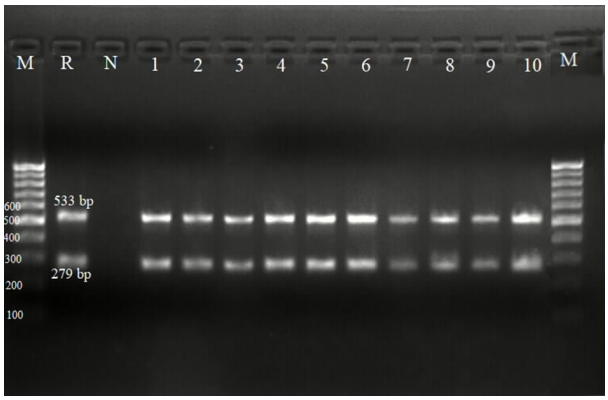

Figure 1. PCR image of strain and reference strains confirmed as MRSA according to the *mecA* gene (M: Marker, ATCC 43300: Positive Control Strain, 1: *mec*A positive 533 bp)

DISCUSSION

Staphylococcal intoxications, mainly caused by *S. aureus*, have an important place worldwide in foodborne intoxications. The fact that MRSA strains, which are thought to be mostly from hospital, have been detected in foods of animal origin in recent years brought serious studies on this issue. In this study, the prevalence, molecular characterization, and antibiotic susceptibility of MRSA were investigated in the samples of beef, chicken, and turkey meat collected from different markets. It was detected in 21.2% (53/250) of *S. aureus* and 4% (10/250) of MRSA in 250 meat samples. When looking at the distribution of meat types, *S. aureus* was found in 31% of the beef samples and MRSA in 6% (Table 1). Looking at the results of studies on raw beef in some countries; In the USA, 3.3% of 30 samples (Pu et al., 2009), in the Netherlands, 10.6% of 395 samples (de Boer et al., 2009), in Nigeria, 14.3% of 77 samples (Nanchi et al. 2014), 0.5% of 847 samples in Korea (Lim et al., 2010); They reported that in 23.3% of 30 samples (Costa et al., 2014) in Brazil, 380 beef samples detected 4.4% (Boost et al., 2013) MRSA. Pu et al. (2009) reported that *S. aureus*, which produces heat-resistant enterotoxins, plays an important role in food-borne intoxications and that the presence of MRSA in meats poses a potential infection threat to those working in the food preparation process. On the other hand, it has been reported that carrier people may play a role in the contamination of meats with MRSA. Bhargava et al. (2011) explained the low prevalence of *S. aureus* and MRSA in raw meat (beef, chicken, and turkey meat) samples in the United States by not using pork in the study. Pork is shown as the largest reservoir of MRSA. Pork was not used as a material in this study. On the other hand, it is reported that differences in percentage rates in a study are directly related to sample size (Jackson et al., 2013).

In this study, the prevalence of MRSA in chicken meat samples was 3% (Table 1). Kwon et al. (2006) detected MRSA in 0.2% of 340 chicken meat samples. Wang et al. (2014) detected MRSA in 2.3% of 264 samples. The results of this study are similar to those obtained by Kwon et al. (2006) and Wang et al. (2014). On the other hand, the results of the study on MRSA prevalence in chicken meat are as follows; Feßler et al. (2011), in 25 of 24 fresh chicken meat samples, in 21.1% of 19 chicken meat products; Karmi et al. (2013), 44% of 25 chicken carcass samples, 52% of 25 chicken piece meat; in 40% of 25 cooked chicken meal (luncheon) samples, 24% of 25 chicken sausage samples and 44% of 25 chicken burger samples, de Boer et al. (2009) 16% of 520 chicken meat samples; Boost et al. (2013) in 6.8% of 455 chicken meat samples and Costa et al. (2014) in 23.3% of 30 chicken meat samples. Considering the results of some similar studies given above, it is seen that our study is much higher than the results. The high prevalence of MRSA in some processed foods (hamburgers and sandwiches) reported that

beef and chicken meat used as raw materials may not be sufficiently cooked or may be due to cross contamination after cooking (Contreras et al., 2015). On the other hand, Kwon et al. (2006) reported that MRSA strains transmitted to humans through chicken meat may cause infections.

In this study, the prevalence of MRSA in turkey meat samples was 2% (Table 1). Feßler et al. (2011) found MRSA in 35.3% of 116 turkey meat samples and 50.0% of 22 fresh turkey meat samples and 52.4% of 21 turkey meat products. Monecke et al. (2013) reported that they detected MRSA in 21.2% of 80 turkeys clinically. Also, contaminated foods can also pose a health risk to food processors. Carcasses obtained from animals colonized with MRSA can be contaminated during slaughter (Lozano et al., 2009). It is reported that the differences between the results obtained, and the previous studies may result from differences in sampling plans and MRSA detection procedures (Feßler et al., 2011). It is seen that the number of studies on the presence of MRSA in turkey meat is limited.

In this study, 4 of the 10 MRSA isolates resisted at least three antibiotics. The transfer of antibiotic resistance from animals to humans can occur by removing antibiotic residues in foods or resistant food-borne pathogens (Pesavento et al., 2007). Accordingly, the detection of MRSA in animals is considered as one of the most important zoonotic origin pathogens in the recently published reports. For eradication of infections caused by MRSA, it is important to identify the vectors causing the contamination and the origin of the agent and their spread throughout the farm and food chain. Again, determining the potential impact of MRSA strains on public health is of great importance. This study does not represent a country-wide prevalence since it was conducted in a province of our country. However, it is important in terms of shedding light on more comprehensive studies on this subject.

CONCLUSION

As a result, in order to reduce and prevent the prevalence of MRSA in meats, the meats to be used in production must first be obtained from healthy animals, and the necessary hygiene rules must be followed during slaughtering, transport and cooling stages. Food Safety Management Systems such as HACCP (Hazard Analysis and Critical Control Points), GMP (Good Manufactured Practice), and GHP (Good Hygiene Practice) should be fully implemented in meat and meat products with the principle of "farm to fork". Controlled and conscious antibiotics should be used in animals used in meat production. On the other hand, this study is the first study on the prevalence of MRSA in raw meats in our country, according to the literature surveys we conducted. Therefore, we believe that this study will shed light on new studies on MRSA prevalence and antibiotic resistance in various animal origin foods in different regions of our country.

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Environmental and behavioral variables associated with lower urinary tract diseases in domestic cats

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ABSTRACT

Objectives: Feline lower urinary tract disease (LUTD) is a frequently seen disease with multiple etiologies. Since it is a complex condition, the aim of this study is to evaluate the factors that may affect the development of the disease.

Materials and Methods: Demographic, clinical, behavioral and environmental parameters of 30 cats diagnosed with lower urinary tract disease and 15 aged-matched healthy cats presented to Ankara University Small Animal Hospital were evaluated.

Results: In the diseased group, the male population was higher, and the cats were overweight. The number of food, water and litter boxes and litter material were found to differ significantly between groups. Daily habits such as playing with a scratching board and self-grooming differed between the groups. Inappropriate behaviors observed by the owners of the cats in the first group were listed as urinating/defecating outside the litter box and showing aggression towards the owner, guests, and/or other animals.

Conclusions: The results of this study show that the detailed evaluation and regulation of the daily needs of cats is important for the emergence or recurrence of the disease and therefore may contribute to the correct management of the treatment process in cats with LUTD.

Keywords: *Behavioral factors, Environmental factors, FLUTD, Lower urinary tract.*

INTRODUCTION

Feline lower urinary system disease (LUTD), which is one of the most common reasons for referral to veterinary clinics, is a general term used to describe diseases affecting the bladder and urethra in cats (Hostutler et al., 2005; Lew-Kojrys et al., 2017). LUTD has multifactorial etiology such as functional and structural disorders of lower urinary system organs, as like crystalluria, urethral plaque formation, urolithiasis, urinary tract infections (UTI), neoplasia, trauma, neurological disorders, behavioral problems and feline idiopathic cystitis (FIC). Each of these diseases causes serious clinical symptoms and intense pain in patients that significantly affects animal welfare (Gunn-Moore, 2003).

In cats, LUTD may cause complete or partial urethral obstruction and obstructive disease may cause varying degrees of clinical manifestations related with the severity of the obstruction. The signs include localized lower urinary tract symptoms such as dysuria, hematuria, frequent and repeated visits to the litter box, increased vocalization and pain may be seen clinically as well as systemic manifestations such as electrolyte disturbances and uremia, anorexia, lethargy, and vomiting (Segev et al., 2011).

Given the widespread prevalence and severity of LUTD in feline populations, there is a pressing need to identify the key factors contributing to the onset of this condition. Beyond the conventional exploration of demographic and clinical parameters, it is imperative to broaden the research to include variables that impact animal welfare, such as environmental and sociological structures. The intricate nature of LUTD implies significant gaps in our current understanding of its pathogenesis. This study is designed to bridge these gaps through a comprehensive analysis, aiming to unveil potential associations between LUTD in domestic cats and a spectrum of demographic, environmental, and behavioral variables. By doing so, our research seeks to amplify comprehension of the intricate complexities that contribute to the widespread prevalence of LUTD in feline populations.

MATERIALS and METHODS

Study population

The study included 45 cats presented to Ankara University Veterinary Faculty Small Animal Clinics. Patient records were reviewed retrospectively by using the hospital software system. The cats were categorically assigned into two groups; the first group included cats diagnosed with LUTD, and the second consisted of healthy cats. Exclusion criteria ensured the omission of cats with endocrine and/or metabolic disorders, as well as those facing joint problems. This study has been reviewed and approved by the Local Animal Ethics Committee (Decision number: 2022-14-131) and the Ethics Committee for Non-Clinical Human Research (Decision number: 17/157) of Ankara University.

The first group consisted of 30 cats that presented to the clinic with the signs of LUTD including anuria/oliguria, stranguria, hematuria, vomiting, lethargy, etc. In these cases, the diagnosis was made after a complete physical examination, radiographic imaging and abdominal ultrasonography of the urinary tract, urine analysis, bacteriologic culture of the urine, and additional testing of blood parameters was performed in some cases if needed. According to the results obtained, cats were classified based on the cause of the disease. The control group included 15 healthy aged-matched cats brought to our clinics for yearly routine examinations. In addition to the applied exclusion criteria in all cats in this group, cats with history of LUTD were also excluded from the study.

Assessment of environmental and behavioral variables

To assess environmental and behavioral variables, the anamnesis obtained from each patient owner was compiled in detail. The data included a standard question set routinely used in our clinics were adapted from the open-access BSAVA Client Information Leaflet Feline Behavior Questionnaire (Horwitz and Mills, 2009). This set of questions has been incorporated into our anamnesis forms, playing a pivotal role in conducting standardized behavioral assessments consistently in our clinics. The clinical findings and the information obtained from the patient's owner were examined under 6 sub-headings. i) demographics, ii) physical environment and housing, iii) social interaction (hiding, interaction with the owner, guests, and foreigners), iv) daily routine (eating and drinking, sleep and wake routines), v) playing and investigative behaviors, and vi) the problem behavior (inappropriate urination, etc.). The following information was obtained from the patient owners in detail: the type of elimination, covering of elimination in the litter box, the type of the litter box and/or litter tray, the number and localization of the food bowl, water bowl, cat litter box and whether she/he used a scratching board.

Statistical analysis

The relationship between categorical variables was evaluated using either the Chi-square test or the Fisher exact test. Chi-square test was employed to analyse the correlation among numeric variables, while the Chi-squared test of association was utilized to assess relationships between categorical variables. For binary variables, summary statistics such as means, medians, and standard deviations were computed. To compare continuous parameters between the study and control groups, as well as between the two groups, either Student's t-test or the Mann-Whitney U test was employed based on the distribution of the data. The analyses were performed in SPSS 22.0 for Windows, SPSS Inc, Chicago, IL, USA, and the significant effect was defined at a value of $p< 0.05$.

RESULTS

Demographic characteristics of the study population

The demographic characteristics of the cats in both groups were examined in detail. In the first group,

18 of the cats were mixed breed while the rest were Scottish fold (n=5), British shorthair (n=3), Angora (n=2), and Sphinx (n=1). The male (n=25) cat population was higher than the female (n=5) cats. Fifteen of the males and 3 of the female cats were neutered. The age of cats ranged from 6 months to 8 years of age (mean 3.7±2.07 years) and they were weighing between 2 to 8.5 kg (mean 4.8 kg±1.34 kg). The second group consisted of healthy cats, the majority of the cats were mixed breed (n=12), followed by British shorthair (n=2) and Persian (n=1) cats. There were 8 female-spayed and 7 male-castrated cats in this group. The age of the cats ranged from 1 year to 9 years (mean 3.96±2.32 years) and they were weighing between 3 to 5 kg (mean 4.12±0.52 kg). The cats in the first group were found to weighted higher than those in the second group (p=0.036). Although no statistical analysis was applied because the second group was selected with a balanced age and gender distribution, male cats were clearly dominant in the first group.

The most common diagnosis in the first group was struvite crystalluria (n=12), and it was followed by struvite crystalluria and UTI (n=4), urolithiasis (n=6; 4 struvite and 2 CaOx uroliths), FIC (n=6), and UTI (n=2). Identified pathogens for bacterial LUTD were, *Streptococcus* spp. (n=2), *E. coli* (n=1), *Staphylococcus* spp. (n=1) for the Struvite crystalluria and UTI cases and *E. coli* (n=1), *Proteus* spp. (n=1) for UTI cases.

Physical environment and housing

The majority of the cats diagnosed with LUTD were housed in an apartment (n=29) and the owners described their home environments as *calm* or *active* equally. In 14 of the houses, there were multiple pets and, in 13 of these, the second pet was also a cat. All but 5 of the cats in the study group had access to the entire house. These remaining 5 cats were restricted from entering the kitchen and/or living room. Twenty-five of the cats in the first group were indoors only while the rest was allowed to leave the house in a controlled manner for maximum of 2 hours a day. Among those cats, 3 were male (2 were castrated and 1 was active) and 2 were female (1 spayed and 1 active).

As in the first group, most of the cats (n=14) were housed in an apartment in the second group. Three of the owners describe their home environment as *active*, while the remaining 12 describe their home as *calm*. Only 3 of the owners owned one cat, and the rest had more than one in their homes. Cats in this group were allowed access to the entire house. One female and one male cat were allowed to leave the house in a controlled manner.

Since the dominant majority in both groups were indoor cats, information about whether they saw cats/other animals on the street through the window were also evaluated within the scope of this study. In both groups, it was determined that about half of the cats can see other animals through the windows, while the other half did not (p>0.05).

In both groups, the cats were fed with commercial food only. While there were as many or fewer food bowls as the number of cats in the first group, there was one more bowl than the number of cats in all the houses in the second group (p<0.001).

Table 1. Details about litter boxes of cats.

	Groups		P value
	1	2	
Box size (n)			
Medium	18	10	NSD
Large	12	5	
Box type (n)			
Closed	13	19	NSD
Open	17	5	
Litter brand routine (n)			
Same brand	15	6	NSD
Different brands	15	9	
Litter types (n)			
Bentonite (clumping)	15	14	
Silica	12	1	0.016
Active Carbon	3	-	
Litter fragrance (n)			
Scented	22	7	NSD
Unscented	8	8	
Cleaning regime (n)			
Every day	18	15	0.001
Every 2 days	12	-	
Cleaning the entire litter box (n)			
<7 days	3	4	
7-15 days	12	6	NSD
>7 days	15	5	

NSD: No significant difference, (-) = Not applicable.

The data regarding the litter boxes were also analyzed (Table 1). It was determined that there were as many or fewer litter boxes as the number of cats in the houses in the first group. In the second group, while the litter box was more than the number of cats in only one house, it was either as many or less than the number of cats in other houses (p<0.001). Litter box size did not differ

between the two groups (p>0.05). While the litter material was found to be statistically significantly different between the groups (p=0.016); the scent of the litter material was not differed.

In the first group, an equal number of owners stated that they always used the same brand or changed brands while 9 of the owners in the second group stated that they change the brand occasionally and the remaining 6 always use the same brand which does not differ significantly from the first group. The cleaning habits were changing regarding the cleaning regimens of the litter box between both groups. While all the litter boxes are reported to be cleaned every day by the owners in the 2nd group (p=0.001), there was not a significant difference between the groups when the entire litter box cleaning habits were evaluated.

The number of scratching boards was also differed between the groups. While 20 cats had a scratching board in the first group, all the cats had it in the second group (p=0.019).

The daily routine of the cat

Eating habits were evaluated in both groups and it was noted that while 23 of the first group ate slowly and the remaining 7 ate fast and in the second group, 10 ate slowly and the rest ate fast (p>0.05). In both groups, it was observed that cats mostly sleep with their owners and wake up between 6-9 am. All the cats in both groups use their litter boxes routinely. When the data on self-grooming behavior were examined, it was observed that the cat in the first group rarely groomed itself, while all the cats in the second group groomed themselves as a routine daily activity as stated by the owners (p=0.001).

A review of data on whether there has been a recent change in home routine showed that 6 cat owners in the first group indicated a routine change due to a new job, new home, or travel. In the second group, it was learned that 3 cat owners had recently moved.

The data on the routine relationship between the guests and the cat showed that 21 animals in the first group were affectionate towards the guests, while 8 cats in the second group.

Playing and investigative behaviors

Owners of 26 cats in the first group described their cats as *playful* while all but 2 owners in the second group did (p>0.05). In both groups the owners of the playful cats reported that they play the games their cats initiate every day. When the owners of the cats in the first group were asked how long the play time was, 4 of them reported that they played less than 10 minutes while 9 of them played for 10-20 minutes and, 13 of them played more than 20 minutes while the daily routine playing time for the cats in the second group is more than 20 minutes (p=0.021). All the owners in the first group noted that the toys were available for the cats during the day and the first-choice toys of the cats for playing were rope (n=11), ball (n=9), soft plush toy (n=6), furry mice (n=2) and laser (n=2). The 4 cats who were stated to do not like to play, were noted as playing games initiated by their owners for less than 10 minutes and were all played with rope. In the second group, cats' favorite toys are listed as follows; balls (n=5), rope (n=4), soft plush toys (n=4), and furry mice (n=1). The cats who do not like to play are played the laser by the owners.

In the first group, it was noted that all but 3 of the cats were prone to discovery. Eleven of all the cats in this group tended to defend their territory. Moreover, while 16 of the cats hunted (bugs, butterflies, etc.) frequently, the rest did not. In the second group, all 15 cats were prone to explore. Except for 2 cats, it was stated that the cats in this group protect their territory. Eleven cats are hunted frequently, while 4 cats are not (p>0.05).

The problematic behavior

When the data is examined on whether the patient owners observed inappropriate behavior in their cats, a total of 14 cat owners in the first group reported that their cat engaged inappropriate urination/defecation problems occasionally as they urinated/defecated outside the litter box while only one cat in the second noted to urinate outside the litter box when the box was not cleaned that day (p=0.003). Half of the 14 cats in the first group who urinated/defecated outside the litter box were seen squatting and the other half were in the standing position. The location of periuria or inappropriate defecation was stated as the floor nearby the litter box. The second most common undesirable behavior was aggression (n=13) toward the owner, guests, and/or other animals (hissing, biting, attacking) (p=0.002).

DISCUSSION

Demographic data findings obtained from the presented study were compatible with the previous studies. Cats included in the first group were aged between 6 months to 8 years and the majority of the cats were male castrated with

increased average body weight (Lekcharoensuk et al., 2001; Piyarungsri et al., 2020). It is estimated that overweight indoor cats are less active as their environments are generally predictable and unchanging (Rochlitz, 2005). Therefore, they are likely to urinate less and drink less water. In addition, obesity may lead to urethral compression with the accumulation of fat around the urethra and penis (Piyanrungsri et al., 2020). Hence, it is important to control the weight as a part of the treatment in cats with LUTD.

In the presented study, almost half of the cats in the first and 80% of the cats in the second group were reported to live in a multi-cat household. However, the number of food bowls and litter boxes was significantly less in the first group than in the second group. Hence, upon evaluating these data, it becomes evident that resource distribution plays a significant role in the development of LUTD in cats. As indicated in this study and supported by previous reports, adhering to the standard rule of providing one additional resource beyond the number of cats can mitigate the development of various issues (Forrester and Towel, 2015). The social structure of domestic cats is primarily shaped by food availability, while environmental factors within households, including human relationships and resource availability, emerge as crucial contributors to feline stress levels (Wojtaś, 2023). Consequently, making simple adjustments to environmental enrichment has the potential to enhance inter-cat relationships and alleviate stress in multi-cat households, thereby promoting the health and well-being of cats.

In line with the previous findings this study revealed that the size and type of litter box, the scented or unscented litter material, or the change in the brand of litter do not seem to contribute to the development of the LUTD (Sung and Crowell-Davis, 2006). Conversely, the material utilized in the litter box emerges as a crucial factor, constituting a risk element in LUTD development. Cats' preferences for litter material likely stem from the domestication process. As far as is known, the desert-dwelling African wildcat used desert sand as its litter, creating a material preference that is thought to have persisted throughout the domestication process (Neilson, 2001; Neilson, 2009). In this study, the preference for clumping bentonite litter in the healthy group may be associated with the preference of the cats for sand-like material that clumped when wet.

In a majority of cases, LUTD is closely linked to issues in litter box management. Inadequate cleaning of the litter box can precipitate the development of inappropriate elimination behavior and/or LUTD in certain cats. This is particularly noteworthy in multi-cat households, where the unfamiliar odors of urine and feces may render a cat vulnerable, especially if it harbors fear of other cats in the same environment. Consequently, this vulnerability may manifest as a reduction in the frequency of elimination or the selection of an inappropriate toileting location (Overall, 1997; Neilson, 2009). The diminished frequency of urine elimination heightens the risk of urinary tract diseases by prolonging the contact time of high-concentration urine with uroepithelial tissue (Forrester and Towell, 2015). Hence, in accordance with the findings of the present study, consistent daily maintenance proves beneficial in preventing LUTD, even when the entire litter box is not thoroughly cleaned.

There are some species-specific behaviors, and the fact that cats mostly live indoors may prevent them from exhibiting these natural behaviors, as they spend most of their time hunting and exploring their territory when living outside (Amat et al., 2016). Therefore, environmental enrichment strategies should be designed by targeting the needs of cats in mind. One of the elements that should be included in these regulations and that supports the natural behavior of cats is the scratching boards. Scratching is one of the natural cat behaviors and plays an important role in leaving both visual and pheromonal regional marks while helping to maintain claw health (Stella and Croney, 2016). This study presents evidence to suggest that healthy cats are strongly motivated to scratch, and this behavior has positive aspects on their well-being, probably by reducing stress and meeting their daily needs.

Grooming is a normal behavior in cats, and they spend about 8% of their active time self-grooming (Eckstein and Hart, 2000). The grooming behavior in cats can serve different purposes such as hair arrangements, removal of foreign bodies, dirt, and parasites, and sensory stimulation of the skin. Many factors that affect the well-being of cats can cause cats to be stressed and unable to express species-specific behaviors, including self-grooming (Stella and Croney, 2016). In the presented study, grooming behavior was significantly reduced in the first group compared to the second group. This

suggests that the absence or decrease of normal cat behaviors may increase the susceptibility to LUTD.

Cats are natural hunters. Hunting behavior is mostly triggered by the seeking system and thus is not directly related to hunger. Solitary play behavior is a manifestation of hunting in cats, which has a critical value in survival (Bradshaw et al, 2012). Cats, with their biology intricately linked to hunting, thrive when given regular opportunities to practice and hone their natural hunting instincts. The absence of play and hunting outlets can lead to considerable stress in domestic cats (Zhang et al., 2022).

The current study indicates that cats in the first group engage in play for a shorter duration compared to those in the second group. Research suggests that solitary-living cats exhibit improved communication with their owners and tend to engage in longer play sessions (Mertens, 1991). The full extent of the impact of early experiences, essential for fostering social bonding in cats with their environment, on their socialization and play behaviors remains incompletely understood (Rochlitz, 2005). Nevertheless, positive effects can be achieved by optimizing environmental arrangements to encourage cat play. The constant availability of many and varied toys providing opportunities to meet hunting and exercise needs can help reduce stress levels. However, it is noteworthy that in this study, the toys provided in both groups were found to satisfy only a limited range of instinctive skills. A study addressing common behavioral problems in cats has demonstrated an association between playtime and behavior issues, highlighting the inadequacy of typical commercially available toys in meeting the complex needs of house cats (Strickler and Shull, 2014). An interesting finding from the current study is that cats in the healthy group engaged in play involving lasers. Although playing cats with lasers constantly triggers the frustration system here it can be inferred that even playing with a laser would be better than not playing at all (Kogan and Grigg, 2021).

Individual differences in the expression of their behavior may stem from a variety of causes. The most common undesirable behaviors are aggression among cats or towards humans, along with inappropriate elimination behavior (Curtis, 2008). Medical problems can change behavior directly or indirectly. Therefore, when monitoring these behavioral changes, the cat's general health status should be examined as well as their pain assessments, and arrangements for their individual needs should be evaluated (Camps et al., 2019). In the present study, it was determined that the cats in the first group showed markedly inappropriate elimination behavior and were aggressive. However, within the scope of the presented study, it is not known whether these behavioral changes are caused by pain or environmental stress. Evaluation of these changes in future studies involving larger numbers of cats will have important implications.

CONCLUSION

This research underscores the critical role of environmental and behavioral factors in LUTD. To evaluate the effectiveness of these factors, it is imperative to collect comprehensive information from owners of affected patients. This includes demographic details, clinical examination findings, and information about the patient's environment. It should be considered that ensuring that the basic daily needs and species-specific social needs of cats are adequately met will play a very important role in preventing the onset or recurrence of the disease, and patient owners should be informed about making the necessary changes.

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Macroscopic and microscopic characteristics of uropygial gland of budgerigars (*Melopsittacus undulatus*)

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ABSTRACT

Objective: This study was carried out to investigate the anatomical, morphometric, topographic, and histological features of the uropygial gland in adult male and female budgerigars (*Melopsittacus undulatus*).

Material-Method: For this study, the uropygial glands of fourteen adult budgerigars (7 male, 7 female) were used in the study. This glandular structure located between the caudal vertebrae and pygostyle was removed by dissection. Morphological and histological characteristics of the dissected uropygial glands were determined. In addition, morphometric measurements and index calculations were performed. Tissue samples were taken to determine the histological structure of the gland, that were stained with Hematoxylin & Eosin (H&E), Masson Trichrome's and Periodic Acid Schiff-Alcian Blue.

Result: In the study, it was determined that the gland structure was heart shaped and consisted of two lobes, a papilla and a draining duct system. Uropygial gland weight was determined as 1.57±0.96 g in male birds and 1.52±0.09 g in female birds. As a result of the statistical evaluation, there were significant differences between the sexes in the parameters of lobe width ($p<0.05$), papilla length and papilla width ($p<0.01$). Also, the GULI value had a statistically significant difference ($p<0.05$). Histological examination revealed that the gland had a two-lobed structure surrounded by a capsule composed of connective tissue. It was determined that the gland had a tubuloalveolar-holocrine structure and the epithelial layer consisted of cellular layers as germinative layer, intermediate layer, secretory layer and degenerative layer from the periphery to the centre.

Conclusion: As a result of the study, it was determined that the morphological and histological structure of the uropygial gland in budgerigars showed similarities with other bird species as well as showing certain species-specific differences in general.

Keywords: *Anatomy, Budgerigar, Histology, Morphology, Uropygial gland*

INTRODUCTION

Unlike mammals, bird skin does not contain sweat and sebaceous glands. In birds, there is a specialised structure called uropygial gland that produces oil (Jacob and Ziswiler, 1982; King and McLelland, 1984; Reynolds, 2013).

The uropygial gland is located dorsally on the last caudal vertebrae (Jacob and Ziswiler, 1982). It has also been reported to be located dorsally in the region between the fourth caudal vertebrae and the pygostyl (Lucas and Stettenheim, 1972; Sawad, 2006). It has been reported to develop from a pair of ectodermal invaginations (Jacob and Ziswiler, 1982)

and to be a compound tubulo-alveolar, holocrine gland similar to the sebaceous glands of mammals (Wagner and Boord, 1975).

The size and shape of the glandula uropygialis, which is usually a two-lobed organ, may vary depending on the species (Stettenheim, 2000; Salibian and Montalti, 2009). The gland consists of two lobes and a papilla. The secretion produced in the lobes is transmitted to the nipple-like papilla by a complex duct system (Jacob and Ziswiler, 1982; King and McLelland, 1984; Salibian and Montalti, 2009). The papilla is located just above the tail (King and McLelland, 1984; Stettenheim, 2000). In most bird species, bundles of soft feathers surround the papilla (Lucas and Stettenheim, 1972; Jacob and Ziswiler, 1982; King and McLelland, 1984; Stettenheim, 2000). The beaks of birds are lubricated by these brush-like feather bundles and the secretion is distributed on the feathers in this way (Schumacher, 1919). The gland structure, which is present in the embryonic stages of all bird species, may atrophy in some adult birds (Johnston, 1988; Salibian and Montalti, 2009). It is completely absent in a few species of the pigeon (Columbidae) and parrot (Psittacidae) families and in some ostrich species (Rheidae) (Johnston, 1988).The uropygial gland, which produces an oily secretion, is also called the preen gland (Sandilands et al., 2004; Harem et al., 2005; Chiale et al., 2016) and secretes its secretion through the uropygial duct that extends to the top of the papillae of the glands opening into the porus ductus uropygialis (Bhattacacharyya and Ghosh, 1971; Lucas and Stettenheim, 1972; King and McLelland, 1984; Shawkey et al., 2003).

Johnston (1988) reported that gland size is larger in aquatic species than in terrestrial species, while gland size is dynamic and may increase with age (Møller et al., 2010; Vincze et al., 2013).

The function of the glandula uropygialis is not fully explained. While it has been reported that this gland has functions such as feather maintenance, defence against predators and/or parasites, waterproofing and intraspecific communication (Reynolds, 2013), it has been reported that the secretion of the gland prevents the colonisation and growth of microorganisms on feathers, skin and eggshells due to its antimicrobial properties (Galván et al., 2008). The change in the weight of this structure, which is thought to be involved in intraspecific communication, during the breeding season is evidence that it is associated with social or reproductive behaviour (Kennedy, 1971). In addition, the uropygial gland performs similar functions to sebaceous glands involved in oil production in mammals (King and McLelland, 1984; Salibian and Montalti, 2009). The chemical composition of the secretory content of the gland may also vary depending on sex (Abalain et al., 1984), age and diet (Zık and Erdost, 2002; Sandilands et al., 2004).

The secretory content of the gland consists of cell debris, enzymes that enable fat synthesis, volatile substances consisting of short-chain fatty acids, aldehydes, aliphatic and heterocyclic aromatic amines, ketones and dimethyl sulphides (Bhattacacharyya and Ghosh, 1971; Burger et al., 2004).

Morphometric measurements were performed on bones and soft tissues in mammals and birds. Measurement data can lead to significant differences between species and breeds (Özüdoğru et al., 2023; Dalga, 2021).

The histological descriptions of the gland have been made for several orders of birds and it has been reported that it is generally surrounded by a dense connective tissue capsule (Jacob and Ziswiler, 1982; Chiale et al., 2016; Carril et al., 2019). The gland epithelium consists of different cellular layers: germinative layer, intermediate layer, secretory layer and degenerative layer (Jacob and Zeman, 1972; Carril et al., 2019). In the parenchyma, secretory tubules are arranged radially from the periphery to the center and open into the central cavity. In addition, the tubules are separated from each other by compartments composed of connective tissue (Yılmaz et al., 2018).

The budgerigar (*Melopsittacus undulatus*) is a domestic bird species all over the world and is included in the parrot family. It is one of the most popular cage birds that has been taken from its homeland Australia to all over the world (Petek, 2004).

There is no detailed study on the macroscopic and microscopic structure of the uropygial gland in budgerigars. This study was carried out to determine the macroanatomical, morphometric and histological structure of the uropygial gland in male and female budgerigars and to establish a basic data source by comparing the recorded results between male and female animals and with data obtained from other bird species.

MATERIALS and METHODS

The uropygial glands of 14 budgerigars (7 females and 7 males) were used as material in the study. The glands were obtained from birds that did not show any clinical signs and died for reasons unrelated to the study. The deceased birds were obtained from a private enterprise where they were sold and the tissues were brought fresh to the laboratory.

Statistical analysis

IBM SPSS Statistics 23.0 programme was used for statistical analysis. The data obtained as a result of normally distributed measurement parameters and index calculations were analyzed with Independent Sample-t Test to determine the differences between genders.

Macroscopic examination and morphometric measurements

On macroscopic examination, the topographic location of the uropygial gland was firstly determined. The glands were separated from the surrounding tissues by dissection. The weights of the glands were measured with a precision balance (Weightlab- WL-303L). The glands were photographed (Canon EOS 2000D, Japan) under a stereomicroscope (Olympus SZ61, Japan). Considering the morphological characteristics of the gland, the images taken under stereomicroscope were transferred to ImageJ (1.4) for morphometric measurements. Jacob and Ziswiler, (1982) were used as a guide for morphometric measurement points. Nomina Anatomica Avium (Baumel et al., 1993) was used for nomenclature of anatomical terms. Seven morphometric measurements and four index calculations were made from the glands. Morphometric measurement parameters and index abbreviations and descriptions were given below. Also, these parameters were shown in Figure 1.

Morphometric parameters:

LW: Live weight

GW: Gland weight

GUW: Glandulae uropygialis width

GUL: Glandulae uropygialis length

PUL: Papilla uropygialis length

PUH: Papilla uropygialis height

Index parameters:

Relative gland weight index (RGWI) = Gland weight (GW) x 100 / Live weight (LW),

Lobus glandulae uropygialis index (LGUI) = Glandulae uropygialis length (GUL) / Glandulae uropygialis width (GUW),

Papilla uropygialis index (PUI) = Papilla uropygialis height (PUH) / Papilla uropygialis length (PUL),

Glandulae uropygialis length index (GULI) = Glandulae uropygialis length (GUL) / Papilla uropygialis length (PUL).

Histological examination

The uropigial glands were fixed in 10% buffered formalin solution for 48 hours. After fixation step, all tissues were dehydrated in ascending grades of ethanol, cleared in xylene, and then embedded in paraffin. Paraffin blocks were cut at a thickness of 4 μm and stained with Haematoxylin-eosin (H&E) for histological examination, Masson's Trichrome (MT) for collagen and smooth muscle fibres, and Periodic Acid Schiff-Alcian Blue (Ph: 2.5) for the character of secretion produced. Chicken intestinal tissue was used as a positive control for PAS-AB staining.

The procedures applied in the present study were approved by the Siirt University Experimental Animals Application and Research Centre with the ethics committee report numbered 2023/01/04.

RESULTS

In our study, the presence of uropygial gland was observed in all budgerigars. The gland was located dorsally in the region between caudal vertebrae and pygostyle. After the feathers were removed, the gland was covered with a thin layer of superficial skin (Figure 2-A). Anatomically, the gland structure consisted of two lobes and a papilla system (Figure 2-B, C). Both dorsal and ventral sides of the lobes showed a distinct convexity. The lobes appeared symmetrical when viewed from the outside. They resembled the heart in shape and there was a short papilla at the caudal junction of the ends of the lobes (Figure 2-C). Numerous holes (*porus glandulae uropygialis*) were observed on the papilla, which allowed the internal secretion to flow out. In addition, many bristle structures were observed on these holes (Figure 2-C). The gland was divided into two lobes by an interlobarseptum (Figure 2-D) formed by connective tissue. Inside the lobes, it was determined that there was a light-yellow secretion material with a dense consistency.

The uropygial gland weights of male and female budgerigars were measured as 1.57±0.96 g and 1.52±0.09 g, respectively. As a result of statistical evaluation, GUW ($p<0.05$), PUL and PUH ($p<0.01$) parameters were significantly different between sexes. GUW, PUL and PUW parameters were found

to be higher in male budgerigars than females. At the same time, as a result of the index calculations, there was no difference between the sexes in terms of relative RGWI, LGUI, and PUI, whereas GULI value was higher in female birds and showed a statistical difference between the sexes ($p<0.05$). Descriptive statistics and p values for the measurement parameters are presented in Table 1 and for the index values in Table 2.

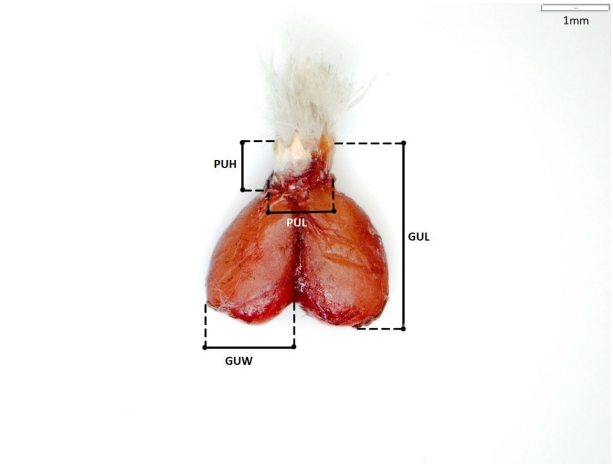

Figure 1. Dorsal view of the uropygial gland in the female budgerigar. PUH: *Papilla uropygialis* height, PUL: *Papilla uropygialis* length, GUL: *Glandulae uropygialis* length, GUW: *Glandulae uropygialis* width.

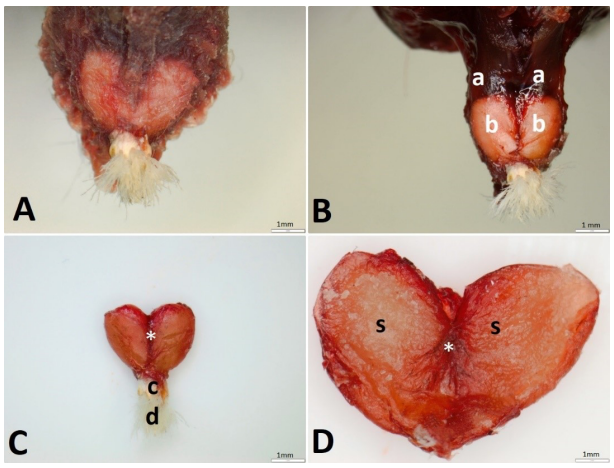

Figure 2. A: Pre-dissection view of uropygial gland in the female budgerigar (dorsal), **B:** Post-dissection view (dorsal), **C:** View of the uropygial gland separated from the surrounding tissues and body (dorsal), **D:** View of the internal structure of the uropygial gland in median section. **a:** tail muscles, **b:** *lobus glandulae uropygialis*, **c:** *papillae uropygialis*, **d:** papillae bristle (*pluma*), **s:** secretion, ***:** interlobular septum (external (C) and internal (D) view).

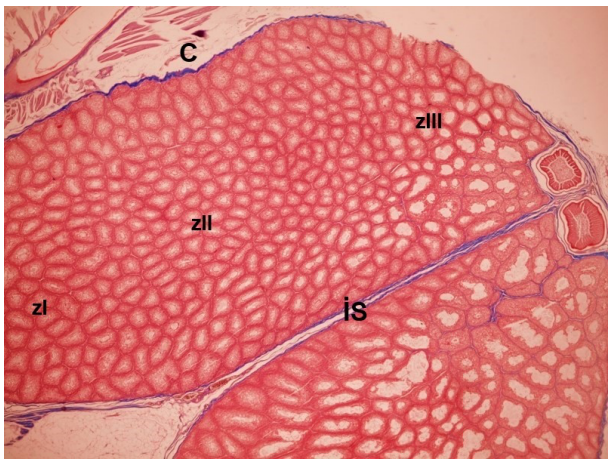

Figure 3. Masson's Trichrome staining X4, **zI:** zone I; **zII:** zone II; **zIII:** zone III, **C:** Capsule, **İS:** Interlobar septum.

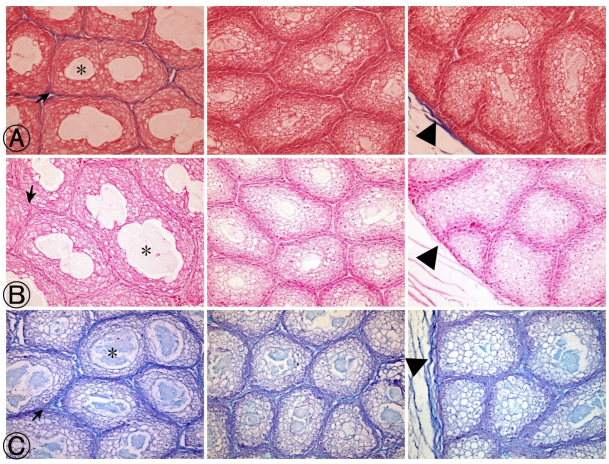

Figure 4. A: The capsule of the uropygial gland. MT staining. x40, **B:** The interstitial connective tissue of the uropygial gland. H&E. x40, **C:** Glycogen deposition in the uropygial gland. PAS-AB staining. X40. ***:** Secretion, **Arrowhead:** Capsule, **Arrow:** Interstitial Connective Tissue (Trabecula).

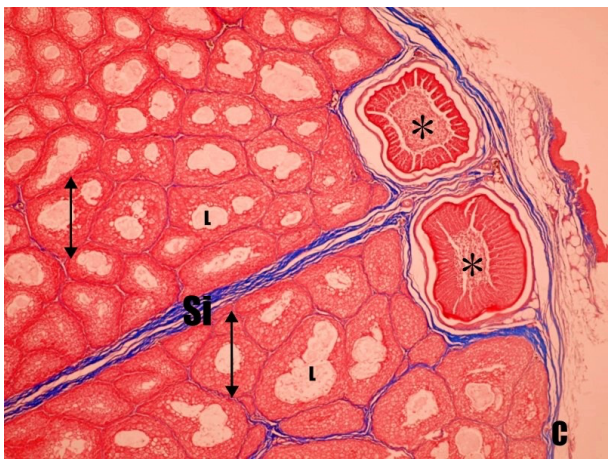

Figure 5. General view of adenomers. MT staining. X10 **Double sided srrow:** Adenomer, **L:** Lumen, **C:** Capsule, ***:***Ductus glandulae uropygialis*, **Si:** *Septum interlobare*.

Table 1. Descriptive statistics and p values of morphometric measurement parameters of uropygial gland in budgerigars. LW: Live weight; GW: Gland weight; GUW: Glandulae uropygialis width; GUL: Glandulae uropygialis length; PUL: Papilla uropygialis length; PUH: Papilla uropygialis height.

	Average			Male	Female	p
Parameters	Mean±SE	Min.	Max.	Mean±SE	Mean±SE	
LW (g)	40.77±0.68	30.40	38.40	43.38±0.84	38.17±1.11	0.58
GW (g)	1.54±0.06	1.18	1.98	1.57±0.96	1.52±0.09	0.70
GUW (mm)	1.39±0.01	1.30	1.47	1.41±0.01	1.36±0.01	*<0.05
GUL (mm)	1.87±0.02	1.73	2.00	1.87±0.39	1.88±0.26	0.88
PUL (mm)	0.53±0.02	0.39	0.65	0.60±0.01	0.45±0.02	*<0.01
PUH (mm)	0.60±0.02	0.45	0.72	0.67±0.01	0.53±0.02	*<0.01

*: p<0.05

Table 2. Uropygial gland index values in budgerigars. RGWI: Relative gland weight index; LGUI: Lobus glandulae uropygialis index; PUI: Papilla uropygialis index; GULI: Glandulae uropygialis length index.

	Average			Male	Female	p
Index parameters	Mean±SE	Min.	Max.	Mean±SE	Mean±SE	
RGWI	0.04±0.001	0.04	0.05	0.04±0.002	0.04±0.001	0.53
LGUI	1.35±0.015	1.21	1.41	1.32±0.027	1.38±0.003	0.08
PUI	1.14±0.011	1.08	1.25	1.11±0.009	1.16±0.018	0.05
GULI	3.05±1.66	1.62	4	2.77±0.083	3.32±0.297	0.02*

*: p<0.05

Histological examination revealed that the gland structure consisted of two lobes and one papilla. MT staining showed that the capsule (Figure 3,4) on the outer part of the glands was stained in a distinctly blue color and branched between the adenomers (Figure 5), limiting them. The interlobular septa (Figure 3) were found to be devoid of fibrocytes. It was determined that the gland had a tubuloalveolar-holocrine structure. The epithelial layer of the gland consisted of four cell layers from the periphery to the center: basal cell, intermediate cell, secretory cell and degenerative cell (Figure 6). H&E staining showed that the nuclei of the cells in the peripheral parts of the adenomeres were centrally located and their cytoplasm contained eosinophilic, secretory material. The cytoplasm of the cells closer to the lumen became light-colored vacuolar and the nuclei shifted to the periphery.

PAS-AB staining showed a weak light blue staining in the cytoplasm of the peripheral cells (glycogen). The cytoplasm of the central cells was not stained and the nuclei were pushed aside (lipid). The secretion in the lumen of the glands was moderate and the thin interstitial connective tissue (*trabeculae* - Figure 4) between the glands was weakly stained light blue (positivity). Histological examination revealed the presence of drainage ducts, which opened and terminated into two large ducts (*ductus glandulae uropygialis*) located caudal to the gland (Figure 5). On the papillae, the presence of numerous holes for the outflow of the secretion was identified (*porus ductus glandula uropygialis*-Figure 7) The presence of Herbst corpuscule was also noted (Figure 8).

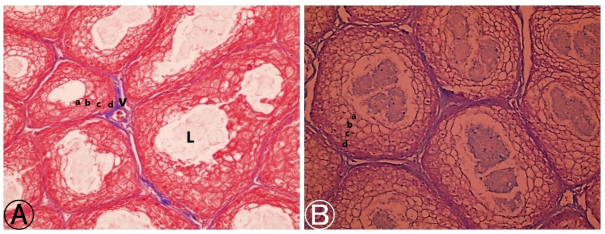

Figure 6. The epithelial layers of the uropygial gland. **A:** MT staining. X40, **B:** PAS-AB staining. X40. **V:** Vena, **L:** Lumen, **a:** Degenerative cells, **b:** Secretory cells, **c:** Intermediate cells, **d:** Basal cells.

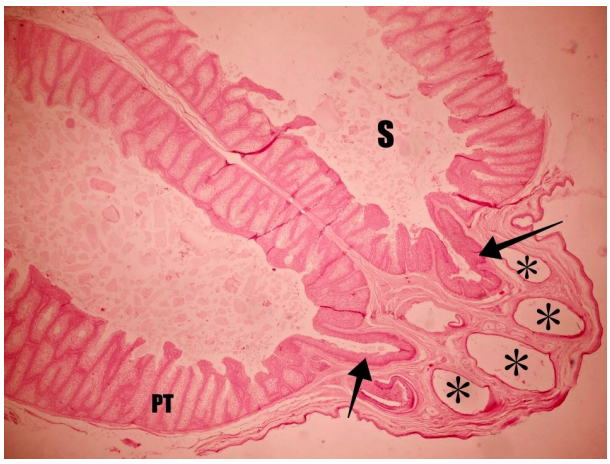

Figure 7. The secretion of the uropygial gland. H&E staining X4. **S:** Secretion, **PT:** Peripheral tubule, **Arrow:** *Ductus glandulae uropygialis*, ***:** *Porus ductus glandulae uropygialis*.

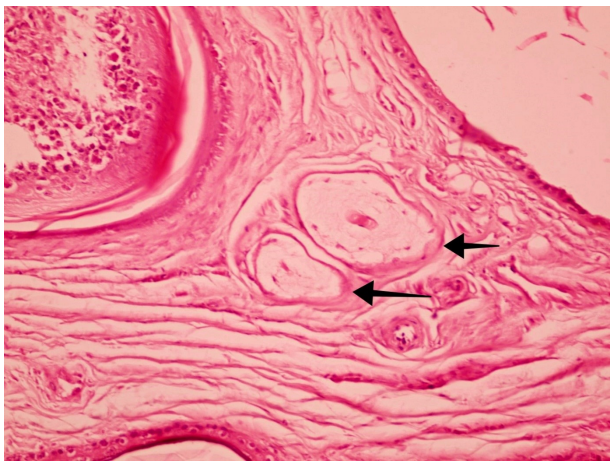

Figure 8. The Herbst corpuscule. H&E staining. X40 **Arrows:** Herbst corpuscules.

DISCUSSION

Although the gland is present in the embryonic stages of almost all bird species, it may atrophy in adults of certain orders, families, genera and species (Johnston, 1988, Salibian and Montalti, 2009). In our study, it was observed that uropygial gland was present in all of the budgerigars examined.

In different birds, the gland is located at the base of the tail, on the pygostyle muscles (Johnston, 1988; Sawad, 2006), between the caudal aspect of the lumbosacral bone and the first coccygeal vertebra (Kozlu et al., 2011). It has been reported to be found on the last caudal vertebra (Jacob and Ziswiler, 1982), in the region between the fourth caudal vertebra and pygostyle (Lucas and Stettenheim, 1972; Yılmaz et al., 2018) and generally on free caudal vertebrae (Moreno-Rueda, 2016; Yılmaz and Yılmaz, 2019). In our study, it was determined that the uropygial gland was located dorsally in the region between the caudal vertebrae and the pygostyle in accordance with the literature.

The size and shape of the uropygial gland have been reported to vary among species (Taşbaş, 1996; Salibian and Montalti, 2009). While the shape of the gland was reported to be heart-shaped in chickens (Yılmaz et al., 2018), it was reported to be similar to the letter "V" in water birds (Gezici, 2002). In the present study, it was detected that the shape of the uropygial gland was heart-shaped and surrounded by a capsule of connective tissue in accordance with what has been reported in gulls (Chiale et al., 2014), monk parrots (Carril et al., 2019) and flamingos (Chiale et al., 2021). The gland was also located directly under the skin.

It has been reported that the gland anatomically consists of two lobes (Jacob and Ziswiler, 1982; Mobini and Ziaii, 2011; Kozlu et al., 2011) and these lobes consist of numerous holocrine secretory alveoli opening into the central cavity (Lucas and Stettenheim, 1972, Menon et al., 1981; Jacob and Ziswiler 1982). It has been stated that the ducts open outwards through a nipple-like papilla located towards the tail, in the midline and dorsally (Gezici, 2002). In the present study, a single papilla structure was observed and the anatomical structure of the gland was similar to the literature. In addition, in accordance with the literature (Chiale et al., 2014; Yılmaz and Yılmaz, 2019), it was identified that there were many bristle structures around the papilla. The papilla structure, which was reported to have a conical shape in Psittaciformes species (Jacob and Ziswiler, 1982), was reported as cylindrical in the Monk parrot (Carril et al., 2019). In our study, the papilla structure was found to have a cylindrical shape. On the caudal end of the papilla structure, the presence of numerous holes (porus glandulae uropygialis), which are involved in the discharge of the incoming secretion through two uropygial gland ducts, was noted.

Chen et al. (2015) reported that the weight of the uropygial gland varied with age, and the gland weights of female and male mule ducks at 49 days of age were 4.76 g and 6.23 g, respectively. In addition, Elder (1954) stated that gland weights can vary greatly seasonally and according to sex. The uropygial gland weights of adult male and female mallard ducks were reported as 4.02±0.26 g and 5.10±0.22 g, respectively (Yılmaz and Yılmaz, 2019). The same value was founded as 0.95±0.15 g and 0.91±0.26 g in Aseel breed roosters and chickens, respectively (Yılmaz et al., 2018). In our study, these values were measured as 1.57±0.96 g and

1.52±0.09 g in male and female budgerigars, respectively.

In previous studies, RGWI was determined as 0.29-0.34 g/100 g in mule ducks (Chen et al., 2015), while it was reported as 0.31 and 0.28 in male and female mallard ducks, respectively (Yılmaz and Yılmaz, 2019). The RGWI was reported as 0.08 in owls (Elder, 1954). In our study, this value was found as 0.04±0.001 on average, close to the value reported in Aseel breed roosters and chickens (Yılmaz et al., 2018) and smaller than the RGWI value determined in Tyto alba (Yılmaz and Yılmaz, 2020).

In a study conducted in endemic bird species in New Zealand, Reynolds (2013) determined the LGUI of glandula uropygialis as 1.4, 1.3, 1.2 respectively. In the same study, the GULI was reported as 4.3, 5, 4.2 respectively. Yılmaz et al. (2018) determined that LGUI values in Aseel breed roosters and hens were 1.64 and 1.87, respectively, and GULI values were 3.35 and 5.4, respectively. The LGUI value determined in our study was smaller than the values reported in male and female mallard ducks (Yılmaz et al., 2018), while the GULI value was higher than these ducks. In our study, the same index values were higher than the values reported in Tyto alba (Yılmaz and Yılmaz, 2020).

In our study, the uropyigial gland had a holocrine gland structure surrounded by a dense connective tissue capsule as reported in many literatures. In addition, thin connective tissue trabeculae branched from the capsule into the organ and bounded around the adenomeres (Yılmaz and Yılmaz, 2020). In histological examinations observed in most bird species such as flamingo (Chiale et al., 2021), parrot (Carril et al., 2019), pigeon (Chiale et al., 2019), cormorant (Stangier et al., 2023), magpie (Balkaya et al., 2016), both lobes forming the gland consist of tubules located around a central lumen (Kozlu et al., 2011; Chiale et al., 2016). In our study, in accordance with the literature, it was founded that the functional part of the gland structure consisted of tubulo-alveolar secretory units (adenomers) covered with stratified epithelium, and the epithelial cells were classified as basal or germinative cell layer, intermediate cell layer, secretory cell layer and degenerative cell layer. It has been reported that the uropygial gland of poultry species such as geese (Hou, 1928), grouse (Sawad, 2006) and starlings (Sadoon, 2011) are completely devoid of smooth muscle cells. However, the presence of smooth muscle cells in the structure of the gland, especially in the interlobar septum and interfollicular septum, has also been reported in studies (Lucas and Stettenheim 1972; Balkaya et al., 2016; Yılmaz and Yılmaz,2019; Madkour et al., 2023). Similarly, smooth muscle cells were found in the magpie, especially in the trabeculae (Balkaya et al., 2016). In our study, smooth muscle cells were found in the interlobular septum and interfollicular septum. In accordance with Balkaya et al. (2016), the presence of fibroblasts, smooth muscle cells and blood vessels were observed in the trabeculae. It was observed that smooth muscle cells were more concentrated especially in the trabeculae around the central lumen. The secretion produced was delivered to the papilla through small and narrow drainage ducts within the gland. Kozlu et al., (2011) and Yılmaz and Yılmaz (2019) determined that the draining duct opening to the glands was located in the centre of the lobe. In our study, similar to Tyto alba (Yılmaz and Yılmaz, 2020), the drainage ducts opened into several collecting ducts caudal to the gland. Yılmaz and Yılmaz (2020) stated that this may be related to the fact that the owl is not an aquatic bird.

In some studies, it can be seen that gland lobules are divided into three regions according to epithelial height and lumen width (Lucas and Stettenheim, 1972; Yılmaz and Yılmaz, 2020). In these zones, which were divided as zone I, zone II and zone III, respectively towards the centre under the capsule, germinative cell layer, intermediate cell layer, secretory cell layer and degenerative cell layers were determined from outside to inside, consisting of different numbers of layers. In agreement with the literature, the intermediate and secretory cell layers were much thinner in zone III compared with the other two zones (Abalain et al., 1984; Chiale et al., 2016; Carril et al., 2019; Chiale et al., 2021;). In addition, the cell layers in all zones consisted of irregularly arranged intermediate cells and secretory cells with a small number of degenerative cell layers at the innermost layer. In accordance with Yilmaz and Yılmaz (2020), intermediate cells have acidophilic cytoplasm and basophilic nucleus. In our study, the lumen of the secretory cells contained numerous large and small, white-coloured fat vacuoles. It was also noted that larger fat vacuoles were present in the cytoplasm of degenerative cells.

Studies on the uropygial gland of Chilean flamingo and Monk parakeet revealed the presence of glycoconjugates containing carboxyl groups and sulphated esters, which reacted positively with PAS- AB (pH:2.5). These compounds are associated

with protective functions in various organs (protecting and maintaining feathers) (Díaz et al., 2008; Yashpal et al., 2014; Chiale et al., 2016). In a study conducted in falcons, the germinative cell layer, germinative membrane and secretion of the adenomere were shown to be PAS-positive (Chiale et al., 2016). The high number of acidophilic cells indicates that there is a large amount of oil synthesis in the gland. (Yılmaz et al., 2018). In our study, staining with Periodic Acid Schiff-Alcian Blue (PAS-AB) showed a weak light blue staining (glycogen) in the cytoplasm of the cells in the peripheral region, while the cytoplasm of the central cells was not stained and the nuclei were pushed aside, indicating a large amount of fat synthesis.

In the light of these results, it was thought that the intracellular secretion in the peripheral region of the glands was mostly glycogen-derived in histochemical staining and changed to lipid character as the secretion pushed the nucleus aside as it descended towards the lumen.

The presence of Herbst's corpuscles, which was revealed for the first time by Harem et al. (2005) in wild ducks, was not determined in mallard ducks by Yılmaz and Yılmaz (2019). In our study, the presence of Herbst's corpuscles was observed similar to laughing dove (Madkour et al., 2023).

CONCLUSION

As a result of macro-anatomical and histological findings, it was determined that the uropygial gland in budgerigars resembles most terrestrial bird species with its characteristics such as being surrounded by a fibrous capsule, consisting of two lobes and a papilla system, being heart-shaped, having draining ducts opening into several collecting ducts located caudal to the gland, the location of lymphoid follicles and the presence of Herbst corpuscles, but it also shows significant differences with aquatic bird species and some endemic bird species.

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Investigating wound healing and antimicrobial activity of terebinth extract and terebinth extract+oxytetracycline mixture in experimental wounds in mice

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ABSTRACT

Objective: The aim of the study is to investigate the wound healing and antibacterial activity of terebinth extract and the mixture of terebinth+3% oxytetracycline in experimental back skin wounds

Materials-Methods: The animal material of the study consisted of 18 mice. The animals were divided into 3 groups as control group (group I, n:6), terebinth group (group II, n:6), terebinth+oxytetracycline group (group III, n:6). Wounds with a 1 cm^2 diameter were induced on the back of the mice and infected with *Staphylococcus aureus* ATCC® 25923 reference strain. Treatment protocols for the groups were applied daily, once a day. Total aerobic mesophilic bacteria and *S. aureus* count was performed in the swab samples taken on days 3, 7, and 14 of the healing process.

Results: In the study, it was found that wound healing process was completed the earliest in Group III (mean duration of 15.67±0.609 days), which was followed by Group II (18±0.73) and Group I (24.67±0.919), respectively. The healing period was statistically significantly shorter in Group II and Group III than in Group I ($p<0.001$). In the evaluation of aerobic mesophilic bacteria and *S. aureus* load, much less live bacteria were found in Group III compared to the other groups. In addition, the number of bacteria measured in group II, in which terebinth extract was used, was found to be significantly lower than the number of bacteria measured in the control group.

Conclusion: Consequently, it was concluded that the extract of terebinth plant grown in Siirt region reduced the bacterial load in the wound area and accelerated the healing process.

Keywords: *Mice, Wound healing, Terebinth extract, Oxytetracycline, Antibacterial*

INTRODUCTION

Wound is defined as the deterioration of the skin integrity and mucous membranes as a result of various diseases, trauma, bites, or stings (Ayyanar and Ignacimuthu, 2009; Sorg et al., 2017). Wounds classified as open or closed can show an acute or chronic course (Biswas and Mukherjee, 2003). As a result of injury, tissue loss and loss of sensation and function as well as bleeding, redness, pain, contraction of tissues and discharge can be observed (Dhifi et al., 2012). Based on the extent of the injury, some cases can heal spontaneously; whereas, serious cases need a rapid and effective intervention. Especially in open wounds, complications characterized by pus may be seen due to bacterial infections (Wang et al., 2018; Balestrin et al., 2022).

Wound healing is a pathophysiological condition that occurs to restore dermo-epidermal integrity (Wang et al., 2018). Various cells of the immune

system play a role in hemostasis, inflammation, neovascularization, fibroplasia and re-epithelialization stages of wound healing (Taş et al., 2003., De Almeida et al., 2022; Wallace et al., 2022). As well several cytokines, released by cells of the immune system, have a critical importance in the wound healing process, (Darby et al., 2014; Chitturi et al., 2015; Darwin and Tomic-Canic, 2018; De Almeida et al., 2022). Infections, some diseases, and medical treatment can affect the healing process positively or negatively (Wernick et al., 2022).

Numerous studies have examined the effects of various plant extracts on wound healing (Budovsky et al., 2015; Yuan et al., 2016; Sharma et al., 2021; De Almeida et al., 2022). These effects of plants are caused by alkaloids, iridoids, flavonoids, tannins, saponins and phenolic compounds they contain in their structures (Thangapazham et al., 2016). The fact that plant extracts contain various combinations of phytocomponents involved in various pathophysiological steps of wound healing (angiogenesis, fibroplasia, and wound contraction) is one of the most important reasons behind why they are used to treat wounds (Ibrahim et al., 2018; De Almeida et al., 2022).

The phenomenon of bacterial antimicrobial resistance poses problems in effectively treating infectious diseases. This situation necessitates searching new alternatives to antimicrobial agents. In this sense, it has been reported that in recent years there has been an increasing interest in the use of plants known to have antimicrobial effects in the treatment of infectious diseases in both humans and animals (Khalil et al., 2007; Tohidi and Hayran, 2011).

Since ancient times, terebinth plant has been used as an antispasmodic, antipyretic, antibacterial, antiviral and stimulant in eczema treatment, throat infections, kidney stones, asthma and stomach diseases in human medicine and is known to contain phenolic compounds and triterpenoids (Kusmenoglu et al., 1995; Tohidi et al., 2011; Dhifi et al., 2012). In addition, terebinth plant has antioxidant, anti-inflammatory, anti-pyretic, antiparasitic, neuroprotective, and anticholinesterase activity properties (Göçer, 2013; Hacıbekiroğlu et al., 2015).

In the treatment of infected wounds, the use of both topical and systemic antibiotics has been shown to be beneficial (Saco et al., 2015; Gerçeker Türk, 2020). Oxytetracycline is a second-generation tetracycline group antibiotic produced by *Streptomyces rimosus*. Oxytetracycline, which has a broad spectrum, is effective against Gram positive and Gram-negative bacteria and mycoplasma species and inhibits protein synthesis in bacterial agents (Augusto and Alves, 2015; Demirseren, 2020).

The aim of the study is to investigate the wound healing and antibacterial activity of the extract of terebinth plant, which is ecologically grown in Siirt region, and the mixture of terebinth extract+3% oxytetracycline in Experimental back skin wounds in mice.

MATERIALS and METHODS

Material

Animal material and selection: The animal material of the study consisted of 30-day-old, 18 male dormouse mice (*Mus musculus*) obtained from Van Yüzüncü Yıl University Experimental Animals Research and Application Center. The mice were divided into three groups and housed in individual mouse cages. They were fed standard feed and water ad-libitum.

Ethics committee approval: The current study was approved by Siirt University Experimental Animals Local Ethics Committee with the decision dated 10.02.2017 and numbered 2017/01/21.

Method

Preparation of the drug material: Terebinth extract was obtained from the Laboratory of Siirt University Research Center. The mixture of terebinth extract+3% oxytetracycline was obtained by mixing 1 gr terebinth extract and 30 mg oxytetracycline in a sterile glass beaker until a homogenous mixture was obtained. The prepared drug material was stored in a light-proof sterile plastic container. The drug material was prepared originally without considering any cream formulation.

Study groups: The study consisted of 3 groups; control group (C) with no drug administration (group I, n:6), terebinth group (T) (group II, n:6), terebinth extract+3% oxytetracycline group (TO) (group III, n:6).

Group I: No treatment was applied on the wound in mice in this group.

Group II: Terebinth extract (0.5 ml) was applied to the wound once every day until healing took place.

Group III: A mixture of terebinth extract+3% oxytetracycline (0.5 ml) was applied to the wound once a day, every day until healing took place.

Wound formation and care: Food intake of the mice was stopped 3 hours before wound was induced. 10 mg/kg dose of xylazine HCl (2% Rompun, Bayer) and 100 mg/kg dose of ketamine (10% Alfamine, Egevet) were administered Intraperitoneal for anesthesia. Shaving and asepsis-antisepsis procedures were performed in the area where the wound was to be induced on the back of the animals. To ensure standardization of the wound size in animals, a template was created by opening a 1x1 cm square area on an A4 paper. An experimental wound with an approximately 1 cm^2 diameter containing skin and subcutaneous connective tissue was induced by using this template with a scalpel on the animals in each of the 3 groups. From the day the wound was induced, the follow-up process was carried out by performing the prescribed applications specific to the groups until healing was formed. Wound dressing placed and renewed daily wound dressing placed and renewed daily.

Measurement of the wound area: In each group, photographs of the wound line were taken before the dressings on days 0, 2, 4, 7, 10, 12, 14, 16, 18, 20, and 24, and the wound areas were measured and recorded on the photographs using ImageJ program on the computer.

Infection of the wound area: *Staphylococcus aureus (S. aureus)* ATCC® 25923 reference strain obtained from culture collection of Van Yüzüncü Yıl University, Faculty of Veterinary Medicine, Department of Microbiology was used for bacterial contamination of the wounds. For this purpose, 1 ml of the bacterial suspension prepared in physiological saline (PS) at a density of 10^8 cfu/ml was inoculated into the wounds in the control and experimental groups. For 24 hours, no treatment protocol was applied, and bacterial colonization was ensured.

Total aerobic mesophilic bacteria and *S. aureus* count: Swab samples were taken from a 1 cm^2 area of the wounds induced in the control and experimental groups on days 3, 7, and 14 of the study. While taking the samples, care was taken to ensure that 12 hours had elapsed since the application of the preparation. The swab samples were sent to the microbiology laboratory in Stuart transport medium in accordance with cold chain requirements. For total aerobic mesophilic bacteria count and *S. aureus* count, samples were placed in tubes containing 10 ml sterile PS and vortexed for a few minutes. One ml of the suspension was taken and transferred to tubes containing 9 ml of PS. Dilution was continued until 10^{-8} dilution.

One ml of each dilution was transferred to two separate petri dishes and 15 ml of Plate Count Agar (Merck, Darmstadt, Germany), which was sterilized and cooled to 45-50°C, was poured into each dish and mixed. After the media was solidified, they were inverted and incubated at 37°C for 48 hours in aerobic environment. At the end of the incubation period, bacterial counts were made in the petri dishes in which 30-300 colonies grew. The geometric mean of the number of bacterial colonies detected in 2 petri dishes of the same dilution was taken and the total count of aerobic mesophilic bacteria in the samples was evaluated as cfu/cm^2 (TS, 2014).

Method given in ISO 6888-1 was used for *S. aureus* count (TS, 2001). For the verification processes of the counted *S. aureus* colonies, typical and/or atypical 5 colonies were selected from the petri dishes and Gram staining, catalase, and coagulase (M43 Microgen™ STAPH, Italy) tests were applied. In the form of Gram-positive cocci, those that gave positive results in catalase and coagulase tests were identified as *S. aureus*.

Statistical analysis: The probability of healing and the mean and median healing periods of the study groups were calculated by Kaplan-Meier survival analysis method. The significance of the difference in healing periods between the study groups was analyzed by Log rank (Mantel Cox) test. To examine the effect of treatment (group) and time on wound healing, a single factor repeated two-way analysis of variance was performed using general linear modelling technique. Treatment (group), time and treatment (group)*time interaction terms were included in the model. Simple effect analysis was performed to analyze the significant interaction terms and Bonferroni correction was applied on the results. SPSS 14.01 software was used for data analysis.

RESULTS

The mean healing time of the subjects was 24.67±0.919 days (95% CI: 18.6-23.4) in group I, 18±0.73 days (95% CI: 21.605-28.395) in group II and 15.67±0.609 days (95% CI: 14.807-17.193) in group III. The healing time was found to be statistically significantly longer in group I compared to group II and group III ($p<0.001$). Although the healing time of the subjects in group III was shorter than group II, no statistically significant difference was found (Table 1, 2) (Figure 1,2).

Table 1. Descriptive statistics of wound areas according to the measuring times.

Period	Control							Terebinth							T+O					
	n	Arithmetic Mean	Std. Error	Std. Deviation	Median	Min.	Max.	n	Arithmetic Mean	Std. Error	Std. Deviation	Median	Min.	Max.	n	Arithmetic Mean	Std. Error	Std. Deviation	Median	Min.	Max.
t0	6	98.5	2.51	6.16	97	93	110	6	95.67	2.97	7.28	94	89	110	6	97.33	2.76	6.77	95.5	91	110
t2	6	81.5	1.48	3.62	80	78	87	6	66.17	4.2	10.28	67	51	80	6	59.67	2.19	5.35	60.5	51	67
t4	6	65.67	1.82	4.46	66	59	71	6	54.5	3.79	9.29	55.5	40	68	6	49.17	0.7	1.72	49.5	46	51
t7	6	59.17	1.35	3.31	59.5	54	63	6	45.33	2.03	4.97	46	37	51	6	39.67	0.56	1.37	39.5	38	42
t10	6	58.33	4.65	11.4	55	50	81	6	37.5	2.05	5.01	37.5	30	45	6	27.5	1.78	4.37	27	22	35
t12	6	51	3.28	8.02	48.5	45	67	6	30.33	2.06	5.05	30	24	37	6	17.83	1.45	3.54	17.5	14	24
t14	6	42	3.51	8.6	40.5	32	58	6	25	2.83	6.93	26.5	12	31	4	13.25	1.8	3.59	12.5	10	18
t16	6	33.83	2.06	5.04	33.5	27	42	4	19	2.86	5.72	20.5	11	24	1	10	.	.	10	10	10
t18	6	27.83	1.6	3.92	28	22	34	2	13.5	0.5	0.71	13.5	13	14	0	.	.	.	.	.	.
t20	6	23.67	1.23	3.01	24	19	28	0	.	.	.	.	.	.	0	.	.	.	.	.	.
t22	6	16.67	1.2	2.94	18	12	19	0	.	.	.	.	.	.	0	.	.	.	.	.	.
t25	4	13.5	0.65	1.29	13.5	12	15	0	.	.	.	.	.	.	0	.	.	.	.	.	.

T: Group treated with terebinth extract, **TO:** Group treated with terebinth extract + oxytetracycline. **Min:** Minimum, **Max:** Maximum

Table 2. Mean and Median Healing Periods

Group	Mean				Median				P*
	Probability	Std. Error	95% Confidence Interval		Probability	Std. Error	95% Confidence Interval		
			Lower Limit	Upper Limit			Lower Limit	Upper Limit	
Control	24.67 [a]	0.919	22.866	26.468	25	1.732	21.605	28.395	
Terebinth	18 [b]	0.73	16.569	19.431	18	1.155	15.737	20.263	<0.001
T+oxy	15.67 [b]	0.615	14.462	16.871	16	0.609	14.807	17.193	
General	19.444	1.014	17.458	21.431	18	1.405	15.245	20.755	

** Log Rank (Mantel Cox) test result (Chi square = 18.602; sd=2; p<0.001)*

T: Group treated with terebinth extract, **TO:** Group treated with terebinth extract + oxytetracycline

Table 3. Total aerobic mesophilic bacteria and *S. aureus* count (cfu/cm²).

Animal No	Day 3		Day 7		Day 14	
	TAMB	***S. aureus***	**TAMB**	***S. aureus***	**TAMB**	***S. aureus***
C-1	0.1×10^7	9.6×10^5	1.8×10^5	0.8×10^5	4.5×10^3	3.4×10^4
C-2	0.9×10^4	0.7×10^4	1.6×10^4	1.7×10^4	2.5×10^4	1.6×10^4
C-3	2.0×10^5	2.0×10^5	1.8×10^5	0.8×10^5	8.5×10^3	2.4×10^4
C-4	6.5×10^5	5.6×10^5	1.8×10^5	2.0×10^5	4.5×10^4	1.4×10^4
C-5	3.4×10^5	3.4×10^5	3.5×10^5	3.5×10^5	1.5×10^4	3.8×10^4
C-6	2.3×10^4	1.7×10^4	2.7×10^4	2.0×10^4	6.5×10^3	0.5×10^5
T-1	1.6×10^4	2.7×10^4	1.5×10^3	1.4×10^3	1.5×10^2	1.5×10^1
T-2	1.1×10^4	0.6×10^4	5.8×10^3	0.6×10^3	2.5×10^2	1.5×10^2
T-3	2.0×10^4	1.7×10^4	1.1×10^4	1.0×10^4	1.5×10^3	1.0×10^3
T-4	1.7×10^4	1.2×10^4	3.9×10^3	1.2×10^3	1.6×10^2	1.1×10^1
T-5	1.6×10^5	1.4×10^5	2.0×10^4	1.8×10^4	1.5×10^3	1.3×10^2
T-6	1.4×10^5	1.2×10^5	5.8×10^4	5.5×10^4	1.4×10^3	1.2×10^3
TO-1	0.3×10^3	0	0	0	0	0
TO-2	2.4×10^3	0.9×10^3	0	0	0	0
TO-3	0.2×10^4	0	1.6×10^3	0	3.0×10^1	0
TO-4	0.8×10^3	0.3×10^3	3.0×10^2	0	0	0
TO-5	0.8×10^3	0	3.2×10^2	0	3.0×10^1	0
TO-6	0.8×10^3	1.7×10^4	0.1×10^3	0	3.0×10^1	0

C: Control group, **T:** Group treated with terebinth extract, **TO:** Group treated with terebinth extract+oxytetracycline, **TAMB:** Total aerobic mesophilic bacteria.

In the microbiological analysis of the samples taken from the wound line, S. aureus count and total aerobic mesophilic bacteria count were performed in all cases. As a result, total aerobic mesophilic bacteria and *S. aureus* counts per unit area in group III were significantly lower than the other groups. However, the bacterial load in group II, in which terebinth extract was used, was less than the control group. In addition, on the days 7 and 14, the *S. aureus* count was 0 in all subjects in group III (Table 3).

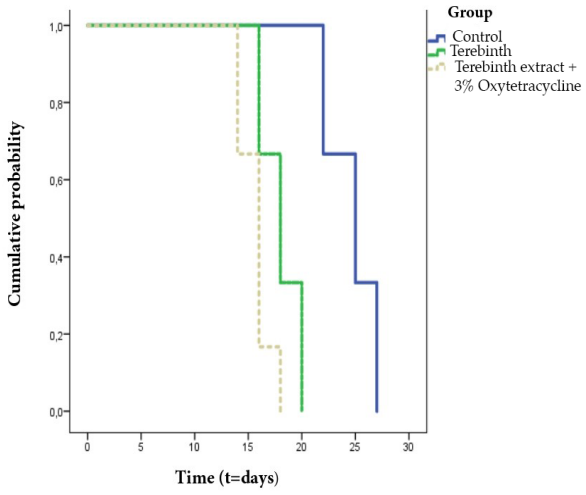

Figure 1. Graph of cumulative survival probability

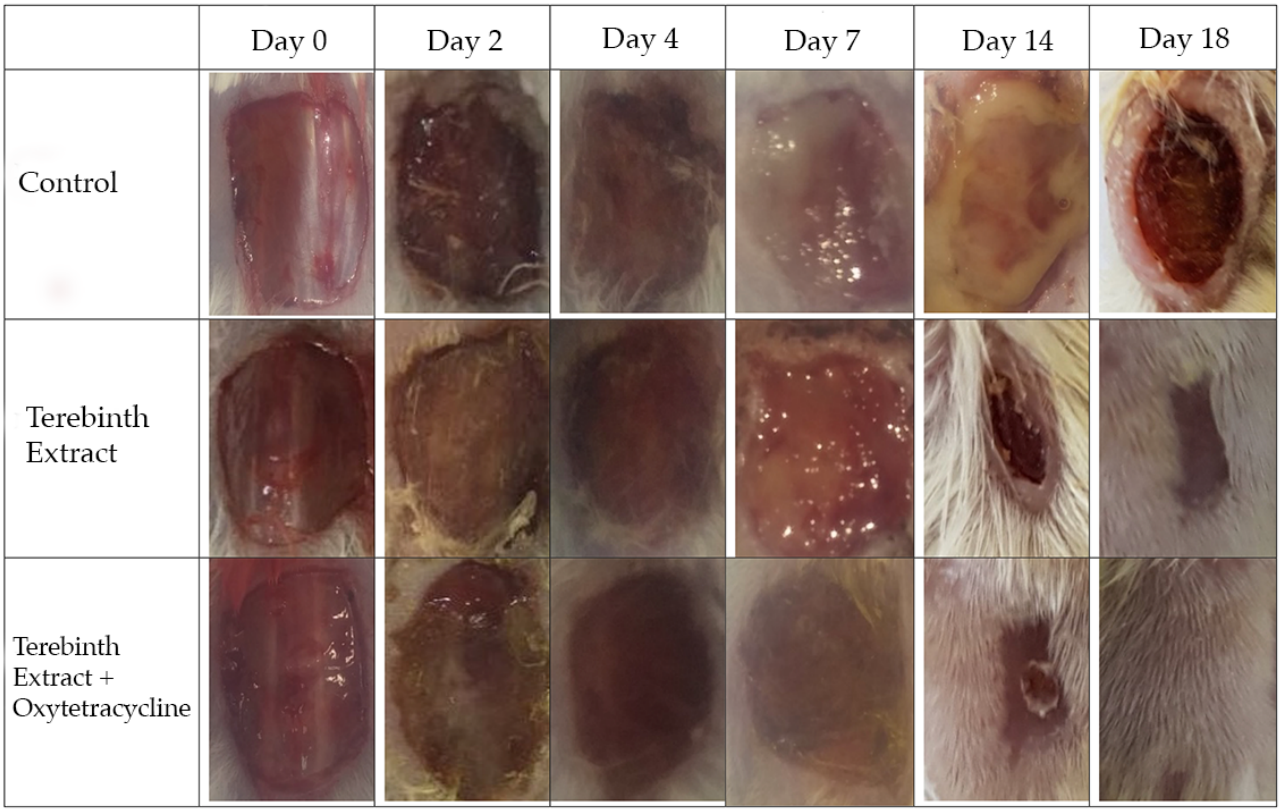

Figure 2. Macroscopic view of the wounds belonging to the groups

DISCUSSION

Wound healing is a pathophysiological condition that includes phases of hemostasis, inflammation, cell proliferation, extracellular matrix synthesis and remodeling; many parts of the process are known in detail, but some parts remain unexplained (Christine and Theoret, 2010; Ayla et al., 2017). The completion of these processes involves various factors such as cytokines, growth factors, proteases, eicosanoids, kinins and cellular metabolites. While in the past years, multiple complications were seen together in wound healing (infection, chronic scarring, etc.), today the frequency of wound healing complications has decreased (Mustoe et al., 2006; Ayla et al., 2017). The aim of wound treatment is to reduce the wound healing times by increasing the effects of factors (inflammatory cells, platelets, cytokines, extracellular matrix, etc.) that are effective in healing and to ensure the formation of scar tissue with appropriate neovascularization (Ayla et al., 2017). Thus, studies have been conducted in order to determine whether or not numerous plants have wound healing potential (Ximenes et al., 2013; Ayla et al., 2017; Balestrin et al., 2022; Sharma et al., 2021; De Almeida et al., 2022). The present study was aimed to scientifically demonstrate the wound healing properties of the extract obtained from the seed of the terebinth plant, which has been widely used by the people of Siirt in the treatment of diseases from past to present, and its combinations with oxytetracycline.

The healing process in wounds can be observed by many methods (macroscopic, microscopic, ELISA methods, measurement of some biomarkers) (Lin et al., 2012; Akdoğan et al., 2022). Macroscopic follow-up of wound closure is important. Recently, measurement of wound size on the computer is important in terms of preventing personal errors (Lucas et al., 2021). In the study, the wound line was photographed before applying the dressings on days 0, 2, 4, 7, 10, 12, 14, 16, 18, 20, and 24 in each group and the areas were measured and recorded with the ImageJ program.

Plants contain bioactive phytochemical structures such as alkaloids, iridoids, flavonoids, tannins, saponins and phenolic compounds (Thangapazham et al., 2016). As various combinations of these phytochemical compounds can be found in a single plant extract, the use of that plant extract alone provides the advantage of acting on various stages of wound healing (angiogenesis, fibroplasia and wound contraction) (Ibrahim et al., 2018; De Almeida et al., 2022). The terebinth plant, consisting of phytochemical compounds such as flavonoids and flavonoid glycosides, has a very important place in medical treatments (Kawasty et

al., 2000; Tohidi et al., 2011;). Flavonoids (alpha pinene, terpinolene, limonene, etc.) are antimicrobial substances that can act on many microorganisms by their binding ability to bacterial cell walls with proteins (Tohidi et al., 2011). Besides, this plant contains phenolic compounds and triterpenoids, and it is reported that such components are active against bacteria (Kusmenoglu et al., 1995). In the study, in order to determine the healing properties of the extract of the terebinth plant grown in the Siirt region in infective wounds, 3 groups were formed as the control group with no treatment (group I, n:6), group treated with terebinth extract (group II, n:6), group treated with terebinth and oxytetracycline combination (group III, n:6). When analyzed in terms of wound healing time; healing time in group I was found to be statistically significantly longer compared to group II and group III ($p<0.001$). Although the healing time of the subjects in group III was shorter than that of group II, no statistically significant difference was found. This was interpreted as the fact that the phytochemical components of the terebinth extract affected wound healing by increasing epithelialization and granulation tissue and thus accelerating wound healing.

In terms of regression of infection, *S. aureus* and total aerobic mesophilic bacteria count was performed in the samples taken from the wound line. As a result, total aerobic mesophilic bacteria and *S. aureus* counts in group III were significantly lower than the other groups. In this case, it can be asserted that terebinth and oxytetracycline combination significantly reduced the bacterial load. In addition, it was observed that the bacterial load in group II, in which terebinth extract was used, was less than the control group. This result shows the antibacterial activity of phenolic compounds and flavonoids in terebinth extract.

The related studies have reported that topical application of antioxidant-containing compounds will be useful for the protection of tissues from oxidative damage (Kumar et al., 2007). In a study investigating terebinth seeds, it was found that they had effective antioxidant properties (Göçer, 2013). When the wound healing time in the study is considered, it was concluded that one of the reasons for shorter healing time in the groups II and III treated with terebinth extract compared to group I was the effectiveness of the antioxidants in the composition of terebinth extract.

In a previous study comparing the effects of topical administration of glycerin solution and terebinth oil on wound healing, it was statistically demonstrated that terebinth oil accelerated wound healing compared to glycerin solution. Histological evaluation in the same study showed increased collagen synthesis and epithelialization in the group treated with terebinth oil, supporting this difference (Akgül et al., 2016). Another study revealed the effects of combinations of terebinth oil with different substances on wound healing. When the healing time was taken into consideration, it was observed that there was a very slight difference between the group treated with terebinth oil only and the group treated with terebinth oil and *Centella asiatica* pomade mixture, but it was observed that the healing time was completed in a much shorter time than all other groups (Şındak et al., 2017). In their study Tohidi et al., (2011) stated that the extract of a species of terebinth plant (*P. khinjuk*) showed faster healing compared to the control groups and its antibacterial activity was very good. In the study conducted by Djerrou et al., (2010) on rabbits by inducing experimentally 3rd degree burn, they reported that *Pistacia lentiscus* oil supported and accelerated the wound contraction and epithelialization process compared to madecassol and Vaseline. In the current study, it was concluded that the extract of terebinth plant grown in Siirt province accelerated the wound healing process and had significant antibacterial activity, which was in parallel with the studies using terebinth plant.

CONCLUSION

Consequently, it was scientifically found that the extract of terebinth plant found in Siirt province has positive effects on wound healing in mice. It was statistically shown that the antimicrobial activity of only terebinth extract and terebinth extract+3% oxytetracycline mixture used locally was higher compared to the control group and shortened the wound healing process. It is thought that the present study would form the basis of studies to convert terebinth extract into a commercial product suitable for topical use in wound treatment.

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Investigation of the healing effectiveness of pine resin in experimentally induced corneal wound in rats

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ABSTRACT

Objective: Pine resin is a product obtained from plants belonging to the Pinaceae family and traditionally used in the treatment of wounds. The aim of this study is to determine the effectiveness of pine resin in corneal wounds.

Material-Methods: In this study, three groups of 7 male Wistar Albino rats (n=7), each 2 months old, were established. To create the corneal wound model, the rats were anesthetized and the borders of the wound to be created on the corneal surface were determined using a 3 mm punch biopsy, then the first two layers of the cornea were removed with a corneal knife. Then, the first group was considered as the control group and no treatment was performed. The second group was determined as the pine resin group and applied once a day. The third group was considered as the drug group and was administered once a day. Fluorescein staining was performed every day for three days and the results were recorded.

Results: Pine resin group showed the fastest recovery. On the third day, the rats were euthanized, and their eyes were enucleated. The collected eyes were sent for histopathologic examination and stained with hematoxylin-eosin. The lesions in the examined specimens were evaluated under microscope for hyperemia, vascularization, cellular infiltration and corneal edema.

Conclusion: As a result of the study, ulceration was observed in the pine resin group. The study concluded that pine resin reduces clinical symptoms and promotes healing in corneal wounds.

Keywords: *Fluorescein, Natural, Rosin, Terpenoids, Ulceration*

INTRODUCTION

The corneal epithelium defends the eye from pathogen entry and is crucial to maintaining corneal clarity. As a result, cornea is extremely susceptible to the blinding effects of serious illness or trauma (Fernandes-Cunha et al., 2019). External traumas to the cornea frequently result in abrasions, lacerations, and corneal ulcers, which impair vision. Pathogens such as viruses, bacteria, and fungi can cause corneal ulceration and infection, which can result in corneal blindness (Demir et al., 2022). Reduced vision is frequently caused by corneal ulceration. Due to the severity of corneal lesions and the ways in which they are repaired during various morbid processes, prompt implementation of efficient therapeutic measures is required (Alfaris et al., 2009).

The squamous epithelium protects the cornea from environmental trauma while preserving optical clarity. The cornea typically re-epithelializes right away after damage to reduce the chance of infection, opacification or perforation. Delay in re-

epithelialization, however, may result in a slow healing of the injured corneal epithelium and ultimately the loss of corneal barrier function in conditions like dry eye syndrome, diabetic keratopathy, and chemical injury. If the injury is not treated properly, it invades the cornea, eventually leading to corneal melting, corneal perforation, and blindness. Healing a corneal wound is a difficult process that requires three ongoing procedures. These are complete re-modelling of the adhesive structure, active migration of superficial cells to cover the wound surface, and cell proliferation, differentiation, and stratification. The first step in the sequential procedures that quickly heal the wounds is corneal epithelial migration. Therefore, it is crucial to create a therapeutic plan to encourage epithelial migration and thus corneal re-epithelialization (Kibar Kurt and Belge, 2021; Hu et al., 2023).

Resins are liquids produced by trees to protect themselves against external factors, to heal wounds on their trunks or to protect newly developing shoots from the effects of rain and sun rays (Dell and McComb, 1979; Alihosseini, 2016). This liquid usually contains substances with important physiological properties such as phenolic compounds, alkaloids, terpenes, saponins, glycosides as secondary metabolites (Alihosseini, 2016). Pine resin is a natural resin secreted by members of the Pinaceae family (Güzel, 2019). It is secreted especially from the injured parts of the trunks of pine trees or during the maturation of the cones and provides protection and healing of the tree. The resin, which is initially secreted as liquid, solidifies white and yellow upon contact with air and dries on the surface (Rodrigues-Corrêa et al., 2012). The chemical composition of pine resin includes many secondary metabolites, mainly terpenoids (Park et al., 2017). Most of these are volatile monoterpenes and sesquipenes (Rodrigues-Corrêa et al., 2012; Güzel, 2019). Many traditional medicine systems use pine resin as medicine in various forms to treat wounds (Khmelnitskii et al., 2002; Shah, 2011; Rodrigues-Corrêa et al., 2012; Park et al., 2017). Turpentine obtained from pine resin is widely used in medicine (Shah, 2011; Sharma et al., 2018). Tar, which is obtained by burning various pine trees and contains a large amount of terpenes, is used locally to treat various wounds on animals (Barnes and Greive, 2017). Apart from wounds, it is used to protect the hooves of animals (Barnes and Greive, 2017; Sinmez et al., 2018). Especially terpenoids and other secondary metabolites in pine resin accelerate wound healing by various mechanisms (Pérez-Recalde et al., 2018; Romo-Rico et al., 2022; Venkata et al., 2022).

Pine resin, also referred to as rosin, is the natural resin secreted by Pinaceae family members including Pinus longifolia Roxb., Pinus sylvestris L., and Pinus palustris Mill. It is also known as Colophony, Pine Resin, and Resina Pini. Many conventional medical systems use pine resin as a drug to treat wounds. Pine resin is used in Unani medicine to treat suppurated wounds because it decreases exudation and boosts local perfusion (Park et al., 2017). Abietanes and pimaranes are the two main types of diterpenoids found in fresh resin. Pimaranes are more stable than abietanes, which are more likely to isomerize and yield abietic acid (Beltran Sanchidrian, 2016).

Studies on the effectiveness of pine resin on corneal wounds could not be found in the literature. Therefore, the aim of this study is to investigate the effectiveness of pine resin on experimentally induced corneal wounds in rats.

MATERIALS and METHODS

The preparation of pine resin

Pine resins to be used in the study were collected from red pine (Pinus brutia Ten.) forests in Muğla region. The collected resin was dried in the shade, then hardened in the freezer and finally homogenized by pulverizing with a grain grinder. The powder resin was weighed 100 g and mixed with 500 ml of ultrapure water. It was shaken for 5 min twice a day for 10 days using a vortex mixer, then filtered with Whatman No. 1 filter paper, transferred to falcon tubes and stored at +4°C until the experimental phase was performed. The pH of pine resin is between 2-7. Since the pH of the eye is 7.4, the pH of the extraction was adjusted to 7.4 with NaHCO3 before the application (Angin and Ertas, 2021).

Animals

Twenty-one male Wistar albino rats (230-250 g/60 day old) were used in study. The rats were fed with ad libitum water and food under a 12/12-hour light/dark environment at a temperature of 22±2°C during the study. Rats were obtained from Muğla Sıtkı Koçman University, Experimental Animals Application and Research Center, Muğla, Turkey. The study was conducted with the approval of the Muğla Sıtkı Kocman University Animal Experiments Local Ethics Committee under permit number 24.08.23/20-23

Experimental procedure

Rats were acclimatized for two weeks prior to the study. After 0. hours in study, all rats were anesthetized by administering 10mg/kg Xlazine hydrochloride (Rompun, Bayer, 23.32 mg/mL, Barmen, Germany) followed by 70 mg/kg Ketamin hydrochloride (Ketalar, Parke-Davis, 50 mg/mL, Brooklyn, New York, ABD) injection intramuscularly. The right eyes of the anaesthetized rats were prepared sterilely for the operation. The borders of the wound to be created with a 3 mm punch biopsy were then determined and the first two layers of the cornea were removed with a corneal knife (Zagon et al., 2000; Nagai et al., 2009). After 21 male Wistar albino rats were divided into three groups randomly. Groups: Control (no medication or treatment was applied to the rats in this group from the time of corneal wound formation), Pine resin (Pine resin was applied one a day with one drop to the rats in this group until the day of sacrifice at the cornea), Drug (The antibiotic drug - Exocin, Allegan, 3mg/mL, Ireland) was applied one times in a day with one drop to the rats in this group until the day of sacrifice at the cornea). Following the operation, the corneal wound site was measured in terms of length and within mm until the day of sacrifice and documented by taking photographs. For this purpose, before the measurements each rat was applied fluorescein test. The corneal wound surface areas were calculated by transferring the photographs to the "Image j" program in the computer environment. Total wound healing was found by looking at the difference between days in terms of wound areas.

Histopathologic analyses

After the formation of the corneal wound, rats were euthanized by cervical dislocation under general anesthesia on third day. The corneal tissue sample was taken from each euthanized rat with enoculation bulbi procedures. The cornea samples taken were fixed in a 10% formaldehyde solution for histopathological examination. After fixation, the tissues were subjected to a tissue processing procedure consisting of alcohol and xylene series, and then embedded in paraffin blocks. 4µm thick sections transferred from the paraffin blocks to slides were stained with hematoxylin-eosin, and then microscopic examination was performed on these sections.

Statistical analysis

All the data was presented as mean ± standard error of mean. Statistical Package for Social Sciences (SPSS) version 22.0 for Windows (SPSS Inc., Chicago, IL) was used for analysis. For corneal wound sizes used to compare data across groups using one-way Analysis of Variance (ANOVA) and post hoc Tukey honestly significant difference tests were conducted. Kruskal-Walli's test was used for histo-pathological examination. Paired comparisons of variables were performed using Mann-Whitney U. P-values below 0.05 were considered as statistically significant results.

RESULTS

Corneal wound sizes

When daily fluorescein staining images were examined macroscopically, a gradual decrease was observed in all groups. The corneas of the rats in the pine resin group showed complete disappearance of dye uptake at the end of the 48th hour (Figure 1).

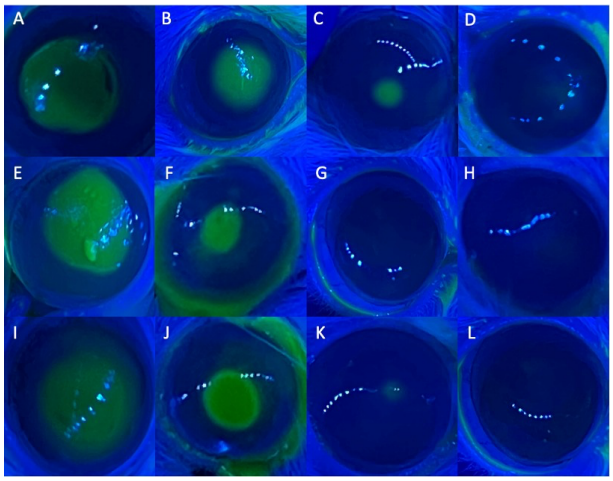

Figure 1. (A) 0.hours in control group (B) 24.hours in control group (C) 48.hours in control group, (D) 72.hours in control groups, (E) 0.hours in pine resin groups, (F) 24.hours in pine resin groups, (G) 48.hours in pine resin groups, (H) 72.hours in pine resin groups, (I) 0.hours in drug groups, (J) 24.horus in drug groups, (K) 48.hours in drug groups, (L) 72.hours in drug groups.

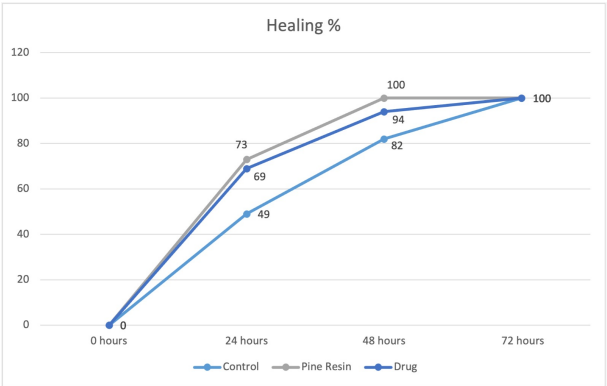

Figure 2. Daily healing percentages of corneal wounds.

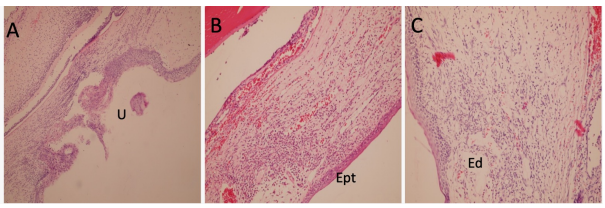

Figure 3. Histopathological images. A. Showed ulceration area in control group. U: Ulceration. B. Showed remodeling area in pine resin group. Ept: Epithelium Layer. C. Showed edema at stroma in drug group. Ed: edema.

The fluorescein staining measurements were subjected to an ANOVA analysis test. According to the analysis, at the 24th hour, the pine resin and drug groups showed significantly faster improvement compared to the control group. At the 48th hour, there is a significant difference among the three groups, with the pine resin group showing the fastest improvement, while the control group exhibited the slowest improvement ($p<0.05$). At 72 hours, all three groups have fully improvement (Table 1).

Table 1: Results of fluorescein staining 0, 24, 48, and 72 hours after surgery of the wound. The values are given in mm^2 (square millimeters).

Time	Control	Pine Resin	Drug	P value
0. hours	9.956±0.227	9.772±0.170	10.009±0.225	0.706
24. hours	5.034±0.197[a]	2.569±0.245[b]	3.038±0.135[b]	<0.001
48. hours	1.759±0.273[a]	0.000±0.000[c]	0.522±0.137[b]	<0.001
72. hours	0.000±0.000	0.000±0.000	0.000±0.000	-

a-c: Different letters in the same line are statistically significant ($p<0.001$).

Table 2: Histopathological examination results (0: none, 1: mild, 2: moderate, 3: severe)

Findings	Control	Pine Resin	Drug	p value
Corneal Edema	2.125 ±0.295[a]	0.875±0.295[b]	1.750±0.250[a]	0.029
Bleeding	1.750±0.250	1.375±0.182	1.750±0.313	0.375
Vascularization	1.875±0.295	1.625±0.263	1.500±0.267	0.728
Cell Infiltration	2.250±0.163	2.000±0.267	1.750±0.163	0.238
Ulceration	0.625±0.182[a]	0.000±0.000[b]	0.500±0.188[a]	0.028

The mean extent of healing in the groups was calculated as percentages. The control group showed an average of 49% healing at 24. hour, 82% at 48 hour and 100% at 72 hours. Pine resin group showed 73% healing at 24. hour and 100% healing at 48 hours. The drug group showed 69% improvement at 24. hour, 94% at 48 hour and 100% at 72 hour (Figure 2). The amounts of corneal wound healing (%) were calculated according to this equation (Nagai et al., 2010);

Corneal wound healing (%) = (wound area0h – wound area24-36-72h) / wound area0h x 100

Histopathological examination

The sections stained with hematoxylin-eosin were taken for microscopic examination. In the histopathological examination, the groups were evaluated semi quantitatively for the presence of bleeding, vascularization, cellular infiltration, corneal edema, and ulcer formation (0: none, 1: mild, 2: moderate, 3: severe).

When histopathological images were examined, microscopic ulceration areas were observed in the control group within the epithelial layer. In the pine resin group, it was noted that the epithelial layer exhibited a smoother healing compared to other groups, and cell infiltration was observed in the subepithelial layer, while microscopic bleeding foci were visible near the endothelial layer. In the drug group, edematous areas were observed in regions close to the epithelium, and bleeding foci were observed in subendothelial layer (Figure 3).

Histopathologically, all animals were examined for corneal edema, bleeding, vascularization, cell infiltration and ulceration. The tests revealed that the pine resin group exhibited significantly better healing in terms of corneal edema and ulceration compared to the control and drug groups. However, there was no difference between the drug group and the control group in terms of corneal oedema and ulceration. No significant differences

were detected among the groups in terms of bleeding, vascularization and cell infiltration (Table 2).

DISCUSSION

Due to its structure, the cornea is extremely important for the continuity of vision. Therefore, corneal wounds should be treated quickly and normal corneal structure should be restored (Martin et al., 2013; Chandler et al., 2019). Physical injuries of the cornea occur in animals due to factors such as foreign bodies, tear problems, loss of eyelid function, anomalies, infection and trauma (Demir et al., 2022). Various methods such as scraping the layers of the cornea (physically) or exposing the cornea to chemical agents can be applied to experimentally create corneal wounds (Ho et al., 2013; Fernandes-Cunha et al., 2019; Kibar Kurt and Belge, 2021). Physically induced corneal injuries in rats have been used in studies for the development of corneal healing drugs (Zagon, 2007; Nagai et al., 2010). Therefore, we physically induced corneal injury to examine the efficacy of pine resin extract on corneal injury.

Resins have important biological activities such as antibacterial, antifungal, antiviral, antiviral and antiparasitic thanks to the substances in their content (Alkan et al., 2016). One of the effective examples of this is propolis, which bees obtain by collecting plant resins (Salatino et al., 2011). Pine resin is a natural resin found in injured areas on the trunks of pine trees (Rodrigues-Corrêa et al., 2012; Güzel, 2019). Traditional medicine systems have used pine resins to treat wounds (Khmelnitskii et al., 2002; Shah, 2011; Rodrigues-Corrêa et al., 2012; Park et al., 2017). Pine resins, which are frequently used in traditional medicine, are now included in ointments used for medicinal purposes. These ointments are seen as a promising treatment for burns, wounds (stage 1 of the wound process), purulent diseases of the skin and subcutaneous tissue (Simbirtsev et al., 2002e). The terpenoids and other secondary metabolites it contains have been reported to accelerate wound healing by various mechanisms (Pérez-Recalde et al., 2018; Venkata et al., 2022).

Studies on the direct wound healing effect of pine resin are quite limited wounds (Khmelnitskii et al., 2002; Shah, 2011; Rodrigues-Corrêa et al., 2012; Park et al., 2017). In addition, there are no studies in the literature on the effectiveness of pine resin on corneal wounds. However, honey, which has been used together with resins for wound healing since ancient times, and propolis, which is also obtained from resins, have been reported to accelerate healing on corneal wounds (Martin et al., 2013; Park et al., 2017; Abd Rashid et al., 2022; Bulut et al., 2023). Based on these results, we also examined the healing accelerating activity of pine resin on corneal wounds.

Alcohol-based substances have been used in previous studies to purify pine resin (Boudjelal et al., 2022) and it is known that good efficiency in the extraction of terpenoids in it is obtained with solvents such as alcohol and benzene (Angin and Ertas, 2021). However, it is not appropriate to use alcohol and benzene in eye application (McDonald et al., 1970) , so we preferred to use pure water for the extraction of pine resin in our study. The normal pH value of pine resin is between 2-7. For this reason, after extraction with pure water, the pH value was adjusted to around 7.4 with NaHCO3, which is suitable for use in the eye. 7.4, which is suitable for use in the eye (Peyman et al., 2007).

Simbirtsev et al. (2002a; 2002b; 2002c; 2002d; 2002f) carried out various studies on pine resin and ointments containing pine resin. As a result of these studies, pine resin increased macrophage, neutrophil and leukocyte activity (Simbirtsev et al., 2002f), showed bacteriocidal activity (Simbirtsev et al., 2002d), inhibited humoral response (Simbirtsev et al., 2002a), suppressed free radicals (Simbirtsev et al., 2002b), showed immunomodulatory activity (Simbirtsev et al., 2002c). Rozbahani et al. (2019) examined the effectiveness of pine resin with their study on the skin and stated that pine resin showed a similar healing rate to the positive control group. However, in our study on corneal wounds, the use of pine resin accelerated healing compared to antibiotic use. Previous studies have indicated that the excipients in antibiotic drops used in the treatment of corneal wounds may affect corneal healing (Lin and Boehnke, 2000). The difference between the two studies is thought to be due to this reason. Pine resin has shown these effects in studies on skin. However, the effects of pine resin on the cornea are not fully known.

This study is pioneering as it is the first study to investigate the efficacy of pine resin extract on physically induced corneal wounds. Therefore, it was not compared with a previous study using pine resin. However, it has been reported in various studies that propolis species accelerate healing in corneal injuries compared to propolis obtained from resins (Ozturk et al., 1999; Martin et al., 2013; Abd Rashid et al., 2022).

Martin et al. (2013) used propolis in corneal wounds caused by alkaline substances in their study and revealed that corneal wounds were completely healed macroscopically at the end of the 120th hour. In our study, corneal wounds in animals treated with pine resin were completely healed macroscopically at the end of the 48th hour. It has been reported in previous studies that physically induced corneal wounds heal faster than corneal wounds induced by alkaline substances (Ho et al, 2013). The difference in healing time between the two studies is thought to be due to the difference in methods of corneal wound creation. In both studies, the fastest healing occurred within the first 24 hours.

Öztürk et al. (1999) compared propolis and dexamethasone on the corneas of rabbits and reported that they showed similar effects in terms of anti-inflammatory activity. Abd Rashid et al. (2022) reported that propolis obtained from honey and resins, which have been frequently used with resins since ancient times, accelerated healing in corneal wounds. The results of our study are compatible with these studies and pine resin, which is also used in propolis production, accelerated healing in physically induced corneal injuries.

When compared histopathologically with Propolis studies, Martin et al. (2013) reported that the use of Propolis at 24 and 48 hours reduced cell infiltration in corneal wounds. Bulut et al. (2023), in their study examining the effects of propolis and nanopropolis on corneal wounds, reported that propolis did not reveal a significant difference in terms of corneal edema, bleeding, vascularization, cell infiltration and ulceration compared to the control group. In our study, pine resin decreased corneal oedema and ulceration, but did not cause a significant change in cell infiltration.

CONCLUSION

In conclusion, in the study, pine resin accelerated the healing of experimentally induced corneal wounds in rats. Histopathologically, corneal oedema and ulceration were reduced. However, there is a need for further studies on the efficacy of pine resin by increasing the amount, frequency and duration of application.

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Evaluation of the effect of daily cow's milk production on liver enzyme levels

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ABSTRACT

Objective: This study aimed to determine the possible effect of the total daily amount of skim milk on bilirubin and liver enzymes through regression analysis.

Materials and Methods: The study included 63 Holstein-Friesian cows. They were formed in 3 groups, based on the amount of daily milk production. First was the group of lower daily milk production n=21, the second group of examined cows was the group of medium daily milk production n=23 and third was the group of higher daily milk production n=19. Peripheral blood was punctured, through which the activities of total bilirubin were analyzed (μmol/L), as well as liver enzymes: alanine aminotransferase-ALT (U/L), aspartate aminotransferase-AST (U/L), lactate dehydrogenase-LDH (U/L) and alkaline phosphatase-ALP (U/L), from the blood plasma of the examined blood.

Results: The lowest concentration of total bilirubin in blood plasma was recorded in the group of cows that have the lowest daily milk production (1.295±0.255 μmol/L), and highest concentration is in cows that produce the most milk (1.855±0.159 μmol/L), but intergroup differences are not significant. Regression analysis found a statistically significant relationship between the amount of produced daily milk and the concentration of total bilirubin (R^2=0.132, p=0.005<0.05). It is determined that the highest activity of ALT, AST, ALP and LDH was found in cows with the highest amount of daily skimmed milk, but intergroup differences were not statistically significant (p>0.05).

Conclusion: The activities of bilirubin and liver enzymes in the examined cows were in physiological balance. This indicates that the cows on the farm are raised in modern and good zootechnical and feeding conditions. In such conditions, dairy cows are able to maintain blood composition and homeostatic integrity within physiological limits and adequate reproductive and productive capacity.

Keywords: *Liver Enzymes, Bilirubin, Lactating Cows, Milk*

INTRODUCTION

Permanent monitoring of the metabolic profile is a necessary prerequisite for successful management of highly productive dairy cows in farm housing conditions. The metabolic profile is important tool for monitoring health and disease prevention in these animals (Eşki and Kurt, 2021). All parameters of the metabolic profile are equally important and it is necessary to interpret them correctly, because through them we manage to reach certain pathophysiological deviations, as well as physiologically conditioned variations (Katica et al., 2019; Andjelić et al., 2022). The functional state of

the liver has a particularly significant influence on biochemical indicators (Jašović et al., 2016; Andjelić et al., 2022).

The negative energy balance of cows leads to the mobilization of fatty acids from the body's reserves, and reaches its maximum in the second week of lactation. Free fatty acids that are mobilized in increased amounts from body depots in the peripartum period in high-milk cows are an important predisposing factor in the etiopathogenesis of fatty infiltration and degeneration of liver cells, as well as ketosis of cows (Mukača, 2015).

Increased energy needs during pregnancy and lactation are often not accompanied by adequate energy intake, and as a consequence there is a violation of the functional state of certain organs, most often the liver. Many researchers consider that elevated values of liver enzymes most often occur when acute or chronic disease is suspected (Hadžimusić and Krnić, 2010; Aladrović et al., 2018).

Increased aspartate aminotransferase (AST) activity in dairy cows is mainly associated with fatty liver syndrome, decreased appetite and the occurrence of ketosis in dairy cows during early lactation (Aladrović et al., 2018). Also, lactate dehydrogenase (LDH) together with AST is often used to diagnose cow liver disease, as well as during infections and inflammatory processes (Tedesco et al., 2004; Klein et al., 2020).

Alkaline phosphatase (ALP) originates mainly from the intestinal mucosa, placenta, kidneys and bones, but also from the liver and is mostly found in cell membrane of hepatocytes, and the determination of this liver enzyme can serve as a tumor marker and as an index of liver disease and bone disease (Sato et al., 2005; Valocky et al., 2007; Yeniyol and Ricci, 2018; Ogunmoyole et al., 2022; Šaljić et al., 2022; Utari et al., 2022).

The activity of alanine aminotransferase (ALT) in blood plasma is influenced by age and muscle activity. The established physiological variations in the activity of this enzyme are related to pregnancy and the beginning of lactation, when the level of ALT activity is reduced (Hadžimusić and Krnić, 2010).

Bilirubin is natural heme degradation product. It binds tightly to albumin and is also rapidly eliminated from the body, mainly by hepatic glucuronidation and elimination by bile (Sane et al., 2014). Hyperbilirubinemia is uncommon in sick cattle and is mostly commonly associated with the inability of the liver to regulate unconjugated bilirubin (McSherry et al., 1984).

Pathophysiological conditions leading to hyperbilirubinemia are related to: increased production of bile pigments (in hemolytic processes, or in resorption of larger hematomas), decreased uptake of unconjugated bilirubin into hepatocytes, inhibited bilirubin conjugation process, impaired bile duct excretion in biliary canals and obstruction bile in intestines (Mukača, 2015). The level of concentration of total bilirubin in the blood serum of cows is considered as one of the safest indicators of the functional state of the liver. In cows, elevated bilirubin concentration occurs during hemolytic crises (Faixova et al., 2012).

There are reports that indicate a certain correlation between the quality and composition of meals in lactating cows, with the parameters of skimmed milk in correlation with the activity of liver enzymes (Šaljić et al., 2022). On the other hand, another similar study found a correlation between liver enzyme activity depending on the reproductive cycle of dairy cows in the circumstances of lactation, dryness and postpartum period depending on the sampling season (Hadžimusić and Krnić, 2010).

Since the trend of modern intensive breeding of dairy cows is based on constant increase in the daily amount of skimmed milk, the aim of our study was to determining the possible impact of total daily amount of skimmed milk on the level of bilirubin and liver enzymes, by regression analysis.

MATERIALS and METHODS

Ethics Committee Approval

This research was approved by the Ethics Committee of the University of Sarajevo, Veterinary Faculty, under registration number 07-03-764-2/23, Sarajevo, Bosnia and Herzegovina.

Animal and general experimental procedure and study group

The study included 63 Holstein-Friesian cows, aged between 2 and 9 years and was carried out at the farm in the northern region of Bosnia and Herzegovina placed in modern and very good zoo-technical conditions. The Radio Frequency ID (RFID) technology was used during the breeding and production in this farm, which implies that each cow owns a chip through which the animal's activities related to reproduction, lactation and

history of the diseases is being monitored and recorded.

The study was carried out during the winter period, and it included 63 cows aged 2-9 years (the largest number of cows was between 3 and 5 years old) in different lactation stages (1-8). The largest number of cows, eighteen of them, were in the second, third and fourth lactation stage, while nine cows were in the first lactation stage. Three groups of cows were formed according to the amount of daily milk produced, based on the control of the amount of daily milk production which was performed during the last seven days, prior to the blood sampling (Table 1).

Table 1. Classification of cow groups in the study according to daily productivity level and daily milk production amount.

Group	Level of daily productivity	Varying the amount of daily skimmed milk
1 (n=21)	Lower level	20-28 L
2 (n=23)	Middle level	29-34 L
3 (n=19)	Higher level	35-52 L

Dietary treatments

Dairy cows were fed with 30 kg of silage, 15 kg of haylage, 10 kg of concentrate mixture containing 18-20% protein. Dry cows were fed with 20 kg of silage, 10 kg of haylage and 3-4 kg of concentrate with 18-20% protein. They consumed water ad libitum.

Blood collection and biochemical analysis

Blood samples were taken by the technique of puncturing the cocigaeal vein, in injectors containing heparin á 4-5 ml. The blood was then centrifuged (LC 320, 2000 rpm/10 min) to extract plasma.

We used the spectrophotometer "Beckman DU-64 UV/VIS" and commercial kits manufactured by "Human", Germany, to assess the levels of bilirubin (μmol/L) and liver enzymes, including alanine aminotransferase-ALT (U/L), aspartate aminotransferase-AST (U/L), lactate dehydrogenase-LDH (U/L) and alkaline phosphatase-ALP (U/L).

Animal care

During the research on dairy cows the usual veterinary diagnostic procedures were used. Blood sampling from the coccigaeal vein was done by an authorized veterinary technician. All procedures on dairy cows during the research were in accordance with the Law on Protection and Welfare of Animals of Bosnia and Herzegovina (Official Gazette of BiH, no. 25/2009 and 9/2018).

Statistical analysis

The results were statistically processed by the method of descriptive statistics. To determine whether there are differences in the arithmetic means of liver enzymes activity levels, a global ANOVA test was used between the groups. Testing of differences in mean values of liver enzyme parameters between the examined groups was performed at the significance level of $p<0.05$. After determining the existence of statistically significant differences, using regression analysis (single linear regression), the possible dependence of liver enzyme parameters on the amount of milk produced was determined. Statistical processing of the results obtained by the research was performed using a software program (IBM Corp. Released, 2016).

RESULTS

Comparing the obtained mean values, with Radostits et al. (2000), we notice that all values are within physiological variations. The exception is the mean value for AST, which is slightly lower than the lower physiological limit.

Significance results of differences in bilirubin and liver enzymes between groups of cows with different amounts of daily skimmed milk.

The average concentration of total bilirubin in blood samples of all examined cows on the farm (n=63) was 1.513±0.117 μmol/L, and individual values varied within the range 0.21-3.42 μmol/L (Table 2) with the distribution of individual values as is shown in Figure 1. The lowest concentration of total bilirubin in blood plasma was recorded in the group of cows that have the lowest daily milk production (1.295±0.255 μmol/L), and the highest in cows that produce the most milk (1.855±0.159 μmol/L), but intergroup differences are not significant (Figure 1).

Although there are differences in daily milk productivity between cows in ALT activity (Figure 2), they statistically significantly differ only between cows of middle and higher levels of daily milk production ($F= 3.659$, $p=0.032<0.05$).

Values for alanine aminotransferase (ALT) activity in 63 cows included in our research ranged from 10.79-44.11 U/L (24.208±0.853) (Table 2 and Figure 2). The highest value of ALT activity was in cows with the highest daily milk production (26.539±1.846

U/L), and the lowest in plasma in cows with daily milk production of 29-34 L of milk (21.371±1.149 U/L) and this difference is statistically significant (F=3.659, p=0.032<0.05) (Figure 2).

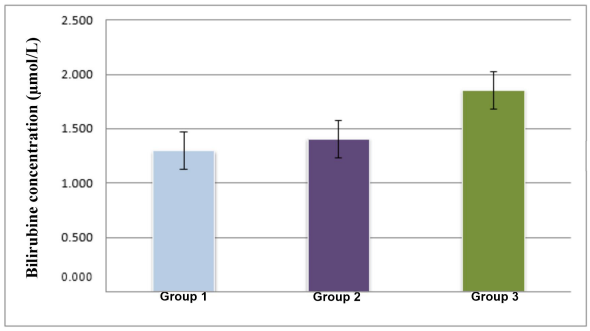

Figure 1. The concentration of bilirubin (µmol/L) in blood plasma of cows with different amounts of daily skimmed milk (Group 1=20-28 L; Group 2=29-34 L; Group 3=35-52 L of milk). All values are represented as $\bar{x} \pm S\bar{x}$.

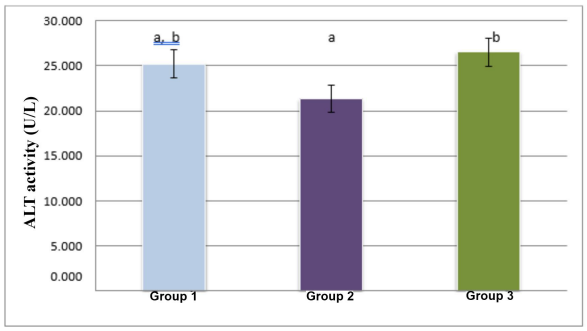

Figure 2. The activity of ALT (U/L) in blood plasma of cows with different amount of daily skimmed milk (Group 1=20-28 L; Group 2=29-34 L; Group 3=35-52 L of milk). All values are represented as $\bar{x} \pm S\bar{x}$. a, b = values that have a different letter are statistically significantly different (p<0.05).

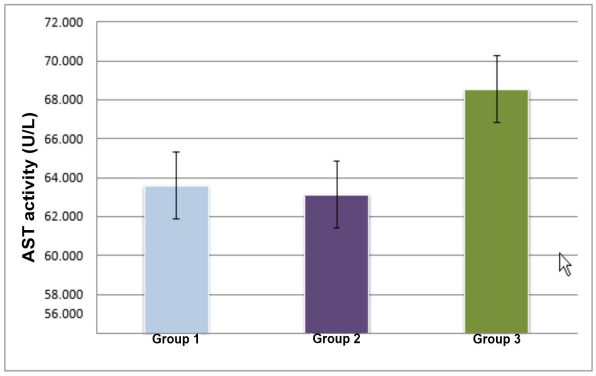

Figure 3. The activity of AST (U/L) in blood plasma of cows with different amount of daily skimmed milk (Group 1=20-28 L; Group 2=29-34 L; Group 3=35-52 L of milk). All values are represented as $\bar{x} \pm S\bar{x}$.

We also found that AST activity varied in range 25.40-94.60 U/L (64.831±2.906; Table 2). The lowest AST activity was recorded in the group of cows with daily milk production in quantity of 29-34 L, and highest in cows with the highest amount of daily skimmed milk. However, the differences between the three examined groups of cows are not significant (p>0.05), (Figure 3).

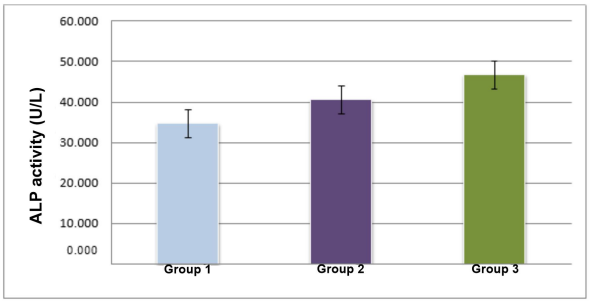

Figure 4. The activity of ALP (U/L) in blood plasma of cows with different amount of daily skimmed milk (Group 1=20-28 L; Group 2=29-34 L; Group 3=35-52 L of milk). All values are represented as $\bar{x} \pm S\bar{x}$..

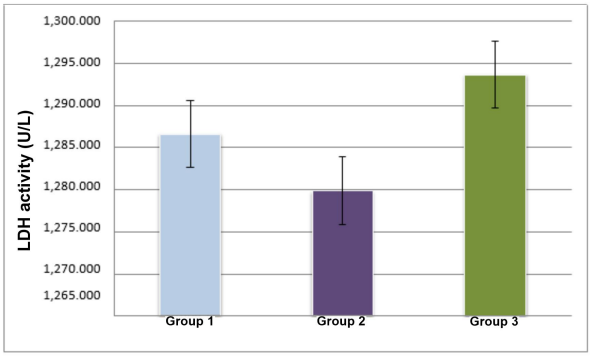

Figure 5. The activity of LDH (U/L) in blood plasma of cows with different amount of daily skimmed milk (Group 1=20-28 L; Group 2=29-34 L; Group 3=35-52 L of milk). All values are represented as $\bar{x} \pm S\bar{x}$..

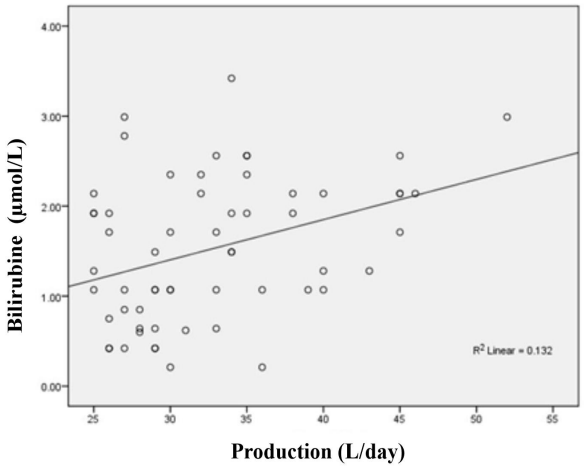

Figure 6. Dependence of bilirubin concentration (µmol/L) on the amount of daily skimmed milk.

The average value of ALP activity in blood samples of all examined cows on the farm (n=63) was 40.515±2.244 U/L, and individual values varied within the range of 5.51-78.12 U/L (Table 2). The lowest activity of ALP in blood plasma was recorded

in the group of cows that have the lowest daily milk production (34.680±3.331 U/L), and the highest is in cows with the highest daily milk production (46.665±3.593 U/L), but intergroup differences are not significant (Figure 4).

The values of lactate dehydrogenase (LDH) activity in our research vary in the range of 299.52-2075.02 U/L (1286.365±53.575 U/L; Table 2). In the case of LDH activity, the highest activity was recorded in cows with the highest daily skimmed milk (Figure 5), and intergroup differences are not significant ($p>0.05$).

Results of the dependence of bilirubin and liver enzymes on the amount of daily skimmed milk

The results of the regression analysis for all examined parameters are shown in Table 3.

Table 2. Results of descriptive statistics for total bilirubin, ALT, AST, ALP and LDH from the blood of the entire sample

Parameters	NVO	Mean	SE	Med.	SD	Var.	SDA	Min.	Max.
Tot. Bilirubin (μmol/L)	63	1.51	0.11	1.50	0.90	0.82	-0.17	0.21	3.42
ALT (U/L)	63	24.21	0.85	24.11	6.77	45.86	0.27	10.80	44.11
AST (U/L)	63	64.83	2.9	63.31	14.69	215.80	0.16	25.40	94.60
ALP (U/L)	63	40.51	2.24	36.99	17.09	292.00	0.60	5.51	78.12
LDH (U/L)	63	1286.36	53.57	1249.32	421.85	17796.52	-0.06	299.52	2075.02

NVO: Number of valid observations, SE: Standard error, Med: Median, SD: Standard deviation, Var: Variance, SDA: Skewness distribution asymmetry. Min: Minimum, Max: Maximum.

Table 3. Results of regression analysis to determine the dependence of the values of the examined parameters on the amount of daily skimmed milk.

Regression parameters (in summary)	Model		ANOVA		Non-standardized coefficients (B)	
	R	R^2	F	Sig.	Constant	Production (L/day)
Bilirubin(μmol/L) *	0.36	0.13	8.65	0.00	0.06	0.04
Alanine aminotransferase (U/L)	0.14	0.02	1.25	0.27	19.44	0.15
Aspartate aminotransferase (U/L)	0.17	0.03	1.68	0.20	51.40	0.42
Alkaline phosphatase (U/L)	0.10	0.01	0.57	0.45	31.91	0.27
Lactate dehydrogenase (U/L)	0.06	0.00	0.23	0.63	1427.69	-3.95

* significantly at the level of 5% ($p< 0.05$)

Using regression analysis (single linear regression), a statistically significant effect of the amount of daily produced milk on concentration of bilirubin was determined the same indicates a statistically significant relationship between the amount of daily skimmed milk and the concentration of total bilirubin (R2=0.132, p=0.005<0.05; Table 3 and Figure 6).

The results of the regression analysis did not show a statistically significant influence of the amount of daily produced milk on the examined parameters: ALT, AST, ALP and LDH (Table 3).

DISCUSSION

Increasing the intensity of milk production i.e. at the maximum utilization of genetically conditioned high milk yield, is aimed with the modern method of breeding. In its full activity, the mammary gland represents the greatest metabolic load for the organism of high-milk cows (Katica et al., 2019).

The activities of the examined enzymes indicate that they represent a morphological and functional hemostatic integrity of the liver, which achieves metabolic activity in extremely demanding conditions of high milk production. Intensive

metabolic efforts of the liver imply that all necessary neurohumoral regulatory mechanisms involved in the control of glycomobilization, lipomobilization and gluconeogenesis, with adequate nutritional conditions, function purposefully to provide metabolic precursors for synthetic processes in the mammary gland (Andjelić et al., 2022).

The period of early lactation of cows is characterized by a negative energy balance, i.e. a condition when the energy intake in the body is less than its needs for milk production and the ability to take food. Until a balance is established between the amount of energy ingested and the amount of milk produced (between the sixth and tenth week of lactation), the difference is compensated by the mobilization of body reserves, first glycogen reserves, then fat, and then protein (Mukača, 2015).

However, in conditions of negative energy balance due to intensification of the lipomobilization process, in cows that accumulated higher fat reserves in the last phase of lactation during the previous pregnancy and during the drought period, an uncontrolled process of mobilization and deposition of non-esterified fatty acids in liver can occur. The consequence of such condition is the appearance of a fatty liver, infiltrative or degenerative in nature (Akgül et al., 2017).

Determination of the activity of enzymes originating from liver cells is used in the examination of the functional state of the liver. If their activity in the blood is elevated, they can be a good indicator of functional liver damage (Jašović et al., 2016). Damage to the liver parenchyma can be caused by various inflammatory agents, drugs, toxins, metabolic disorders and autoimmune diseases. The values for ALT activity obtained in our research correspond to the results (Krnić et al., 2003; Hadžimusić, 2010). The value of ALT activity is significantly higher during the winter, compared to the summer period. Higher values of ALT activity in the plasma of cows with the highest daily milk yield can be explained by the high variability of liver enzymes, which may indicate the intensification of metabolic processes in high milk production (Reynolds et al., 1991).

Examination of the value of aspartate aminotransferase (AST) activity is considered a relevant indicator in the assessment of liver function (Krnić et al., 2003; Andjelić et al., 2022). Increased activity of the AST enzyme is found in acute hepatitis, liver cirrhosis, toxic liver necrosis, but also in muscle injury, acute hemolytic anemia, muscular dystrophy, etc.

Like ALT, higher values of AST activity in the plasma of cows with the highest daily milk production may indicate an intensification of metabolic processes at high milk production. Such differences can be explained by the high variability of enzymes, which results in their instability in blood plasma (Reynolds et al., 1991).

Lactation has a great influence on the intensity of metabolism and metabolic parameters in the blood and this agrees with the results of Filipejova and Kovačik (2009), who found that AST in dairy cows at the beginning of lactation was significantly higher compared to the dry period (Ghada, 2014).

The increase in AST activity after birth could be explained by the degradation of muscle cells caused by the mobilization of body reserves. Furthermore, increased AST values may often be due to mobilization of muscle proteins for gluconeogenesis (Ghada, 2014.).

Single linear regression analysis showed that the amount of daily milk production did not have a significant effect on activity of AST in blood plasma.

In this study ALP is a very widespread enzyme in the body and hydrolyzes phosphoric acid esters. The level of activity of this enzyme in the blood changes in various physiological conditions, as well as in many diseases, especially when a metabolic bone and liver disease is suspected. In younger individuals, the activity in blood plasma mainly originates from bone, and in adults from hepatic ALP. The regression model did not determine a statistically significant effect of the amount of daily skimmed milk on the activity of ALP in blood plasma.

Study of Yokus and Cakir (2006) showed different mean ALP values depending on the production and reproduction status of the animas, and lactating cows had a mean ALP value that were consistent with the results of our research. The results closest to our established values of ALP activity are the results of Hadžimusić (2010) during the winter lactation period. It is possible that these differences are related to the variability of nutrient composition related to climate, nutrient type, soil type and climatic conditions defined by different altitudes Belic et al. (2018), and Yokus and Cakir (2006) state that ALP activity varies depending on the physiological condition, but also due to seasonal variations.

The values obtained for LDH in our research ranged within physiological limits (Radostits et al., 2000; Kaneko, 2008). In a research conducted by Sako et al., (2007) recorded slightly LDH values in moving cows, in the range of 1230-2074 U/L. The research conducted by Hodžić et al., (2007) also showed high mean values of this enzyme in clinically healthy cows.

Such wide variations in LDH activity are often due to the different physiological and production statues of the cows (Yokus and Cakir, 2006). Increased activity of this enzyme can occur during infectious, inflammatory, toxic or metabolic liver damage. It shows the greatest activity in the kidneys, heart, skeletal muscles, pancreas, spleen, liver, lungs and placenta. By damaging the cells, it easily passes into the blood. In chronic liver disease or slower-progressing liver disease, the activity of this enzyme may fall below the lower reference value if a small number of hepatocytes are damaged and the hepatocellular mass is significantly reduced (Lechtenberg and Nagaraja, 1991). The amount of daily produced milk has no statistically significant effect on LDH activities.

When assessing the activity of liver enzymes in lactating cows, the sampling season, the age of animal and the energy status should be taken into account. All of the above can to a greater or lesser extent affect the values of liver enzymes (Stojević et al., 2005).

Increased concentration of bilirubin in the blood plasma is mainly with clinically manifest signs of icteric skin and other visible mucous membranes. Occurrence of hyperbilirubinemia over 8.55 μmol/L (Radostits et al., 2000), is considered a pathophysiological condition and is usually due to impaired morphological and functional integrity of liver cells (Faixova et al., 2012). Significantly higher concentration of total bilirubin was found in cows with ketosis compared to blood values in healthy cows (Jovanović et al., 1991; Đoković, 1998; Bugarski, 2002). Total bilirubin in the blood of cows is therefore an indispensable test for examining the functional state of the liver (Reid et al., 1982).

Differences in the value of total bilirubin concentration are evident even for individual stages of cow production in farm breeding. According to Jovanović et al. (1997), values for total bilirubin in the blood of cows 10-15 days before calving is 4.7 μmol/L, 10 days after calving is 5.4 μmol/L, in the second month of lactation is 4.0 μmol/L, while in the fifth month of lactation is about 3.9 μmol/L.

The bilirubin concentration values in our research were within physiological limits, (Radostits et al., 2000), and which is in collision with the research of Krnić et al., (2003). According to Krnić et al., (2003), established hyperbilirubinemia in lactating cows of 9.67 μmol/L, link to the present problem of nutrition in a longer period before blood sampling on the farm, and state that the finding indicates a violation of liver function or increased hemolytic processes. In our research, the cows used in the research were bred in modern and very good zootechnical conditions, and were fed balanced meals that could ensure normal liver function within physiological balance. However, single linear regression values indicate a statistically significant relationship between the amount of daily skimmed milk and the total bilirubin concentration in blood plasma. This indicates that when the amount of daily milk produced increases, the concentration of total bilirubin also increases. The stated positive correlation between the amount of daily milk production and the concentration of bilirubin indicates that the liver with its function can meet the needs of the organism in conditions of high milk productions.

CONCLUSION

The activities of bilirubin and the activities of the enzymes ALT, AST, LDH and ALP in the examined cows were in physiological balance. This indicates that high milk cows are kept and bred on the examined farm under modern and very good zootechnical and feeding conditions. In such conditions, dairy cows are able to maintain blood composition and homeostatic integrity within physiological limits and adequate reproductive and productive capacity.

A positive correlation between the amount of daily milk production and the concentration of bilirubin indicates that the liver with its function can meet the needs of the organism in conditions of high milk production.

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Evaluation of cryptorchidism in cats and dogs

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ABSTRACT

Objective: Cryptorchidism, the failure of one or both testes to descend into the scrotum, is a common congenital condition in male cats and dogs. This study aimed to assess the incidence and characteristics of cryptorchidism in neutered cats and dogs.

Materials and Methods: A retrospective analysis was conducted on 1622 animals (348 dogs and 1274 cats) brought for neutering between 2015 and 2023. Cryptorchid was diagnosed by clinical examination and its location was determined by ultrasound examination. Animals diagnosed with cryptorchid were castrated. Breed, age, weight, and location of the retained testicle of all patients were recorded.

Results: Dogs exhibited a higher prevalence (8.91%) compared to cats (3.30%). The affected testes were predominantly subcutaneous in both species (61.29% in dogs, 73.81% in cats). Unilateral cryptorchidism was more common, with equal distribution between the right and left sides in dogs and a higher incidence on the left in cats. Purebred animals, particularly small-sized breeds, showed a higher susceptibility to cryptorchidism.

Conclusion: Our study provides valuable insights into the prevalence, localization, and characteristics of cryptorchidism in cats and dogs, emphasizing the importance of detection and intervention to safeguard the health of affected animals. The findings highlight the higher susceptibility of purebred cats and dogs.

Keywords: *Cryptorchidism, Cat, Dog*

INTRODUCTION

The failure of one or both of the testes to descend into the scrotum is known as cryptorchidism (Davidson, 2020; Gradil and McCarthy, 2023). Cryptorchidism is considered the most common congenital disease in male cats and dogs (Villalobos-Gomez et al., 2023). In embryonic life, the testes are located caudal to the kidneys (Romagnoli, 1991; Englar, 2019) in the abdominal cavity. The right testicle is slightly more cranial than the left testicle (Spangenberg, 2021). The testes, which complete their development before the inguinal canal closes, descend from the abdominal cavity into the scrotum through the inguinal canal (Romagnoli, 1991; Englar, 2019; Spangenberg, 2021). The gubernaculum, a peritoneal fold also known as the genito-inguinal ligament, is a long, cylindrical, mesenchymal structure that connects the testes to the scrotum at one end. This structure mediates the descent of the testicle from the inguinal canal to the scrotum (Romagnoli, 1991; Gier and van Sluijs, 2010; Spangenberg, 2021). The testicles descend into the scrotum in 3 stages; (a) abdominal testicular translocation: testes translocate from the lower part of the kidneys to the

entrance of the inguinal canal, (b) trans inguinal testicular migration: within the inguinal canal, (c) inguinoscrotal testicular migration: refers to the process of displacement from the outer ring of the inguinal canal into the scrotum (Amann and Veeramachaneni, 2006; Gier and van Sluijs, 2010). Insulin-like peptide 3 produced by fetal leydig cells mediates the descent of the testis from the caudal aspect of the kidney into the inguinal canal (Gier and van Sluijs, 2010; Davidson, 2020). Inguinoscrotal descent is mediated only by testosterone (Davidson, 2020). While the inguinal canal closes in dogs at around 6 months of age (Yates et al., 2003), it is reported to close in cats at 7-8 months of age (Little, 2011).

Although cryptorchidism in dogs is inherited and transmitted as a sex-limited autosomal recessive trait (Gier and van Sluijs, 2010; Davidson, 2020; Gradil and McCarthy, 2023), it is probably caused by more than one gene (Gier and van Sluijs, 2010). Since the gene(s) responsible for testicular descent is autosomal, cryptorchidism can be carried by both males and females (Gradil and McCarthy, 2023). Cryptorchidism in cats is assumed to be inherited and a polygenic mode of inheritance has been proposed (Davidson, 2020; Gradil and McCarthy, 2023). The prevalence of cryptorchidism in dogs varies between 1.2% and 10% (Gier and van Sluijs, 2010; Gradil and McCarthy, 2023). In cats, it has been reported as 0.37-3.8% (Gier and van Sluijs, 2010; Little, 2011; Gradil and McCarthy, 2023) and this rate is generally lower than the prevalence in dogs (Little, 2011). Unilateral cryptorchidism does not lead to infertility due to the presence of one scrotal testicle. Bilateral cryptorchid cats and dogs are infertile due to lack of normal spermatogenesis resulting from intra-abdominal temperature (~5° > scrotal). In both cases, libido and secondary sex characteristics are normal as interstitial cells continue to produce testosterone (Davidson, 2020; Griffin, 2020). Especially older dogs with abdominal cryptorchids are at high risk for the development of Sertoli cell tumors and spermatic cord torsion (Englar, 2019; Griffin, 2020). Although unilateral cryptorchidism is common in cats and dogs (Little, 2011; Griffin, 2020; Gradil and McCarthy, 2023; Villalobos-Gomez et al., 2023; Runge et al., 2024), bilateral cryptorchidism also appears (Griffin, 2020). Left and right sides are equally affected (Villalobos-Gomez et al., 2023). However, it is stated that it is more common in the right testicle in dogs (Moon, 2014; Griffin, 2020; Gradil and McCarthy, 2023).

Cryptorchidism is diagnosed by examination and palpation. Those in the inguinal region can sometimes be palpated, but in young animals, it is difficult to reliably determine the position of the testes due to their small size in the first weeks of life. Testicles cannot be palpated in abdominal cryptorchids (Gier and van Sluijs, 2010). Ultrasound examination is the preferred method to determine the localization of cryptorchidism in suspected cats and dogs (Davidson, 2020). Bilateral castration is recommended for all cryptorchid dogs and cats (Griffin, 2020). The aim of this study is to describe the results of an investigation to determine the incidence and nature of cryptorchidism in neutered cats and dogs. This research is the first and most comprehensive study on this subject in our country.

MATERIALS and METHODS

This study was approved by the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (2023/14-05). Healthy male cats and dogs brought to Van Yuzuncu Yil University Animal Hospital Surgery Clinic and private clinics for neutering between 2015 and 2023 were included in the study. Breed, age and weight data of all patients were recorded.

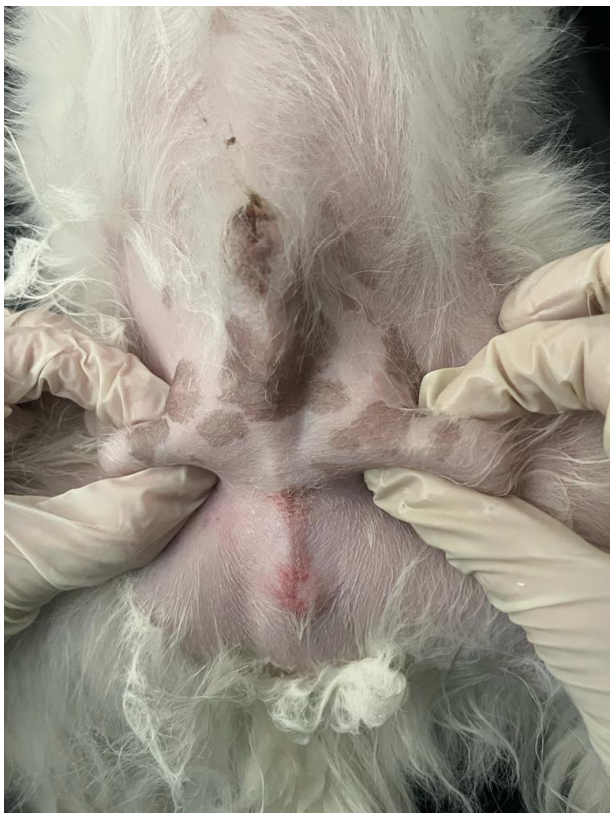

Figure 1. Examination and diagnosis of subcutaneously retained testicles by palpation.

Scrotal examination was performed in all animals. Patients in whom one or both testes were absent in the scrotal sac during scrotal examination by palpation were considered as cryptorchids (Figure 1). Ultrasonographic examination was performed in all patients diagnosed as cryptorchids to determine the localization of the undescended testicle. Cryptorchid animals were classified according to the affected testes (right, left or bilateral) and their localization (abdominal, inguinal or subcutaneous).

All patients were sedated and intubated following induction. Surgical approach was performed with standard surgical techniques according to the localization of the cryptorchid testicle. In abdominal cryptorchidism, the testicle was removed from the abdomen by caudal paramedian laparotomy in dogs and midline median laparotomy in cats. In inguinal cryptorchidism, it was removed through a skin incision over the inguinal canal. Subcutaneous cryptorchidism was removed by skin incision just above the testicle (Figure 2).

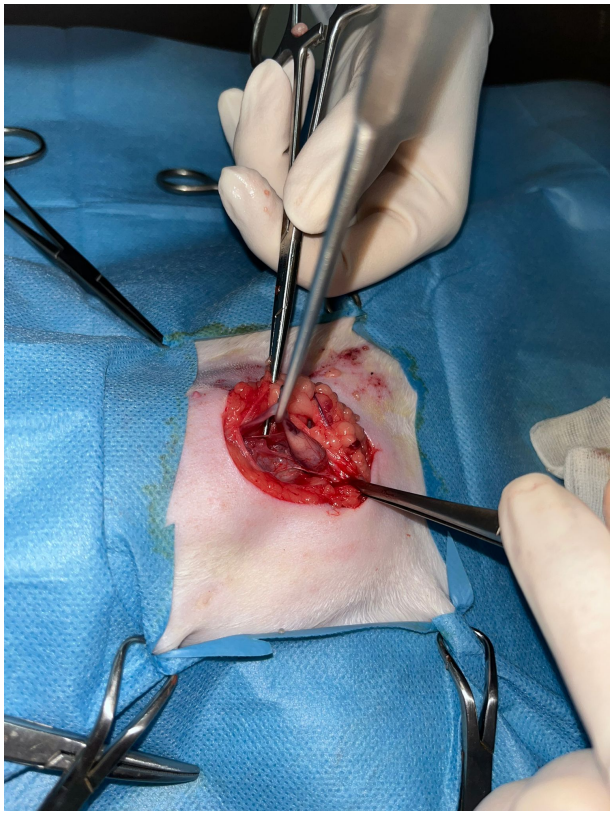

Figure 2. Subcutaneously retained testicle was removed by skin incision just above the testicle.

Statistical Analysis

The breed, age, weight, presence of cryptorchidism and localization data of all animals brought for castration were evaluated using descriptive statistical analysis.

RESULTS

In this retrospective study, the presence of cryptorchid was detected in 73 (4.50%) of the 1622 cats and dogs brought to the clinic for castration as a result of clinical examination, palpation and ultrasonographic examination imaging (Table 1).

Table 1. Animal species, numbers and rates of the cryptorchid animals

	Cat	Dog
Castrated animal number	1274	348
Number of cryptorchids	42	31
Total cryptorchid rate (%)	3.3	8.91
Subcutaneous cryptorchids rate (%)	73.81	61.29
Abdominal cryptorchids rate (%)	11.9	25.81
Inguinal cryptorchids rate (%)	14.29	12.9

Of 348 dogs brought for castration, 31 (8.91%) were found to have cryptorchids. The distribution of cryptorchid dogs according to breeds was 27 (9.75%) in purebreds and 4 (5.63%) in crossbreds (Table 2). The distribution of cryptorchid cases according to body weight was 16 (7.21%) in small breeds, 3 (15.79%) in medium breeds and 12 (11.21%) in large breeds. Mean age (months±SD) was 40.59±40.58 and mean weight (kg±SD) was 13.92±11.31. The localizations of the testes in cryptorchid dogs were subcutaneous (61.29%), abdominal (25.81%) and inguinal (12.9%), respectively (Table 1). In animals with unilateral subcutaneous cryptorchids, 42.1% of the cryptorchid testicle was the right testicle and 52.6% was the left testicle. Bilateral subcutaneous cryptorchidism was seen in 5.3%. In dogs with abdominal cryptorchidism, right and left cryptorchid testes were equally affected. In animals with unilateral inguinal cryptorchidism, the rate of right testicle was 75% while the rate of left testicle was 25%. No case of bilateral inguinal cryptorchid was observed. The dog breeds with the highest number of cryptorchids were Poodle, Maltese Terrier, and Chihuahua.

Of the 1274 cats brought for castration, 42 (3.30%) were found to be cryptorchid (Table 1). The distribution of cryptorchid cats according to breeds was 29 (3.90%) in purebreds and 13 (2.45%) in crossbreds. Mean age (months±SD) was 21.29±21.42 and mean weight (kg±SD) was 4.26±0.77 (Table 2). The localizations of cryptorchids were subcutaneous (73.81%), inguinal (14.29%) and abdominal (11.9%), respectively (Table 1). In

animals with unilateral subcutaneous cryptorchid, 32.3% of the cryptorchid testicle was the right testicle and 45.2% was the left testicle. Bilateral subcutaneous cryptorchidism was seen in 22.6%. In animals with unilateral inguinal cryptorchidism, right and left testes were equally affected. In cats with unilateral abdominal cryptorchidism, the rate of right testicle involvement was 40% while the rate of left testicle involvement was 60%. No case of bilateral abdominal cryptorchid was observed. The cat breeds were British Shorthair, Van Cat, Chinchilla, and Domestic Shorthair.

Table 2. Distribution of the cryptorchidism among crossbred and purebred animals**.**

Species	Breed (Crossbred / Purebred)	Total Number	Number of Cryptorchids	Rates (%)
Cat	Crossbred	530	13	2.45
	Purebred	744	29	3.90
Dog	Crossbred	71	4	5.63
	Purebred	277	27	9.75

DISCUSSION

In veterinary medicine, cryptorchidism is a hereditary condition and one of the most common congenital defects seen in small animals. (Runge et al., 2024) The testes are the male reproductive organs responsible for sperm production and secretion of sex hormones. Normally, the testes descend from the abdominal cavity into the scrotum shortly after birth in cats and from day 10 (Gier and van Sluijs, 2010; Little, 2011; Griffin, 2020; Gradil and McCarthy, 2023) to day 35 **(**Pretzer, 2008**)** in dogs. If one or both testes are not present in the scrotum by 2 months of age, the animal is considered cryptorchid. It has been reported that testes are unlikely to descend into the scrotum after this age (Birchard and Nappier, 2008; Millard, 2018). However, in some literature, it is reported that the inguinal canal closes at approximately 6 months of age in dogs (Yates et al., 2003), while it closes at 7-8 months of age in cats (Little, 2011). It is stated that it is necessary to wait until the end of the inguinal canal closure period for the testes to descend into the scrotum (Little, 2011; Yates et al., 2003). Affected young animals usually do not show signs of disease unless an associated pathology such as torsion or neoplasia develops in the involved testicle. Because they do not show any clinical signs, the disease often goes unrecognized by the owners (Birchard and Nappier, 2008). On inspection and especially palpation of the scrotum, the animal is easily diagnosed as cryptorchid if one or both testes are absent from the scrotum (Birchard and Nappier, 2008; Villalobos-Gomez et al., 2023). During palpation, a sebaceous gland and lymph node in the scrotum may be perceived as a testicle. It is easily differentiated from the sebaceous gland and lymph node by the free movement of the testicle in the scrotum and the detection of an epididymis attached to the testicle (Miller et al., 2004; Spangenberg, 2021). In this study, cryptorchids were diagnosed in animals in which one or both testes were not in the scrotum by palpation examination of the scrotum of animals brought for castration.

Testicular descent from the abdominal cavity to the scrotum is regulated by both androgenic and non-androgenic factors and mediated by the gubernaculum, a gelatinous tissue of mesenchymal origin. The pathogenesis of defects in testicular descent is poorly understood. However, the physical size and/or growth rate of the epididymites and gubernacular may be involved in abnormal testicular descent. It is hypothesized that lack of regression of the cranial suspensory ligament may prevent overgrowth of the gubernacular and subsequent descent of the testes (Gradil and McCarthy, 2023). As a result of disruption in the descent of the testes to the scrotum, the testes are retained in the abdominal cavity, inguinal canal and subcutaneously after passing the inguinal canal without reaching the scrotum (Amann and Veeramachaneni, 2006; Birchard and Nappier, 2008; Millard, 2018; Griffin, 2020; Spangenberg, 2021). In determining the location of the involved testicle, palpation and ultrasonographic examination are important. Subcutaneously involved testicles can be easily detected by palpation and/or ultrasonographic examination (Miller et al., 2004). Abdominal retained testes may be difficult to detect by ultrasound because they may be smaller in size and in a wider range in location. However, ultrasound is usually more successful in locating testes retained in the inguinal canal (Spangenberg, 2021). In one study, it was reported that ultrasound was used with 96.6% success in the detection of abdominal cryptorchids and 100% success in the detection of inguinal cryptorchids and that it is a sensitive diagnostic technique for locating retained testicles in domestic animals (Felumlee et al., 2012). In our study, palpation and ultrasound examination were used together to locate the retained testes. In

particular, the location of the abdominal and inguinal retained testicle was determined by ultrasound examination and this was later confirmed during surgery. Thus, we suggest that ultrasound examination can be successfully used to localize retained testicles preoperatively.

Currently, it is accepted that cryptorchidism has many causes including genetic, epigenetic and environmental components. However, the role of environmental factors has not yet been established (Amann and Veeramachaneni, 2006). In addition, the presence of umbilical infections and failure to increase abdominal pressure as a result of late closure of the umbilical canal or inability of the testes to reach the scrotum due to adhesion caused by inflammation have been shown as predisposing factors (Romagnoli, 1991). We think that the cases of cryptorchidism in our study had a genetic origin. In addition, we did not find any predisposing factor such as umbilical hernia or infection in the dogs and cats diagnosed with cryptorchidism.

Because cryptorchid testicles usually have significantly higher temperatures than normal descending testicles, unilateral cryptorchids reduce semen quality or fail to produce normal sperm. A male with bilateral cryptorchids does not produce normal sperm and is infertile. Both unilateral and bilateral cryptorchid dogs produce testosterone, so most show sexual desire. In cryptorchid cats, the testes produce testosterone and the cats show typical secondary sex characteristics such as urine marking, aggressive behavior and urine odor (Spangenberg, 2021; Gradil and McCarthy, 2023). Abdominally retained testes move more freely than testes within the scrotum and are therefore more susceptible to testicular torsion. Furthermore, the incidence of testicular neoplasia has been found to be 13 times higher in abdominally retained testes than in descending testes (Birchard and Nappier, 2008). There is no proven treatment that causes the retained testicle to descend into the scrotum. It is considered unethical to attempt to treat an animal for reproductive purposes (Miller et al., 2004; Little, 2011). Since cryptorchidism is inherited in all the animals we diagnosed as cryptorchids in our study, we preferred treatment with castration to prevent the transmission of this defect to offspring and because retained testicles are prone to develop neoplasia or torsion (Little, 2011; Davidson, 2020; Griffin, 2020; Spangenberg, 2021; Villalobos-Gomez et al., 2023).

Previous studies have reported the incidence of cryptorchidism to be between 0.8% and 12.9% in dogs (Spangenberg, 2021; Runge et al., 2024) and between 1.3% and 6.2% in cats (Little, 2011; Villalobos-Gomez et al., 2023; Runge et al., 2024). Yates et al. (2003) found a 4% prevalence of cryptorchid in cats and dogs brought for neutering over a 54-month period. While the number of dogs with cryptorchids was 240 (6.8%), this number was 50 (1.3%) in cats (Yates et al., 2003). In a study in which 4924 dogs underwent ultrasound scanning, cryptorchid was found in 8.2% of dogs (Tannouz et al., 2019). In a study evaluating congenital anomalies in puppies, the incidence was found to be 2.6% (Ruble and Hird, 1993). In a previous study, cryptorchids were found in 72 (1.7%) of 4140 neutered cats (Little, 2011). In a 10-year period, 23 (1.7%) of 1345 cats admitted for orchiectomy were found to have cryptorchid (Millis et al., 1992). In a large-scale study in stray cats, the prevalence of cryptorchidism in cats was found to be 1.3% (Wallace and Levy, 2006) and 1.9% in a similar study (Scott et al., 2002). In our study conducted on 1274 cats and 384 dogs brought to our clinics for neutering, the prevalence of cryptorchidism was found to be 8.91% in dogs and 3.30% in cats. The prevalence of cryptorchidism in dogs was higher than in cats and this finding is similar to other studies. We think that the prevalence of cryptorchidism in cats and dogs varies greatly according to the number of animals and animal breeds in the studies.

Testes retained in cryptorchid animals are reported to be localized as 33% abdominal, 49% inguinal and 14% subcutaneous in cats (Richardson and Mullen, 1993) and 59.8% abdominal, 40.2% inguinal and subcutaneous in dogs (Yates et al., 2003). In a study in which testicles retained in the inguinal canal and abdomen were removed by inguinal canal surgery, it was found that 68.18% were retained in the abdominal and 31.82% in the inguinal canal in dogs and 75% in the abdominal and 25% in the inguinal canal in cats (Steckel, 2011). In studies conducted in cats, 48% (Scott et al., 2002), 70% (Yates et al., 2003) abdominal, 52% (Scott et al., 2002), 30% (Yates et al., 2003) inguinal and subcutaneous involvement was reported. In a study conducted to determine the prevalence of cryptorchidism in dogs, it was reported that 38.4% of the involved testes were located in the abdominal region and 61.6% in the inguinal region (Tannouz et al., 2019). In this study, it was determined that cryptorchid testes were located in 25.81% abdominal, 12.9% inguinal and 61.29% subcutaneous regions in dogs and 11.19% abdominal, 14.29% inguinal and 73.81%

subcutaneous regions in cats. It was determined that the testes retained in cats and dogs were mostly localized in the subcutaneous region.

It is reported in the literature that the testes are usually unilaterally involved in cryptorchid cats and dogs. In studies, the rate of unilateral cryptorchid in cats was reported to be 51.6% to 86% (Scott et al., 2002; Yates et al., 2003; Little, 2011; Steckel, 2011; Villalobos-Gomez et al., 2023) and in dogs it was reported to be 70% to 81.8% (Yates et al., 2003; Steckel, 2011; Tannouz et al., 2019). In the literature, it is stated that unilateral cryptorchid cases occur equally in the right and left testicle (Romagnoli, 1991; Scott et al., 2002; Little, 2011; Moon, 2014). It is suggested that right cryptorchidism is more common because of the longer distance that the right testicle has to travel to reach the scrotum due to the fact that the right testicle is located in the abdomen in the embryonic period in the right testicle is located in a more cranial position than the left testicle (Moon, 2014; Villalobos-Gomez et al., 2023). In previous studies, the rate of involvement of the unilateral right testicle was 57.9% (Villalobos-Gomez et al., 2023) and 52% (Yates et al., 2003) in cats. In dogs, the rate of involvement of the unilateral right testicle was 77.7% (David et al., 2023), 68.1% (Yates et al., 2003), 60.4% (Felumlee et al., 2012), 59.5% (Tannouz et al., 2019) and the rate of involvement of the left testicle was 66.6% (Runge et al., 2024). In the present study, the rate of involvement of the unilateral left testicle in cats was 57.14%, the rate of involvement of the right testicle was 42.86%, while the rates of involvement of the right and left testicles in dogs were found to be equal. While the equal rates of right and left testicular involvement in dogs are similar to many literatures (Romagnoli, 1991; Little, 2011; Moon, 2014), the higher rate of left testicular involvement in cats is consistent with the study by Yates et al. (2003).

The pathogenesis of cryptorchidism is not fully understood. It is emphasized that mostly small breeds and smaller animals within a breed are generally at high risk for cryptorchidism (Gradil and McCarthy, 2023). In a study by Tannouz et al. (2019) investigating the size and breed relationships of cryptorchidism in dogs, it was reported that 280 of 403 cryptorchid dogs were small-size, 62 were medium-size and 57 were large-size. In the same study, 377 of 403 dogs with cryptorchidism were purebred and 26 of them were crossbreds. In this study, cryptorchids were found in 7.21% of small-size, 15.79% of medium-size and 11.21% of large-size dogs brought for neutering. In addition, 16 of the 31 dogs with cryptorchid were small-sized, 3 were medium-sized and 12 were large-sized. Among the 31 dogs diagnosed with cryptorchid, the number of small-sized animals was high. This finding we obtained is in parallel with the studies (Tannouz et al., 2019; Gradil and McCarthy, 2023) emphasizing that cryptorchidism is more common in small-sized breeds.

In addition, it is emphasized that the incidence of cryptorchidism is more common in purebred animals than in crossbred animals (Gier and van Sluijs, 2010; Griffin, 2020). However, there are some breeds such as English Bulldog, Boxer, Chihuahua, Shetland Sheepdog, Chihuahua, Shetland Sheepdog, and Yorkshire Terrier which show a higher incidence and thus are at greater risk for the development of cryptorchidism. (Spangenberg, 2021) In studies, it has been reported to be common in dog breeds such as German shepherd dog, Yorkshire terrier, Boxer, Poodle, (Yates et al., 2003; Tannouz et al., 2019), and cat breeds such as Persian, Ragdoll, Siamese, domestic shorthair, (Villalobos-Gomez et al., 2023; Runge et al., 2024). In the present study, the proportion of purebred breed cryptorchid dogs among 348 dogs brought for castration was 9.75% and the proportion of crossbred cryptorchid dogs was 5.63%. Among 1274 cats brought for castration, the proportion of purebred breed cryptorchid cats was 3.90% and the proportion of crossbred cryptorchid cats was 2.45%. The higher incidence of cryptorchid cases in purebred breeds compared to crossbreds in our study can be explained by the high level of cryptorchidism as a result of the mating of animals with inbreeding, which is a more intensive form of pure breeding (Gradil and McCarthy, 2023). In our study, more cryptorchids were observed in Poodle, Maltese Terrier, Chihuahua dog breeds and cat breeds such as British Shorthair, Van Cat, Chinchilla, Domestic Shorthair.

CONCLUSION

In conclusion, cryptorchidism presents a significant health concern for both cats and dogs. This condition, characterized by the failure of one or both testicles to descend into the scrotum, can lead to various complications, including an increased risk of testicular cancer and potential fertility issues. Neutering is often recommended as the primary course of action for cryptorchid pets, involving the surgical removal of the retained testicle(s). This not only helps prevent future health issues but also

reduces the risk of passing on genetic predispositions.

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