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KOCATEPE VETERINARY JOURNAL 2025 18 (3) September

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RESEARCH ARTICLE

The Effect of Carob Aqueous Extract on Oxidative Stress, Proinflammatory Cytokines, Glucose and Lipid Concentrations in the Blood of Diabetic Rats

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ABSTRACT

Herbal treatments offer potential benefits in controlling blood glucose levels and preventing diabetes-related complications. This study was conducted to determine the effects of application of an aqueous extract of carob fruit prepared by ultrasonic-assisted extraction (UAE) method on oxidative stress, glycemic level, pro-inflammatory cytokines and lipid profile levels in the blood of rats induced experimental diabetes with streptozotocin-nicotinamide (STZ-NA) model. Forty male Wistar Albino (200-250 g live weight) animals used in the study were divided into four groups as normal control, diabetic control, normal group given 200 mg.kg^{-1} carob aqueous extract and diabetic group given 200 mg.kg^{-1} carob aqueous extract. At the end of the 21-day study, plasma glucose, hemoglobin A1C (HbA1c), insulin, homeostatic model assessment of insulin resistance (HOMA-IR), malondialdehyde (MDA), reduced glutathione (GSH), proinflammatory cytokines interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), total cholesterol, triglyceride, leptin and 25-hydroxy vitamin D levels were measured in blood samples taken from all animals. Treatment of carob aqueous extract did not cause a significant decrease in high glucose, HbA1c and HOMA-IR values caused by diabetes. The blood insulin levels were not affected by the treatments. Diabetes increased MDA, IL-6 and triglyceride levels ($p<0.05$), while treatment of carob aqueous extract to diabetic animals did not affect these parameters. However, treatment of carob aqueous extract to diabetic animals increased GSH levels ($p<0.05$). The treatments had no effect on IL-1β, TNF-α, total cholesterol and leptin levels. We concluded that the carob fruit aqueous extract treatment had no effect on controlling hyperglycemia.

Keywords: Carob aqueous extract, Cytokines, Diabetes, Dyslipidemia, Hyperglycemia, Oxidative stress

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Diyabetik Sıçanların Kanında Keçiboynuzu Sulu Ekstraktının Oksidatif Stres, Proinflamatuar Sitokinler, Glikoz ve Lipid Düzeylerine Etkisi

ÖZ

Bitkisel tedaviler kan glikoz seviyelerinin kontrol altına alınması ve diyabetle ilişkili komplikasyonların önlenmesinde potansiyel faydalar sunmaktadır. Bu çalışma; streptozotosin-nikotinamid (STZ-NA) modelle deneysel diyabet oluşturulan sıçanlara ultrasonik-destekli ekstraksiyon (UAE) yöntemiyle hazırlanmış keçiboynuzu meyvesi sulu ekstraktı uygulamasının kan oksidatif stres, glisemik düzey, proinflamatuar sitokin ve lipit profil düzeylerine etkilerinin belirlenmesi amacıyla yapıldı. Çalışmada 40 adet erkek Wistar Albino (200-250g canlı ağırlık) hayvan kullanıldı. Deneydeki hayvanlar normal kontrol, diyabetik kontrol, 200 mg.kg^{-1} keçiboynuzu ekstraktı verilen normal ve 200 mg.kg^{-1} keçiboynuzu ekstraktı verilen diyabetik grup olmak üzere dört gruba ayrıldı. Toplam 21 gün süren çalışmanın sonunda tüm hayvanlardan alınan kan örneklerinde; plazma glikoz, hemoglobin A1C (HbA1c), insülin, insülin direncinin homeostatik model değerlendirmesi (HOMA-IR), malondialdehid (MDA), indirgenmiş glutatyon (GSH), proinflamatuar sitokinler interlökin-1beta (IL-1β), tümör nekroz faktörü-alfa (TNF-α), interlökin-6 (IL-6), total kolesterol, trigliserit, leptin ve 25-hidroksi vitamin D düzeyleri ölçüldü. Keçiboynuzu ekstraktı uygulaması, diyabetin yol açtığı yüksek glikoz, HbA1c ve HOMA-IR değerlerinde önemli bir düşüşe neden olmadı. Uygulamaların kan insülin düzeylerine etkisi olmadı. Diyabet; MDA, IL-6 ve trigliserit düzeylerini artırırken ($p<0.05$), keçiboynuzu sulu ekstrakt uygulamasının bu değerlere etkisi gözlenmedi. Bununla birlikte, diyabetli hayvanlara keçiboynuzu sulu ekstraktı verilmesi, GSH düzeylerini yükseltti ($p<0.05$). Uygulamaların IL-1β, TNF-α, total kolesterol ve leptin düzeylerine etkisi bulunmadı. Sonuç olarak, keçiboynuzu meyvesi sulu ekstraktı uygulamasının hiperglisemiyi kontrol altına almada etkili olmadığı kanaatine varıldı.

Anahtar kelimeler: Dislipidemi, Diyabet, Hiperglisemi, Keçiboynuzu sulu ekstraktı, Oksidatif stres, Sitokinler

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INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder, is increasingly common around the world and a growing public health problem (Kottaisamy et al. 2021; Cloete 2022). The two main types that constitute the majority of diabetics are type 1 diabetes mellitus (T1DM) and T2DM, which have unique pathophysiological features. Interventions aimed at controlling hyperglycemia, which is the primary line of treatment for diabetic patients have recently brought new insights into the understanding of DM. Current treatments for lowering blood glucose levels are only temporarily effective. However, they can not completely prevent the development of DM and its complications. Also, another burden for patients is side effects of most antidiabetic drugs such as gastrointestinal symptoms, weight gain, edema, heart failure, impaired renal function, pancreatitis and genital infections (Cloete 2022). Thus, natural plant products with multidimensional modes of action and various biologically active secondary metabolites are of interest for the management and treatment of diabetic patients due to their significant therapeutic potential and better safety profile. In numerous studies, bioactive compounds from herbal products, such as polyphenols, flavonoids and alkaloids have been shown to have beneficial effects on the prevention and treatment of T2DM through their effects on mechanisms related to glucose tolerance (Behl et al. 2022; Rodriguez et al. 2022; Hazra et al. 2023; Riaz et al. 2024; Shrivastav et al. 2024). In previous studies, bioactive compounds from plants have been reported to control blood glucose levels through various mechanisms such as modulation of antioxidant enzymes (Ighodora et al. 2017), stimulation of insulin secretion (Uehara et al. 2023) or alpha-glucosidase inhibition (Kumar et al. 2011).

Carob (*Ceratonia siliqua L.)* is also among the plants that have an effect on the lowering blood glucose concentrations (Rtibi et al. 2017; Qasem et al. 2018). Applications of carob fruit may have an antidiabetic effect due to their bioactive compounds, which may have glucose-lowering properties (Brassesco et al. 2021; Moumou et al. 2023). Previous studies have also shown that applications of carob fruit may have antioxidant, lipid-lowering and anti-inflammatory effects due to their bioactive compounds (Moumou et al. 2023; Laaraj et al. 2024). Because oxidative stress and low-grade inflammation are associated with diabetes, alleviating oxidative stress and preventing the elevation of chronic inflammation markers may benefit against diabetes-related complications (Dludla et al. 2023). Rtibi et al. (2017) evaluated the reduction of intestinal glucose absorption by carob aqueous extract, confirming that mainly polyphenols and flavonoids, as well as the high content of fiber and complex carbohydrates in carob pods may have hypoglycemic effects. We hypothesized that if carob aqueous extract has a hypoglycemic effect in diabetes, the oral administration of carob aqueous extract to experimental diabetic rats may alleviate oxidative stress, dyslipidemia and proinflammatory response caused by diabetes. Therefore, this study aimed to determine the effects of carob aqueous extract on glucose and insulin concentrations, proinflammatory cytokines, lipid peroxidation, glutathione levels, and lipid parameters in rats with experimental diabetes induced by streptozotocin (STZ)-nicotinamide (NA).

MATERIALS and METHODS

Plant Material and Preparation of Extract

The mature carob plant fruits were obtained fresh from a seller of medicinal herbs whose products can be used for scientific study purposes. This plant material (pulp and seed) was dried and ground into powder with a mesh size of 80-100 in a high-speed rotor mill (ZM 200, Retsch GmbH, Haan, Germany). Aqueous extract of carob fruit powder, consisting of 90% pulp and 10% seed by weight, was prepared by an ultrasonic bath (425 W, 40 kHz) (Daihan WUC-D06H, Wonju, South Korea) using 100 g carob powder and 500 ml ultrapure water, for 30 min at 30 °C (Roseiro et al. 2013). The extract was then filtered through white band filter paper with 6 micron pore size. The extract was given to the experimental animals immediately after preparation. The ultrasound-assisted extraction method used in this study can effectively preserve the antioxidant and anti-inflammatory properties of phytochemicals (Demesa et al. 2024).

Animals

A total of 40 male Wistar Albino rats (200-250g) used in the study were obtained from Afyon Kocatepe University Experimental Animal Application and Research Center. All rats were kept at 22±2 °C, 50±10% humidity, 12 hours:12 hours light/dark cycle and in a regularly ventilated environment. Rats were fed with standard pellet diet ad libitum and had free access to water. The study protocol was approved by Afyon Kocatepe University Animal Experiments Ethics Committee (protocol no: 49533702/150).

Induction of Diabetes

Diabetes was induced in animals with the streptozotocin-nicotinamide (STZ-NA) model according to the method of El-Beih et al. (2019) with some modifications. A single intraperitoneal (i.p.) injection of 80 $mg.kg^{-1}$ freshly dissolved STZ (AB352315, abcr GmbH, Karlsruhe, Germany) in ice-cold citrate buffer (0.1 M, pH 4.5) was administered to rats fasted from previous day, 30 min after i.p. administration of 100 $mg.kg^{-1}$ NA (Thermo Scientific Chemicals, Leicestershire, UK). 10% glucose was administered to the rats 6–24 h after STZ-

administration. Hyperglycemia was confirmed at 72 h and on day 10 after injection by Contour Plus Blood Glucometer (Ascensia Diabetes Care, Basel, Switzerland). Ten days after STZ-NA injection, almost all rats had non-fasting blood glucose levels >300 $mg.dl^{-1}$ and were considered diabetic.

Experimental Design

In the experiment, animals were divided into four groups: control group, diabetic control group (diabetes group), normal rats given carob aqueous extract (carob group) and diabetic group given carob aqueous extract (carob+diabetes group). 200 $mg.kg^{-1}$ carob aqueous extract was given to the carob and carob+diabetes groups and 0.5 ml physiological saline was given to other groups orally as a single dose for 21 days. As ensuring proper administration to animals by oral gavage, the extract was diluted to three times its volume with physiological saline and administered daily to animals.

Group 1: Control group: Normal+0.5 ml physiological saline

Group 2: Diabetes group: Diabetic+0.5 ml physiological şaline

Group 3: Carob group: Normal+200 mg.kg-1 carob aqueous extract

Group 4: Carob+Diabetes group: Diabetic+200 mg.kg-1 carob aqueous extract

Blood Collection for Parameter Determination

The next day of the 21-day period, blood were collected from rats fasted from previous day under ketamine (87 mg.kg-1) and xylazine (13 mg.kg-1) anesthesia. Blood was taken from the hearts of rats using syringes and placed in tubes containing dipotassium ethylenediaminetetraacetic acid (K2EDTA) and heparinised plasma. Blood samples collected in K2EDTA tubes were used to measure hemoglobin A1C (HbA1c) levels. Blood samples taken in plasma tubes were immediately separated in a Heraeus Megafuge 8 R refrigerated centrifuge (Thermo Fisher Scientific, MA, USA) at 4 °C and 4000 rpm for 10 minutes. The separated plasma was collected in Eppendorf tubes. Glucose, total cholesterol and triglyceride levels were determined by Cobas c-702, HbA1c levels by Cobas c-502 and 25-hydroxyvitamin D levels by Cobas e-801 analysers (Roche Diagnostics, Basel, Switzerland) in the Afyonkarahisar Health Sciences University Health Application and Research Center Medical Biochemistry Laboratory. Immediately afterwards, cytokines; interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), insulin and leptin hormone levels were determined by Enzyme-Linked Immunosorbent Assay (ELISA) method (BT LAB, China). Determination of plasma malondialdehyde (MDA) and reduced glutathione (GSH) levels were performed using the methods described by Draper and Hadley (1990) and Beutler et al. (1963), respectively, and the results were obtained on a Shimadzu 1601 UV-vis spectrophotometer (Tokyo, Japan). Calculation of homeostatic model assessment of insulin resistance (HOMA-IR) was performed using the formula; fasting glucose (mg.dl-1) x fasting insulin (mIU.l-1) / 405 (Matthews et al. 1985).

Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) 20.0 (IBM Corp., Armonk, NY, USA). Because the data obtained from the study did not show normal distribution, Kruskal Wallis test was used to determine whether there was a difference between the groups, and Dunn's test was used for pairwise comparisons when there was a difference. Data were expressed as mean±standard deviation. A p value of <0.05 was considered statistically significant.

RESULTS

As a result of the loss of animals during the experiment, the experiment was completed with 7, 8, 7 and 7 animals in the Control, Diabetes, Carob and Carob+Diabetes groups, respectively.

The effects of treatment of carob aqueous extract to experimentally diabetic animals on plasma fasting blood glucose, HbA1c, plasma insulin and HOMA-IR levels are shown in Table 1. Fasting blood glucose, HbA1c and HOMA-IR values in the Diabetes and Carob+Diabetes groups increased significantly compared to the rats in the Control group ($p<0.05$). There was no improvement in these parameters in diabetic rats with the treatment of carob aqueous extract ($p>0.05$). In insulin levels, there was not significant difference between the groups.

The effects of treatment of carob aqueous extract to experimentally diabetic animals on plasma MDA, GSH, IL-1β, TNF-α and IL-6 cytokine levels are shown in Table 2. While MDA levels increased significantly in the diabetic groups compared to the non diabetic groups ($p<0.05$), the oral administration of carob aqueous extract to diabetic animals did not decrease this increase ($p>0.05$). Plasma GSH levels increased significantly with treatment of carob aqueous extract in diabetic rats compared to the other groups ($p<0.05$). There was not significant difference in IL-1β levels between the diabetic groups and the Control group ($p>0.05$). However, the IL-1β levels of the Diabetes and Carob+Diabetes groups were found to be significantly higher than in the Carob group receiving carob aqueous extract ($p<0.05$). There was no statistically difference in TNF-α levels between the groups. While there was a significant increase in IL-6 levels in the Diabetes and Carob+Diabetes groups compared to the Control and Carob groups ($p<0.05$), the oral administration of carob aqueous extract to diabetic rats did not improve these levels ($p>0.05$).

Table 3 shows the effects of oral carob aqueous extract administration to diabetic animals on plasma total cholesterol, triglyceride, leptin and plasma 25-hydroxy

vitamin D levels. Diabetes significantly increased triglyceride levels ($p<0.05$), while the oral administration of carob aqueous extract to diabetic rats did not improve this increase ($p>0.05$). Total cholesterol and leptin levels did not differ significantly between the groups. Vitamin D levels decreased significantly in the Carob+Diabetes group compared to the Control and Carob groups ($p<0.05$).

Table 1. The effects of carob aqueus extract on plasma fasting blood glucose, HbA1c, insulin and HOMA-IR levels in diabetic animals (mean±SD).

	Control (n=7)	Diabetes (n=8)	Carob (n=7)	Carob+Diabetes (n=7)
FBG (mg.dl^{-1})	167±35^{b}	364±46^{a}	190±26^{b}	370±98^{a}
HbA1c (%)	3.88±0.14^{b}	7.70±0.45^{a}	3.92±0.10^{b}	7.98±0.55^{a}
Insulin (mIU.l^{-1})	2.34±0.29	2.62±0.45	2.11±0.27	2.65±0.65
HOMA-IR	**0.98±0.24^{b}**	**2.34±0.42^{a}**	**0.98±0.11^{b}**	**2.55±0.46^{a}**

FBG: Fasting blood glucose, HbA1c: Hemoglobin A1C, HOMA-IR: Homeostasis model assessment of insulin resistance, SD: standart deviation.
^{a,b}:The difference between groups with different letters on the same line is statistically significant ($p<0.05$).

Table 2. The effects of carob aqueus extract on plasma oxidative stress, proinflammatory cytokine parameters in diabetic animals (mean±SD).

	Control (n=7)	Diabetes (n=8)	Carob (n=7)	Carob+Diabetes (n=7)
MDA (nmol.ml^{-1})	5.96±1.40^{b}	9.37±0.83^{a}	4.84±1.11^{b}	8.72±1.30^{a}
GSH (mg.dl^{-1})	3.38±0.61^{b}	4.48±0.97^{b}	4.80±1.15^{b}	15.29±4.93^{a}
IL-1β (ng.ml^{-1})	4.94±0.42^{ab}	6.23±1.01^{a}	3.91±0.44^{b}	6.41±2.22^{a}
TNF-α (ng.l^{-1})	109±18	158±51	112±23	135±44
IL-6 (ng.l^{-1})	1.43±0.32^{b}	3.03±0.68^{a}	1.37±0.37^{b}	3.02±1.23^{a}

MDA: Malondialdehyde, GSH: Reduced Glutathione, IL-1β: Interleukin-1beta, TNF-α: Tumor necrosis factor-alpha, IL-6: Interleukin-6, SD: standart deviation.
^{a,b}:The difference between groups with different letters on the same line is statistically significant ($p<0.05$).

Table 3. The effects of carob aqueus extract on plasma total cholesterol, triglyceride, leptin and 25-hydroxy vitamin D levels in diabetic animals (mean±SD).

	Control (n=7)	Diabetes (n=8)	Carob (n=7)	Carob+Diabetes (n=7)
Total Cholesterol (mg.dl^{-1})	52±10	53±8	45±6	51±6
Triglyceride (mg.dl^{-1})	51±9^{b}	85±12^{a}	46±7^{b}	83±14^{a}
Leptin (ng.ml^{-1})	0.99±0.44	1.19±0.48	1.03±0.54	1.15±0.36
Vitamin D (µg.l^{-1})	13.08±2.06^{a}	11.11±1.32^{ab}	14.05±2.47^{a}	9.31±2.11^{b}

^{a,b}: The difference between groups with different letters on the same line is statistically significant ($p<0.05$).

DISCUSSION

The experimental diabetes animal models play an important role for studying the pathophysiological mechanisms and treatment methods of DM (Akinlade et al. 2021). Due to the high genetic similarity between mice, rats and humans (especially in terms of pancreatic structure and function), these animals are among the most widely used animal models in DM disease research (Kottaisamy et al. 2021). Carob pulp and seeds are reported to have natural biologically active compounds with beneficial biological actions

against diabetic conditions (Rtibi et al. 2017; Brassesco et al. 2021; Laaraj et al. 2023). Therefore, this study was conducted to evaluate the efficacy of an aqueous extract from carob pulp and seeds on oxidative stress, insulin and glucose levels. Since Rtibi et al. (2017) showed that 200 mg.kg-1 carob aqueous extract has hypoglycemic effects in the alloxan-induced diabetic rats, this dose level of carob aqueous extract was used in this study. Streptozotocin (STZ) is widely used to induce experimental diabetes in rats and mice because it produces a diabetes model with changes similar to those in human diabetes (Eleazu et al. 2013; Akinlade et al. 2021). Because nicotinamide has been shown to be protective against STZ damage to beta cells (Kuchmerovska et al. 2012), STZ and nicotinamide were used together in this study.

Although Rtibi et al. (2017) showed the hypoglycemic effect of 200 mg.kg-1 carob aqueous extract at 2 weeks in alloxan-induced diabetes, we did not observe the hypoglycemic effect of 200 mg.kg-1 carob aqueous extract at 3 weeks in STZ+NA-induced diabetes. In the present study, the blood glucose levels in the diabetic animals were significantly higher than in the non-diabetic animals ($p<0.05$), which is consistent with the report that blood glucose levels increased in rats with experimental diabetes induced with the STZ-NA model (Szkudelski 2012). In the present study, the treatment of carob aqueous extract did not decrease the blood glucose levels in the diabetic animals. This result is not consistent with the report that aqueous extract of immature carob pulp showed control of glucose concentration in alloxan-induced diabetic rats (Rtibi et al. 2017). The different results between studies may be due to the use of different chemicals (alloxan or streptozotocin) to induce experimental diabetes (Rtibi et al. 2017) or different extraction methods and dose levels of the carob (Qasem et al. 2018). Alloxan and streptozotocin as a diabetogenic agent are the most commonly used chemicals to induce an experimental diabetes (Ighodaro et al. 2017). However, they have different effects in terms of their ability to cause insulin deficiency and type 1 diabetes, and type 2 diabetes with insulin resistance (Singh et al. 2024). Alloxan-induced diabetes produces a situation similar to human T1DM (Federiuk et al. 2004). However, co-administration of STZ and NA causes insulin resistance and induces partial depletion of pancreatic insulin, inducing a condition reflecting T2DM (Nakamura et al. 2006). In this study, while fasting blood glucose increased in STZ-nicotinamide-treated animals compared to non-diabetic animals, there was no significant difference in insulin levels of the groups, indicating that T2DM developed in diabetic animals. In fact, Szkudelski (2012) reported that co-administration of NA with STZ may protect from insulin deficiency caused by only STZ administration. The lack of glucose-lowering effect of carob aqueous extract in diabetic animals in the present study is also not consistent with the report that the high dose of carob methanolic extract reduced glucose levels in STZ-NA-induced rats (Qasem et al. 2018). These results suggest that extraction type (aqueous or methanolic) may also be one of the factors influencing the results of glucose levels with the carob extract treatment to diabetic animals.

In the present study, HOMA-IR levels, an indicator of insulin resistance (Mughni et al. 2023), increased significantly in the diabetes and carob+diabetes groups induced with STZ-NA compared to animals in the control group. This result showed that insulin resistance developed in animals administered STZ-NA. However, the carob aqueous extract treatment did not affect HOMA-IR values in the diabetic animals. This result may also suggest that the administration of carob aqueous extract to the diabetic animals did not improve the beta cell function.

In this study, increasing MDA levels, a product of lipid peroxidation, in the diabetic rats is consistent with the report that MDA, as a marker of oxidative stress, increased in diabetes (Fatani et al. 2016). The oral carob aqueous extract administration to diabetic rats did not decrease the MDA levels. This result indicates that the aqueous extract of carob fruit was ineffective in reducing oxidative stress in diabetes. However, this is not consistent with the report that biologically active natural compounds such as flavonoids found in plants reduce the level of lipid peroxidation in blood plasma and tissues (Aloud et al. 2018). The increase of GSH levels in the carob+diabetes group in this study may support the result of Laaraj et al. (2024) reported that the carob fruit has valuable antioxidant properties. This shows that oral administration of carob aqueous extract to diabetic rats may increase GSH synthesis and enhance cellular defense against oxidative stress. This result supported the report of Nzekwe et al. (2020) found that a plant extract containing phenolic compounds increased GSH levels in the liver of diabetic rats, while GSH levels were not affected in diabetic control. In addition, polyphenols can show antioxidant effects by stimulating antioxidant enzymes and producing a synergistic effect with other antioxidant compounds (Lv et al. 2021). Therefore, the components in the carob aqueous extract used in this study may have stimulated GSH synthesis against oxidative stress caused by diabetes or reduced its use in diabetic rats through such mechanisms. Further studies to determine the underlying mechanisms of the increase in GSH levels with carob aqueous extract in diabetes are needed.

Considering the fact that diabetic patients usually exhibit irregular blood glucose levels, which leads to long-term hyperglycemia and low-grade tissue cytokine production (King 2008), in this study, IL-6 levels were observed to increase in the diabetes and carob+diabetes groups. IL-6, a pivotal cytokine in innate immunity (Ridker et al. 2021), was observed to be elevated in the blood of diabetic patients (Kado et al. 1999). Thus, the determination of blood IL-6 concentrations in diabetic patients is considered a valuable indicator of the low-grade inflammation of

diabetes at the systemic level (Pellegrini et al. 2024). However, the carob aqueous extract in the diabetic rats did not affect the blood IL-6 levels. This result may suggest that the carob aqueous extract can not alleviate the low-grade inflammation caused by diabetes at the systemic level.

In addition to hyperglycemia in DM, lipid metabolism disorders are often accompanied (Kane et al. 2021). In the study, diabetes increased blood triglyceride levels while total cholesterol levels were unchanged in the blood. This result is consistent with the report that total cholesterol levels can remain normal while blood triglyceride levels are elevated in diabetic dyslipidemia (Sugden and Holness 2011). The administration of carob aqueous extract to the diabetic rats did not affect triglyceride and total cholesterol levels. These results are not consistent with the results reported by Macho-Gonzalez et al. (2019). The discrepancy between studies may be due to differences in the diets and doses of carob fruit or extracts used in the studies. In fact, Nemet et al. (2022) reported that the efficacy of carob extracts in preventing diabetes-related dyslipidaemia varies depending on the extraction method and dose. In this study, levels of the leptin hormone released from the fat tissue and provided information about the size of the fat tissue (Schwartz and Porte 2005), did not differ significantly between the groups. This suggests that the change in blood lipid levels caused by diabetes did not affect fat tissue. In fact, the decrease in body fat stores in insulin-deficient diabetes causes a significant reduction in plasma leptin levels (German et al. 2010). In this study, plasma insulin and leptin levels in diabetic animals is consistent with the report of Soliman (2001) showed that there was a positive correlation in serum leptin and insulin levels of diabetic animals.

In the study, diabetes decreased the plazma 25-hydroxy vitamin D levels. This result is consistent with the results of Aly et al. (2016) reported that diabetes decreased the plasma vitamin D levels. Although the carob fruit is rich source of vitamin D (Laaraj et al. 2023), the oral administration of carob aqueous extract to diabetic animals did not improve the vitamin D concentration. This result may indicate that the use of carob fruit aqueous extract does not have a beneficial effect on vitamin D deficiency caused by diabetes.

CONCLUSION

The results of the study indicate that oral administration of the carob aqueous extract to experimentally diabetic rats does not have the ability to restore glycemic balance in DM. Also, the oral administration of carob aqueous extract to the rats with experimental diabetes did not exert activity to alleviate diabetes-induced oxidative stress, dyslipidemia, and pro-inflammatory response. However, the application method, extraction method, and dose factors may affect the effectiveness of carob fruit on diabetes. The results of the study will allow new research on medicinal plants to examine in depth the selection of chemical substances used to induce experimental diabetes, the metabolic pathways that cause diabetes, the underlying mechanisms of these metabolic pathways, and the evaluation of therapeutic interventions.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: The data of this study were taken of MTA's Phd thesis. AE contributed to the study as a supervisor.

Ethical approval: This study was carried out at Afyon Kocatepe University Reserch Animals Application Center. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, Afyon Kocatepe University (AKUHADYEK, Ref No: 49533702/150, Date: 11/26/2021)

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RESEARCH ARTICLE

Evaluation of Tumor Necrosis Factor-Alfa, Cholesterol, BHBA, NEFA and Acetylcholinesterase Levels in Cows with Ketosis

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ABSTRACT

In this study serum beta-hydroxybutyric acid, glucose, total protein, triglyceride, cholesterol, non-esterified fatty acids, acetylcholinesterase and tumor necrosis factor-α were investigated in cows with ketosis. The study material consisted of 10 control and 10 group with ketosis, total 20 holstein dairy cows of 10 control and 10 groups with ketosis, a total of 20 holstein dairy cows aged between 3 and 7 years. In this study serum concentration was measured for nonesterified fatty acids, beta-hydroxybutyric acid, aspartate aminotransferase, total protein, cholesterol, triglyceride, acetylcholinesterase and tumor necrosis factor-α in the healthy group and cows with ketosis. Serum glucose, triglyceride, nonesterified fatty acids, acetylcholinesterase and tumor necrosis factor-α did not differ significantly between the two groups. Aspartate aminotransferase and beta-hydroxybutyric acid increased, while cholesterol and total protein concentration decreased in ketotic cows compared with healthy cows. Consequently, acetylcholinesterase and tumor necrosis factor-α concentrations may prove beneficial biochemical findings in cows with ketosis.
Keywords: Acetylcholinesterase, Beta-hydroxybutyric acid, Cow, Ketosis, Tumor necrosis factor-alfa

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Ketozisli İneklerde Tümör Nekrosis Faktör-Alfa, Kolesterol, BHBA, NEFA ve Asetilkolinesteraz Düzeylerinin Değerlendirilmesi

ÖZ

Yapılan bu çalışmada ketozisli ineklerde serum beta hidroksi bütirik asit, glikoz, total protein, trigliserit, kolesterol, nonesterifie fatty acids, asetilkolinesteraz ve tümör nekroz faktör-α düzeyleri araştırıldı. Yapılan çalışmanın materyalini 3-7 yaş aralığında 10 adet ketozisli ve 10 adet sağlıklı olmak üzere toplam 20 adet holstein ırkı inek oluşturdu. Ketozis hastalığı bulunan ve sağlıklı gruptaki ineklerde serum nonesterifie fatty acids, beta hidroksi bütirik asit, aspartat aminotransferaz, total protein, glikoz, kolesterol, trigliserit, asetilkolinesteraz ve tümör nekroz faktör-α düzeyleri ölçüldü. Ketozisli ineklerde asetilkolinesteraz, glikoz, tümör nekroz faktör-α, nonesterifie fatty acids, trigliserit düzeyleri sağlıklı kontrol grubuna göre istatistiksel olarak önemli çıkmadığı, ketozisli ineklerde total protein ve kolesterol düzeyleri düşük çıkarken, beta hidroksi bütirik asit ve aspartat aminotransferaz seviyeleri sağlıklı ineklere göre yükseldiği belirlendi. Sonuç olarak ketozisli ineklerde asetilkolinesteraz ve tümör nekroz faktör-α düzeylerinin araştırılması hastalığın biyokimyasal bulgularına faydalı olabileceği kanaatine varıldı.
Anahtar kelimeler: Asetilkolinesteraz, Beta hidroksi bütirik asit, İnek, Ketozis, Tümör nekrosis faktör-alfa

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INTRODUCTION

Ketosis is a metabolic disorder in dairy cows, characterized by elevated levels of ketone bodies in the blood, urine and milk during early lactation. The source of ketone bodies can be either exogenous through dietary intake or endogenous from adipose tissue mobilisation (Zhang & Ametaj, 2020). During the transition period, a cow experiences a relative difference in energy demand between the day before parturition and the first day postpartum. Following these rapid metabolic changes, energy requirements double within a single day. Consequently, meeting these increased energy demands solely through feed intake becomes challenging after calving (Darckley, 2011). As a result, the animal compensates for the energy deficit by utilising body reserves. This metabolic adaptation leads to physiological changes in the organism, including an increase in somatotropin hormone levels and the activation of lipolysis in adipose tissue, which is mediated by norepinephrine signalling (Grum et al., 1996). Consequently, the rise in non-esterified fatty acids (NEFA) facilitates the conversion of these lipids into ketone bodies in the liver to meet the increased energy demand (Darckley, 2011).

The mobilisation of non-esterified fatty acids (NEFA) subsequently triggers glucose utilisation to compensate for the energy deficit. However, in response to glucose conservation, the organism converts NEFAs into acetyl-CoA and subsequently into ketone bodies (Zhang & Ametaj, 2020). In cows, the brain is entirely dependent on glucose. In ruminants, when ketone body concentrations increase, peripheral tissue utilization remains limited, leading to an accumulation of ketone bodies in the blood. Consequently, ketone bodies also appear in milk and urine. The elevation of ketone bodies in the blood results in decreased blood pH, reduced feed and water intake and suppressed immune system (Reece, 2008). In ketosis, fat depots in the body undergo breakdown due to glucose deficiency, resulting in elevated NEFA levels (Goff & Horst, 1997; Grummer, 1995).

Cholinesterase exists in two different forms in mammals: acetylcholinesterase and pseudocholinesterase. Acetylcholinesterase is produced in the liver, and its levels in the blood have been reported to decrease in liver diseases and hepatic degeneration (Stojevic et al., 2005). Pseudocholinesterase is primarily synthesized in the liver and found in serum, though its physiological function remains unclear (Kaplay, 1976). In humans, pseudocholinesterase is directly associated with insulin resistance, serum lipid profiles, and obesity (Iwasaki et al., 2007).

Tumor necrosis factor-alpha (TNF-α) is produced in response to various stimuli, including tumors, normal cells, bacteria, viruses, parasites, and cytokines, and exhibits biological activity in different cell types and tissues. It is crucial in systemic and local tumor-related effects, including cachexia and neoplastic tissue degradation (Çömez, 2006). Several studies have reported that cows with ketosis exhibit higher TNF-α, serum amyloid A, interleukin-6, and lactate concentrations during the prepartum period (4–8 weeks before calving) compared to healthy cows (Oetzel, 2007; Tehrani et al., 2011; Zhang & Ametaj, 2020).

This study aims to evaluate the relationship between β-hydroxybutyrate (BHBA), NEFA, TNF-α, acetylcholinesterase (AchE), cholesterol, glucose, total protein, and triglyceride levels in both ketotic and healthy cows.

MATERIALS and METHODS

Animal Material and Study Groups

This study was conducted on a dairy farm with a total of 70 lactating cows located in Bigadiç, Balıkesir Province. A total of 20 Holstein dairy cows, aged between 3 and 7 years and in the first four weeks of lactation, were included in the research group. Among these, 10 cows were diagnosed with clinical ketosis, while 10 healthy cows were assigned to the control group. All animals in the study and control groups were housed under the same feeding, management, and environmental conditions within the early lactation group of the farm. The cows included in the study were selected based on their metabolic health status, with an effort to ensure similarity in age averages and lactation stages (between 1 and 4 weeks postpartum). A detailed clinical examination was performed on all ketotic and healthy cows.

To prevent the inclusion of cows with secondary ketosis, animals diagnosed with metabolic disorders commonly observed in early lactation, such as milk fever, mastitis, abomasal displacement, metritis, retained placenta (RPT), or retained fetal membranes (retentio secundinarum), were excluded from the study. The study was ethically approved by the Animal Experiments Local Ethics Committee of Balıkesir University (Decision Number: 2021/8-5).

Diagnosis of Ketotic and Healthy Cows

Ketotic cows were identified based on a decreased body condition score (BCS) of more than 0.5 within the first four weeks postpartum. The milk yield of these cows was monitored, and those exhibiting a sudden drop in milk production and loss of appetite underwent clinical examination. Urinary ketone bodies were assessed using urine dipstick tests and blood sample collection to measure β-hydroxybutyrate (BHBA) levels. Cows with BHBA levels exceeding 1.5 mmol/L and positive ketonuria were diagnosed with ketosis and included in the study.

Healthy cows were selected based on their feed intake, stable milk production, and body condition score reductions of no more than 0.5. These cows underwent a comprehensive clinical examination to confirm the absence of any metabolic or systemic diseases. Following clinical assessments, blood BHBA levels were measured, and cows with BHBA concentrations below 1.0 mmol/L were classified as healthy and included in the control group.

Sample Collection

Blood samples were collected from the jugular vein following clinical examinations using sterile, single-use needles and 10 mL vacuum tubes. Samples for NEFA analysis were obtained before morning feeding, whereas samples for BHBA analysis were collected four hours after feeding. After collection, blood samples were left to stand briefly before being centrifuged at 5,000 rpm for 5 minutes to obtain serum. Three aliquots from each serum sample were transferred into Eppendorf tubes and stored at -20°C until analysis.

Laboratory Analyses

Serum samples were analysed in a specialised biochemical laboratory using an automated Randox analyser. The levels of TNF-α and AchE were measured using ELISA (Enzyme-Linked Immunosorbent Assay) kits (Bovine TNF-α, AchE SunRed ELISA Kit, Cat. No: E90440, Eastbiopharm, China) (Table 1).

Biochemical parameters were analysed using a Randox Daytona model automated analyser (United Kingdom). The biochemical parameters assessed in this study included BHBA, aspartate aminotransferase (AST), total cholesterol, glucose, NEFA, total protein, and triglycerides. The respective methodologies for these parameters were as follows: BHBA (Cat. No: RB1007) – Enzymatic kinetic method, AST (Cat. No: AS3804) – UV method, Total cholesterol (Cat. No: CH3810) – Enzymatic endpoint method, Glucose (Cat. No: GL3815) – Colorimetric method, NEFA (Cat. No: FA115) – Colorimetric method, Total protein (Cat. No: TP38669) – Biuret reagent endpoint method, Triglycerides (Cat. No: TR3823) – Lipase/GPO-PAP method. (Table 2)

Statistical analysis

Statistical analyses were performed using the SPSS 20 software package for Windows. The relationships between serum biochemical parameters were evaluated using an independent samples t-test, and a p-value of<0.05 was considered statistically significant.

RESULTS

The clinical parameters, including body temperature, heart rate, and respiratory rate, of the ketotic cows included in the study were within normal ranges. However, clinical signs observed in ketotic cows included anorexia, reduced milk yield, depression, constipation, teeth grinding, firm faecal consistency, reluctance to consume concentrate feed, reduced feed intake, acetone odour in the breath in some cases, and loss of body condition.

Biochemical analyses of serum samples revealed that although TNF-α and AchE levels were elevated in ketotic cows compared to healthy cows, the differences were not statistically significant (Table 1).

Table 1. AchE and TNF-α levels in ketotic and healthy cows.

Parameters	Healthy Cows (n=10)	Ketotic Cows (n=10)	p Value
TNF-α (µg/ml)	31.50±17.47	33.76±12.58	NS
AchE (ng/ml)	6.33±5.73	7.06±3.67	NS

NS- Not Significant

Biochemical analyses of serum samples revealed that BHBA levels were significantly higher in ketotic cows than healthy cows ($p<0.001$). Although serum glucose levels were lower in ketotic cows, the difference was insignificant compared to the control group. (Table 2)

The study also showed that serum AST levels were significantly higher in ketotic cows compared to the control group ($p<0.05$). Additionally, ketotic cows serum cholesterol levels were significantly lower than healthy cows ($p<0.01$). However, there was no significant difference in NEFA and triglyceride levels between the ketotic and healthy groups. (Table 2)

Table 2. Biochemical parameters in ketotic and healthy cows.

Parameters	Healthy Cows (n=10)	Ketotic Cows (n=10)	p Value
BHBA mmol/L	0.77±0.41	2.54±0.61	***
Glucose mg/dL	35.3±11.61	28.20±11.42	NS
Cholesterol mg/dL	61.80±13.51	44.80±8.57	**
Triglycerides mg/dL	12.10±7.63	11.50±5.21	NS
NEFA mmol/L	0.98±0.78	0.88±0.25	NS
Total protein g/dL	6.87±0.50	5.68±0.75	***
AST U/L	109.70±8.87	167.20±72.34	*

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS- Not Significant

DISCUSSION

Ketosis in dairy cows predisposes them to secondary diseases such as abomasal displacement, mastitis, metritis, and infertility (Civelek, 2011; Çatık, 2015; Radostits et al., 2006; Sevinç & Başoğlu, 2011). The diagnosis of ketosis is based on clinical and laboratory findings, including elevated BHBA levels in the blood and ketonuria detected using urine test strips. Clinical signs of ketosis in cows include anorexia, reduced milk yield, depression, decreased feed intake, reluctance to consume concentrate feed, increased roughage consumption, dry feces, reduced rumen motility, acetone odor in the breath, and body condition loss (Herdt, 2000; Herdt, 2005; Oetzel, 2007). In this study, cows were carefully examined, and those with secondary ketosis were excluded. Only cows in the first four weeks postpartum that exhibited primary ketosis symptoms were included. The clinical findings observed in ketotic cows in this study were consistent with previous reports, including anorexia, decreased milk production, depression, constipation, teeth grinding, firm faecal consistency, reluctance to consume concentrate feed, reduced feed intake, acetone odour in breath in some cows, and body condition loss.

Liver diseases, subclinical and clinical ketosis, hepatobiliary disorders, acute and chronic hepatitis, liver damage, hepatic lipidosis, biliary diseases, and muscle tissue injuries can lead to increased AST enzyme levels in animals (Civelek, 2011; Sevinç & Başoğlu, 2011; Steen, 2001; Zhang et al., 2018). Increased AST levels have been reported due to hepatic lipidosis (Bogin et al., 1988; Cebra et al., 1997), and elevated AST and GGT levels have been associated with liver damage (Civelek, 2011; Sevinç & Başoğlu, 2011; Steen, 2001). Several studies have found elevated AST, GGT, ALP, and ALT levels in clinically ketotic cows (Zhang et al., 2018), as well as increased AST enzyme levels in subclinical and clinical ketosis cases (Li et al., 2016; Cao et al., 2017). The findings of this study align with these previous reports, as AST levels in ketotic cows were significantly higher than those in healthy cows (Table 2).

It has been reported that total protein levels decrease in cows with hepatic lipidosis (Radostits et al., 2006; Turgut, 2000) and serum albumin levels decrease due to the utilization of body reserves in ketotic cows (Austin & Wilde, 1985). Some studies suggest that reduced urea levels in postpartum cows may be associated with decreased protein anabolism due to hepatic fat infiltration (Elitok et al., 2006). However, others have found that albumin and BUN levels remain within normal ranges in subclinical and clinical ketosis cases (Akgül et al., 2018). Additionally, no significant differences in total protein levels between healthy, subclinical, and clinical ketosis cows have been reported (Çatık, 2015; Li et al., 2016). Similarly, although total protein levels were slightly lower in ketotic cows compared to healthy cows in this study the difference was not statistically significant (Table 2).

In primary ketosis, decreased blood glucose levels lead to mobilising fat reserves. High-yielding dairy cows manage their negative energy balance primarily through glucose metabolism, followed by NEFA and ketone body utilization (Lean et al., 1992; Reynolds et al., 2003; Turgut, 2000). During early lactation, insulin production declines, reducing glucose utilization in fat and muscle tissues, necessitating alternative energy sources. As a result, body fat is mobilized, increasing NEFA levels (Drackley, 1999). Low postpartum blood glucose levels have been associated with negative energy balance or ketosis (LeBlanc, 2010). Several studies have reported increased BHBA levels coincide with decreased glucose concentrations in ketotic cows (Andre et al., 1987). However, blood glucose levels are considered less relevant than BHBA levels for ketosis diagnosis. Studies have found decreased glucose levels in cows with hepatic lipidosis and ketosis (Gilbert et al., 1998; Katoh, 2002; LeBlanc, 2010). Both subclinical and clinical ketosis cases show lower glucose levels than healthy cows (Li et al., 2016). However, some studies found no significant difference in glucose levels between clinically ketotic and healthy cows (Akgül et al., 2018; Çatık, 2015). Although glucose levels were lower in ketotic cows, the

difference was not statistically significant compared to healthy cows (Table 2).

Ketone bodies are intermediates of fat oxidation, with BHBA being a key diagnostic marker for ketosis due to its relative stability compared to acetoacetate and acetone (Herdt, 2005; Duffield, 2000; Ospina et al., 2010b). Studies have classified BHBA levels between 1.2–1.4 mmol/L as subclinical ketosis (Duffield, 2000; Walsh et al., 2007) and have used BHBA levels≥1.4 mmol/L to diagnose clinical ketosis. Several studies have found significantly higher BHBA levels in clinical ketosis cows compared to subclinical and healthy cows (Çatık, 2015; Sun et al., 2015; Xia et al., 2012; Shen et al., 2020). In agreement with these findings, this study also confirmed that BHBA levels were significantly higher in ketotic cows than healthy ones (Table 2).

Cows mobilize body fat during early lactation to compensate for energy deficits, leading to increased NEFA levels in circulation and accumulation in various tissues, including the liver (Ingvartsen, 2006; Oetzel, 2007). Excessive hepatic fat accumulation impairs liver function, contributing to hyperketonemia. Studies have reported that postpartum NEFA levels range between 0.70–1.0 mEq/L, with values above 0.7 mEq/L often associated with ketosis (Chapinal et al., 2011; LeBlanc, 2010; Ospina et al., 2010b). Several studies have reported increased NEFA levels in ketotic cows (Li et al., 2016; Cao et al., 2017; Sun et al., 2015; Xia et al., 2012). However, some studies found no significant difference between NEFA levels in subclinical and clinical ketosis cases (Çatık, 2015; Akgül et al., 2018). Similarly, no significant difference was observed in NEFA levels between ketotic and healthy cows (Table 2).

Serum cholesterol levels are reduced in chronic liver failure, cirrhosis, diabetes mellitus, hepatic lipidosis, and conditions involving hepatocellular damage (Turgut, 2000; Quiroz-Rocha et al., 2009; Nakagawa & Katoh, 1998). While some studies have reported elevated cholesterol levels in ketotic cows (Simonov & Vlizlo, 2015), others have found decreased cholesterol levels in clinical ketosis (Nakagawa & Katoh, 1998; Li et al., 2016). In agreement with the latter, this study found that cholesterol levels were significantly lower in ketotic cows than in healthy cows (Table 2).

Triglycerides are synthesized from dietary fats in the gastrointestinal tract and other liver lipids. Severe hepatic lipidosis impairs lipoprotein (VLDL) formation and fat mobilisation, leading to reduced triglyceride levels (Turgut, 2000; Katoh, 2002; Kennerman, 2011). Studies have found that triglyceride levels are lower in ketosis cases, particularly in early postpartum cows (Kessler et al., 2014). However, some reports found no significant difference in triglyceride levels between ketosis and healthy cows (Çatık, 2015; Li et al., 2016). Consistent with these findings, this study found no significant difference in triglyceride levels between ketotic and healthy cows (Table 2).

TNF-α, a key inflammatory cytokine, has been associated with metabolic disturbances in ketosis. Some studies found elevated TNF-α levels in ketotic cows (El-Deep & El-Bahr, 2017; Zhang et al., 2018), while others reported no significant difference (Brodzki et al., 2021). In this study, TNF-α levels were slightly higher in ketotic cows but not statistically significant (Table 1).

CONCLUSION

In conclusion, this study contributed to the biochemical diagnosis of ketosis by determining the levels of beta-hydroxybutyric acid (BHBA), non-esterified fatty acids (NEFA), aspartate aminotransferase (AST), tumor necrosis factor-alpha (TNF-α), acetylcholinesterase (AchE), glucose, total protein, triglycerides, and cholesterol in both ketotic and healthy cows. Given the limited research on the relationship between cholinesterase enzymes and TNF-α in ketotic cows, we believe further detailed studies are necessary to understand these associations better.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: YY and İA contributed to the article idea, design and execution the study. YY collected datas. YY and İA analyzed data. All outhors contributed to the critical revision of the manuscript and have read and approved the final version.

Ethical approval: This study was carried out at Balıkesir University Reserch Animals Application Center. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, Balıkesir University (BAUNHADYEK, Ref No: 2021/8-5, Tarih: 30/09/2021).

Explanation: We have presented as a (oral, poster, abstract vs.) at the In Oral Presentation, Recognition and Appreciation of Research Contribution to Avrasya 9TH International Conference on Applied Sciences November 24-26 (2023) Tbilisi.

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RESEARCH ARTICLE

Spermatological and Biochemical Examination of New Zealand Rabbit Semen After Thawing

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ABSTRACT

In this study, the impact of caffeic acid on the quality of frozen-thawed rabbit semen was evaluated, with trehalose as a positive control. Four male and one female rabbit were used. Semen samples were obtained twice weekly over a four-week period using an artificial vagina. The collected ejaculates were pooled and allocated into four experimental groups, each diluted with a basic extender containing 6% DMSO: (1) supplemented with 25 µM caffeic acid, (2) 50 µM caffeic acid, (3) 50 mM trehalose (positive control), and (4) no additive (control). Following dilution, semen was loaded into straws and equilibrated at 4°C for one hour, then cryopreserved in liquid nitrogen vapor and stored in liquid nitrogen. Post-thaw analysis showed that the highest sperm motility was recorded in the group containing 50 mM trehalose (46.25±1.25%), which was significantly higher than the control group (35.63±1.99%)($p<0.05$). Evaluation of sperm plasma membrane integrity using SYBR-14/PI staining revealed significantly higher values in the supplement groups: 47.11±0.96% (25µM caffeic acid), 49.05±0.85% (50µM caffeic acid), and 49.79±1.04% (50mM trehalose), compared to the control (39.01±1.21%) ($p<0.05$). Similarly, acrosomal integrity assessed via FITC-PNA staining was highest in the trehalose group (48.70±1.03%), with a significant difference observed relative to the control (41.48±0.80%). Additionally, better results were achieved in the trehalose group compared to the control in terms of total antioxidant status and total oxidant levels ($p<0.05$). As a consequence, it was determined that the inclusion of caffeic acid in the semen extender at higher concentrations, enhanced protective effects on spermatological parameters during cryopreservation.

Key Words: Floresan boyama, Kafeik asit, Kriyoprezervasyon, Oksidatif stres, Tavşan sperması

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Yeni Zelanda Tavşanı Spermasının Çözdürme Sonrası Spermatolojik ve Biyokimyasal Yönden İncelenmesi

ÖZ

Bu çalışmada, dondurulup-çözdürülen tavşan spermasının kalitesi üzerinde kafeik asidin etkisi pozitif kontrol olarak trehaloz kullanılarak değerlendirilmiştir. Dört erkek ve bir dişi tavşan kullanılmıştır. Sperma örnekleri dört haftalık bir süre boyunca haftada iki kez suni vajen kullanılarak toplanmıştır. Toplanan ejakülatlar birleştirilerek dört deney grubuna ayrılmış ve gruplar %6 DMSO içeren temel sulandırıcıya: (1) 25 µM kafeik asit, (2) 50 µM kafeik asit, (3) 50 mM trehaloz (pozitif kontrol) ve (4) katkı maddesi içermeyen (control) eklenerek oluşturulmuştur. Sulandırmanın ardından sperma örnekleri, payetlere aspire edilmiş ve 4°C'de bir saat ekilibrasyon uygulanmıştır, ardından sıvı nitrojen buharında dondurulmuş ve sıvı nitrojen içinde saklanmıştır. Çözüm sonu analizde, en yüksek sperma motilitesinin 50 mM trehaloz içeren grupta (%46,25±1,25) olduğu, bunun kontrol grubu ile (%35,63±1,99) karşılaştırılmasında anlamlı derecede yüksek olduğu görülmüştür ($p<0,05$). Sperm plazma membran bütünlüğünün SYBR-14/PI boyama yöntemi kullanılarak değerlendirilmesinde, katkı gruplarında kontrol grubuna (%39,01±1,21) kıyasla anlamlı derecede daha yüksek sonuçlar ortaya konuldu: %47,11±0,96 (25 µM kafeik asit), %49,05±0,85 (50 µM kafeik asit) ve %49,79±1,04 (50 mM trehaloz) ($p<0,05$). Benzer şekilde, FITC-PNA boyama yöntemiyle yapılan akrozomal bütünlük değerlendirmesinde trehaloz grubunda en yüksek değere ulaşıldı (%48,70±1,03) ve kontrol grubuna göre önemli fark gözlendi (%41,48±0,80). Ek olarak, toplam antioksidan durumu ve toplam oksidan seviyelerinde trehaloz grubunda kontrole kıyasla daha iyi sonuçlara ulaşılmıştır ($p<0,05$). Sonuç olarak, sperma sulandırıcısına daha yüksek konsantrasyonlarda eklenen kafeik asitin, kriyoprezervasyon sırasında spermatolojik parametreler üzerine koruyucu etkiyi arttırdı belirlendi.

Anahtar Kelimeler: Floresan boyama, Kafeik asit, Kriyoprezervasyon, Oksidatif stres, Tavşan sperması

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INTRODUCTION

Sperm cryopreservation is an important biotechnological method that enables long-term preservation of germ cells (Martínez-Páramo et al. 2009). Establishing and maintaining cryopreserved sperm banks is a valuable approach for preserving the genetic diversity of rabbit breeds at risk of extinction and not only increase semen export but also provides a valuable opportunity for biomedical studies. Indeed, the rabbit is an important laboratory animal that is considered a model organism for the study of various processes related to human reproduction (Foote and Carney 2000). Artificial insemination is a widely used assisted reproductive technology that supports both reproductive efficiency and genetic improvement programs. For the procedure to be successful, the semen must be cooled and frozen while preserving sperm quality or viability (López-Gatius et al. 2005). In rabbits, sperm viability after cooling and freezing is not at the desired levels, and current methods do not provide sufficient success (Sarıozkan et al. 2012; Rusco et al. 2022).

Oxidative stress (OS) induces damage to spermatozoa primarily through lipid peroxidation, resulting in a decline in seminal quality parameters. Elevated levels of reactive oxygen species (ROS) contribute to the deterioration of sperm parameters through both lipid peroxidation and protein oxidation (Takalani et al. 2023). It is widely accepted that the disruption of the balance between ROS level and the antioxidant defense system is an important factor causing infertility. Scavenging enzymes within the cytoplasm and antioxidants present in seminal plasma are integral components of the antioxidant defense system, mitigating the deleterious effects of ROS (Agarwal et al. 2014).

Dimethyl sulfoxide (DMSO) is among the most commonly utilized cryoprotective agents in the field of cryopreservation, primarily because of its high protective efficiency (Mandumpal et al. 2011). Its effectiveness has led to widespread use across various biological samples. Research shows that oxygen atoms in the hydrophilic sulfoxide portion of DMSO can form hydrogen bonds with hydrogen atoms in the hydroxyl groups of water. This damages the hydrogen bonds between water molecules at sub-zero temperatures and prevents the formation of ice crystals. Consequently, DMSO plays a pivotal role in reducing cellular damage during cryopreservation procedures (Iwatani et al. 2006; Notman et al. 2006).

Trehalose, a disaccharide not naturally abundant in large vertebrates, can be hydrolyzed into glucose molecules by the enzyme trehalase, which is present in the human small intestine and kidney (Jain and Roy, 2009). Trehalose is considered a powerful bioprotectant that plays a role in many biological processes. One of its main functions is to protect cells from damage under adverse conditions such as freezing and dehydration. In addition, trehalose protects proteins from oxidative damage, increasing the stability of therapeutic proteins (Richards et al. 2002; Dovgan et al. 2017). As a naturally occurring disaccharide found in a wide range of animals, and microorganisms, trehalose exhibits cryoprotective properties by shielding cells from damage induced by ice crystal formation during cryopreservation (Ntai et al. 2018). Moreover, it is considered one of the most effective cryoprotectants for stem cells, offering protection to cells and cellular proteins against oxidative stress-induced free radical damage (Benaroudj and Goldberg, 2001). Trehalose has demonstrated protective effects in the cryopreservation of mesenchymal stem cells (Budgude et al. 2021) and spermatogonial stem cells (Valdivia et al. 2021).

Caffeic acid (CA) and caffeic acid phenethyl ester (CAPE) are naturally occurring compounds present in a variety of foods. In recent years, they have garnered significant attention for their potential health benefits, particularly their protective effects against both natural and chemical toxins. These protective properties position CA and CAPE as promising candidates for novel therapeutic strategies and the development of functional foods (Ehtiati et al. 2023). CAPE has been shown to exhibit antioxidant activity by reducing levels of free oxygen radicals and enhancing the function of endogenous antioxidant enzymes, thereby contributing to the maintenance of the cellular antioxidant defense system (Kus et al. 2004; Ogeturk et al. 2005). Furthermore, Namula et al. (2018) reported that the supplementation of boar semen with 100 μM caffeic acid significantly improved sperm parameters, including motility, viability, and plasma membrane integrity. Furthermore, Soleimanzadeh et al. (2020) reported that the same concentration of caffeic acid reduced malondialdehyde levels, indicating a protective effect against lipid peroxidation and suggesting the potential for enhancing post-thaw semen quality in water buffaloes.

In the present study, the antioxidant properties of caffeic acid, when added to a semen extender, were investigated for their potential to reduce oxidative damage and improve spermatological parameters. Trehalose was used as a positive control, and the protective effects of various doses of caffeic acid were evaluated through biochemical and spermatological analyses.

MATERIALS and METHODS

Animals

In this study, four healthy male rabbits and one female New Zealand rabbit, aged between 24 and 36 weeks and with confirmed fertility status, were used. Animals were housed under standard breeding conditions at the Experimental Animal Application and Research Center of Aksaray University.

Study Groups

Tris-based stock solutions were prepared by modifying the solutions used by Öztürk and Ataman (2018) and Zhu et al. (2017). It contains Tris (0.25 M/100 mL), citric acid (88 mM/100 mL), glucose (47 mM/100 mL), sucrose (50 mM/100 mL), streptomycin (1 mg/mL) and penicillin G (100 IU/mL). The stock solution was prepared in sufficient quantity for the entire study and stored at +4 °C. On the day of sperm collection, 20% fresh egg yolk was added to the stock solution (basic extender). To remove the sediment from the egg yolk, the mixture was centrifuged in 15 mL Falcon tubes at 6000 rpm for 15 min. Finally, 6% dimethyl sulfoxide (DMSO) was added, and the mixture was used as an extender.

After the artificial vagina was filled with 44–45 °C water to 2/3, graduated sperm collection tubes were placed. Ejaculates were collected twice week over a four-week via the artificial vagina. The ejaculates were evaluated according to the criteria of Boiti et al. (2005). Samples with a sperm density of ≥200–600 × 106 spermatozoa/mL and motility of ≥60% were considered suitable for the dilution process. Before the dilution process, the sperm taken from the animals were mixed and divided into four equal parts. The sperm were kept in a water bath until the mixing process was complete.

Experimental groups

Group 1: Basic extender + 6% DMSO + caffeic acid (25 μM)

Group 2: Basic extender + 6% DMSO + caffeic acid (50 μM)

Group 4: Basic extender + 6% DMSO + trehalose (Positive Control) (50 mM)

Group 5: Basic extender + 6% DMSO (Control)

After the dilution process, 0.25 mL straws were aspirated from each group at room temperature. The straws were subsequently equilibrated for 1 h at +4 °C. The samples were frozen in liquid nitrogen vapor (–110 °C to –120 °C) for 15 min, 5 cm above the liquid nitrogen. The frozen straws were stored in liquid nitrogen (–196 °C).

Spermatological and Biochemical Measurements

The straws were thawed in a water bath at 38 ±2 °C for 25–30 s. Spermatozoa motility was assessed by examining five microscopic fields in a drop of the sperm sample placed between a slide and coverslip, using a phase contrast microscope equipped with a heated stage maintained at 37 °C at 40× magnification. The average motility value across the fields was recorded as the percentage motility rate. Spermatozoa density was determined by counting a diluted sperm sample prepared with Hayem solution, using the hemocytometric method on a Thoma slide across a total of ten counting chambers (Soylu et al. 2023).

Spermatological Parameters

Membrane integrity

Sperm viability was evaluated using SYBR-14/PI dual fluorescent staining. For this procedure, 30 μL of diluted semen was combined with 6 μL of SYBR-14 and 2.5 μL of propidium iodide (PI) in an Eppendorf tube, followed by incubation in a water bath at 37 °C for 15 min in the dark. The staining process was terminated by the addition of Hancock solution. Subsequently, 3 μL of the stained sample was placed on a microscope slide, covered with a coverslip, and examined under a fluorescence microscope at 200× magnification. Spermatozoa with intact plasma membranes (viable cells) exhibited bright green fluorescence in the head region, while those with compromised membranes (non-viable cells) displayed red fluorescence. (Garner and Johnson, 1995).

Acrosome Integrity

FITC-PNA/PI fluorescence staining was applied for acrosome evaluation. Fitch staining was performed under a fluorescence microscope in the dark to examine acrosome damage in the sperm. Then, 10 μL of lectin and 2.5 μL of PI dye were added to 60 μL of sperm, which was allowed to equilibrate for 15 min in a water bath set at 37 °C. Following termination of the staining process with Hancock solution, acrosome damage was assessed by evaluating a minimum of 200 spermatozoa at 200× magnification. Spermatozoa exhibiting green-stained acrosomes were classified as having damaged acrosomes, whereas those with unstained acrosomes were considered intact (Nagy et al. 2003).

Biochemical Measurements

Preparation of Sperm Samples

To isolate spermatozoa, diluted samples were centrifuged at 800 rpm for 15 min at +4 °C, with the process repeated three times. After each centrifugation, the resulting pellet was collected, washed with phosphate-buffered saline (PBS), and subsequently resuspended in PBS to a final volume of 0.5 mL. For homogenization, the sperm suspension was transferred into a 2 mL chamber placed in an ice bath and sonicated under cold conditions for 8 s. Each sonication cycle was followed by a 30-s interval on ice, and the procedure was repeated five times in total.

Total antioxidant capacity (TAS) and serum total oxidant (TOS)

The total antioxidant capacity (TAS) and serum total oxidant level (TOS) in sperm were studied spectrophotometrically from serum samples with commercial kits in accordance with kit procedures (Erel 2005; Erel, 2014).

The total antioxidant capacity (TAS) of sperm samples was evaluated using the method proposed by Erel (2004). This technique is recognized for its high

precision and rapid application across a range of biological fluids. The assay is based on a colorimetric reaction in which the sample develops a dark blue-green hue, and this color shift is quantified via absorbance measurements. Calibration was conducted at 660 nm using a stable antioxidant reference solution. Total oxidant status (TOS) in semen samples was measured according to the protocol described by Erel (2005). Under acidic conditions, oxidants present in the sample oxidize a ferrous ion–chelator complex into its ferric form. The resulting change in color intensity, determined spectrophotometrically, directly correlates with the cumulative concentration of oxidant molecules. The outcomes were expressed as micromoles of hydrogen peroxide equivalent per liter (μmol H_2O_2/L), measured at 530 nm.

Statistical Analyses

All raw data obtained from the analyses were expressed as group means ±standard error (SE). Differences among groups were evaluated using one-way analysis of variance (ANOVA). For variables exhibiting statistically significant differences, Duncan's multiple range test was employed as a post-hoc analysis. All statistical evaluations were conducted at a 5% significance level ($p<0.05$) using the SPSS 21.0 statistical software package.

RESULTS

Table 1 presents a comparison of sperm membrane integrity, acrosome integrity, and motility percentages among the experimental groups. The results indicate that the administration of caffeic acid (25 μM and 50 μM) and trehalose (50 mM) led to a significant improvement in all the assessed parameters compared with those of the control group ($p<0.05$). Notably, the 50 mM trehalose group presented the highest values for membrane integrity (49.79 ±1.04%), acrosome integrity (48.70 ±1.03%), and motility (46.25 ±1.25%). Table 2 compares the total antioxidant capacity (TAC) and total oxidant level (TOS) values of the different experimental groups. As a result, 50 μM caffeic acid (16.46 ±2.41) and 50 mM trehalose (13.75 ±2.59) caused significant changes in the TOS values ($p<0.05$). The highest TAC value was determined in the 50 mM trehalose group (1.86 ± 0.52 mmol/L) and differed significantly from the control group (0.55±0.11) ($p<0.05$).

Table 1. Motility, membrane, and acrosome integrity results

Groups	Membrane integrity (%)	Acrosome integrity (%)	Motility (%)
Control	39.01±1.21^{b}	41.48±0.80^{c}	35.63±1.99^{b}
Caffeic Acid 25 μM	47.11 ±0.96^{a}	44.20 ±0.77^{b}	41.00 ±2.27^{a}
Caffeic Acid 50 μM	49.05 ±0.85^{a}	46.84 ±1.05^{ab}	43.13 ±1.62^{a}
Trehalose 50 mM	49.79 ±1.04^{a}	48.70 ±1.03^{a}	46.25 ±1.25^{a}
p	*	*	*

*p<0.05

Table 2. Total Antioxidant Capacity (TAC) and Total Oxidant Level (TOS) Values

Groups	TAC (mmol/L)	TOS (μmol/L)
Control	0.55 ±0.11^{b}	32.34 ±3.59^{a}
Caffeic Acid 25 μM	1.08 ±0.28^{ab}	22.26 ±5.32^{ab}
Caffeic Acid 50 μM	1.70 ±0.50^{ab}	16.46 ±2.41^{b}
rehalose 50 mM	1.86 ±0.52^{a}	13.75 ±2.59^{b}
p	*	*

*p<0.05

DISCUSSION

Caffeic acid phenethyl ester (CAPE) is one of the main components of propolis. It is known to have antioxidant and anti-inflammatory properties. CAPE provides protection against oxidative stress by preventing the formation of free radicals within the cell and suppressing inflammatory pathways (Akyol et al. 2015). Compared with those in the control group, the sperm motility, total motile sperm count, and total functional sperm fraction (TFSF) in male rabbits treated with propolis were significantly greater (Khaled et al. 2016). Furthermore, cotreatment with propolis markedly increased sperm concentration. These results are consistent with previous findings indicating that propolis supplementation enhances sperm quality and fertility in male rats exposed to chlorpyrifos-induced toxicity (El-Mazoudy et al. 2011). Similarly, Yousef and Salama (2009) reported that propolis reduced aluminum chloride-induced reproductive toxicity by improving the overall quality of sperm cells. The protective effect of propolis is attributed to its antioxidant capacity, particularly its ability to neutralize free radicals, thereby stabilizing the sperm membrane and reducing oxidative damage, including the formation of thiobarbituric acid-reactive substances (Russo et al. 2006). In alignment with these findings, Namula et al. (2018) demonstrated that the addition of 100 μM caffeic acid to semen extenders improved post-thaw motility, viability, and membrane integrity in cryopreserved boar spermatozoa, although no significant effect was observed on acrosome integrity. Further advancements in boar semen research have shown that supplementation with 210 μmol/L CAPE yielded the highest levels of total and progressive motility, as well as optimal functional integrity, including mitochondrial activity, plasma membrane stability, and acrosomal integrity. Notably, despite the presence of H2O2, the inclusion of 210 μmol/L CAPE significantly enhanced sperm parameters and higher levels of CAT, SOD and GSH-Px enzymes strengthened the defense against oxidative stress (Lan et al. 2022). The findings of our study align with those reported in previous studies. An examination of motility parameters indicated that the addition of caffeic acid to the extender, especially at higher doses, resulted in improved outcomes and had a statistically significant positive effect compared with those of the control group ($p<0.05$). Moreover, evaluations of membrane and acrosome integrity demonstrated that the groups treated with caffeic acid exhibited a protective effect, with a significant difference compared with the control group ($p<0.05$).

Rosato and Iaffaldano (2013) examined the cytotoxic effects of commonly used permeable cryoprotectants in the cryopreservation of rabbit sperm, taking into account both concentration and incubation time. Their study revealed that the addition of bovine serum albumin (BSA), in combination with optimal concentrations of sucrose or trehalose, to the cryopreservation medium significantly enhanced post-thaw sperm survival in both slow freezing and vitrification protocols. More recently, Petričáková et al. (2024) reported that supplementation of the freezing extender with 100 mM trehalose markedly improved total and progressive motility, as well as plasma membrane and acrosome integrity in cryopreserved rooster spermatozoa ($p<0.05$). This group presented the highest post-thawing quality compared with the other trehalose concentrations. Although the in vivo fertilization rate did not differ significantly, a relatively high value (23.21%) was obtained in the 100 mM group. These results indicate that trehalose may be an effective additive against cryoprotective damage. When the effects of trehalose on acrosome and membrane integrity were evaluated, 100 mM trehalose was shown to have a significant protective effect on both parameters compared with those of the control group ($p<0.05$). While acrosome integrity remained high at similar levels in applications between 75–150 mM, this effect was observed to decrease with 200 mM trehalose. These findings indicate that 100 mM trehalose represents the optimal concentration for preserving both acrosome and plasma membrane integrity (Zhu et al. 2017). In the present study, groups supplemented with additives demonstrated a statistically significant enhancement in subjective motility compared to the control group ($p<0.05$). Among the additives tested, trehalose conferred the most pronounced protective effect on both membrane and acrosome integrity, with all supplemented groups showing significant differences from the control ($p<0.05$).

Cryopreservation-induced cellular damage is primarily attributed to several factors, including osmotic imbalance, cold shock, intracellular ice formation, elevated production of reactive oxygen species (ROS) (Khan et al. 2021), and the impairment of endogenous antioxidant defense mechanisms (Bilodeau et al. 2000). Osmotic stress arises from fluid and solute exchange across the sperm plasma membrane, leading to fluctuations in cellular volume and increased ROS production during the freezing process (Ball 2008). The inclusion of antioxidant compounds and protective agents in cryopreservation media has been shown to attenuate ROS-related damage, thereby enhancing sperm viability and functionality post-thaw (Mehdipour et al. 2022). Propolis supplementation has been reported to reduce thiobarbituric acid-reactive substances (TBARS) and increase glutathione S-transferase (GST) activity in rabbit seminal plasma, underscoring its antioxidative potential (Yousef et al. 2010). Similarly, trehalose supplementation has been shown to upregulate antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD), as well as total antioxidant capacity (T-AOC), while simultaneously reducing ROS and malondialdehyde (MDA) levels in post-thaw spermatozoa (Zhu et al. 2017). The application of 100 mM trehalose in extenders has proven particularly effective, resulting in

improved motility, mitochondrial membrane potential, and preservation of both acrosomal and plasma membrane integrity. Thus, the inclusion of trehalose in freezing extenders offers significant benefits for the rabbit breeding industry. In the present study, supplementation with 50 μM caffeic acid and 50 mM trehalose significantly reduced oxidative stress, as indicated by lower TOS values compared to the control group ($p<0.05$), whereas 25 μM caffeic acid did not yield a significant effect ($p>0.05$). With respect to TAS, only the 50 mM trehalose group exhibited a significant increase in total antioxidant capacity ($p<0.05$). These findings indicate that trehalose is more effective than caffeic acid in enhancing antioxidant defense mechanisms and that the efficacy of caffeic acid is dose-dependent.

CONCLUSION

In conclusion, the incorporation of caffeic acid into the freezing extender demonstrated a dose-dependent cryoprotective effect on rabbit spermatozoa, with higher concentrations yielding greater preservation of membrane integrity. However, trehalose, employed as a positive control, provided superior protection relative to caffeic acid. These findings suggest that future research should investigate the application of higher concentrations of caffeic acid to further optimize its cryoprotective potential.

Conflict of interest: The authors confirm that they have no conflicts of interest that could have influenced the outcomes of this research.

Authors' Contributions: CÖ and MED contributed to the conception of the project, as well as the design and execution of the study. CÖ, MED, and NHA were involved in data collection and contributed to data analysis. CÖ and MED drafted and wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Ethical approval: This study was conducted at the Experimental Animal Application and Research Center of Aksaray University. The research protocol was approved by the Aksaray University Local Ethics Committee for Animal Experiments (Approval date: 11/2021; Decision No: 2021-7/14).

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RESEARCH ARTICLE

Evaluation of Postbiotics Belonging to Industrial Kefir-Derived Exopolysaccharide-Producing *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus paracasei* as Anti-Candidal Biofilm Agents

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ABSTRACT

Candidal biofilm is a concern in both medical and industrial fields. This study aims to reveal the effect of postbiotics on the transcriptional level of biofilm-associated genes of *Candida albicans* in exopolysaccharide-producing industrial kefir-derived isolates and to determine their biofilm prevention and treatment properties. In the study, the exopolysaccharide production capacity of lactic acid bacteria obtained from kefir samples of eight different brands from supermarkets was determined. The transcriptional effect of postbiotics of exopolysaccharide-producing isolates on *C. albicans* biofilm-related genes was carried out via reverse transcription-polymerase chain reaction. The biofilm prevention and biofilm treatment effects of postbiotics were determined in abiotic media (polystyrene microplate). The minimum inhibitory concentration (MIC) values of *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus paracasei* (exopolysaccharide-producing isolates) postbiotics were determined as 12.5% and 25%, respectively. While 2xMIC and above doses of *L. rhamnosus* and *L. paracasei* postbiotics were effective in prevention, MIC and above doses were effective in biofilm treatment. *als1, als3,* and *bcr* genes were down-regulated at 2xMIC and MIC doses of *L. rhamnosus* postbiotic. At exposure to a 2xMIC dose of *L. paracasei* postbiotic, all genes examined were down-regulated. On average, 0.03, 0.15, and 0.79-fold downregulation of *als1*, *als3* and *bcr* genes was determined at 24 hours at 2xMIC dose of *L. rhamnosus* postbiotic; 0.31, 0.55, and 0.77-fold downregulation was determined at 24 hours at MIC doses. A 0.39, 0.05, 0.91, and 0.04-fold downregulation of *L. paracasei* postbiotic at 2xMIC dose at 24 hours was determined in *als1*, *als3*, *bcr* and *hwp* genes, respectively. In conclusion, the discovery of new approaches that can prevent or treat biofilms could stimulate the emergence of novel bio-control agents.

Keywords: Biofilm, Candida, Exopolysaccharide, Kefir, *Lacticaseibacillus*, Postbiotics

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Endüstriyel Kefir Orijinli, Ekzopolisakkarit Üreten *Lacticaseibacillus rhamnosus* ve *Lacticaseibacillus paracasei'ye* ait Postbiyotiklerin Anti-Candidal Biyofilm Ajanları Olarak Değerlendirilmesi

ÖZ

Candidal biyofilm hem tıbbi hem de endüstriyel alanlarda ciddi sorunlara neden olmaktadır. Bu çalışmada, ekzopolisakkarit üreten endüstriyel kefir izolatlarına ait postbiyotiklerinin *Candida albicans*'ın biyofilm ilgili genlerinin transkripsiyon düzeyine etkisinin ortaya konulması ve biyofilm önleme ve tedavi özelliklerinin belirlenmesi amaçlanmıştır. Çalışmada, süpermarketlerden alınan sekiz farklı markanın kefir örneklerinden elde edilen laktik asit bakterilerinin ekzopolisakkarit üretim kapasitesi belirlenmiştir. Ekzopolisakkarit üreten izolatların postbiyotiklerinin *C. albicans* biyofilmle ilişkili genleri üzerindeki transkripsiyonel etkisi Ters Transkripsiyon-Polimeraz Zincir Reaksiyonu ile gerçekleştirilmiştir. Postbiyotiklerin biyofilm önleme ve biyofilm tedavi etkileri abiyotik ortamda (polistiren mikroplak) belirlenmiştir. *Lacticaseibacillus rhamnosus* ve *Lacticaseibacillus paracasei* (ekzopolisakkarit üreten izolatlar) postbiyotiklerinin minimum inhibitör konsantrasyon (MİK) değerleri sırasıyla %12.5 ve %25 olarak belirlenmiştir. *L. rhamnosus* ve *L. paracasei* postbiyotiklerinin 2xMİK ve üzeri dozları önlemede etkili iken, MİK ve üzeri dozları biyofilm tedavisinde etkili bulunmuştur. *L. rhamnosus* postbiyotiği 2xMİK ve MİK dozlarında als1, als3 ve bcr genlerini aşağı regüle etmiştir. *L. paracasei* postbiyotiği 2xMİK dozuna maruziyette incelenen tüm genleri aşağı regüle etmiştir. *L. rhamnosus* postbiyotiğinin 2xMİK dozunda 24. saatte *als1*, *als3* ve *bcr* genlerinde ortalama olarak sırasıyla 0.03, 0.15 ve 0.79 kat aşağı regülasyon belirlenirken; MİK dozlarında 0.31, 0.55 ve 0.77 kat aşağı regülasyon belirlenmiştir. *L. paracasei* postbiyotiğinde 24. saatte 2xMİK dozunda *als1*, als3, bcr ve hwp genlerinde sırasıyla 0.39, 0.05, 0.91 ve 0.04 kat aşağı regülasyon belirlenmiştir. Sonuç olarak, biyofilmleri önleyebilen veya tedavi edebilen yeni yaklaşımların keşfi, yeni biyolojik kontrol ajanlarının ortaya çıkmasını teşvik edecektir.

Anahtar kelimeler: Biyofilm, Candida, Ekzopolisakkarit, Kefir, *Lacticaseibacillus*, Postbiyotikler

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INTRODUCTION

Candida albicans leads to a spectrum of diseases that can range from mucosal to systemic infections and are associated with biofilm formation on host or abiotic surfaces, resulting in considerably higher morbidity and mortality (Tsui et al. 2016). Progressive *C. albicans* biofilms can protect the fungi living in them, thus making *C. albicans* resistant to most antifungal drugs (He et al. 2020). Although it mostly poses a risk in the medical field, *C. albicans* can be potentially hazardous in the food industry due to biofilm formation on abiotic surfaces (Dishan et al. 2025). Genes belonging to the agglutinin-like sequence (*als*) family and hypha wall protein (*hwp*) encode cell surface-associated glycosylphosphatidylinositol-linked glycoproteins that participate in the adhesion of *C. albicans* to mucosal surfaces (Dishan et al. 2025). The biofilm and cell wall regulatory gene (*bcr*) encoded a C_2H_2 zinc finger protein that has an essential part in the establishment of biofilms (Nikoomanesh et al. 2016). Identification of new approaches that can prevent or eliminate biofilms could stimulate the emergence of novel bio-control agents.

Kefir is a natural reservoir of potential probiotic strains with antimicrobial and antibiofilm activity; immune system stimulation; anti-inflammatory, antioxidant, anti-obesity, and anti-proliferative, hypocholesterolemic, and stress-modulating effects; and intestinal bacterial microbiota improvement functions (Bengoa et al. 2021). Postbiotics have been defined as metabolites or non-living bacterial products derived from probiotics that have biological activity in the host (Butrungrod et al. 2023). Postbiotics are better alternatives to probiotics due to their similar beneficial health effects and avoidance of the concerns arising from live microorganisms (Dishan et al. 2022). Exopolysaccharides (EPS) included in the postbiotic classes are divided into homopolysaccharides, which consist of a single sugar molecule, and heteropolysaccharides, which consist of several different sugar molecules (Zhang et al. 2024). Many *Lactobacillus* species that produce EPS have shown anti-biofilm activity (Sarikaya et al. 2017), and EPS is also considered GRAS and has special physical and rheological properties for industrial use (Dailin et al. 2022). Some authors have concentrated on the impact of probiotics or their supernatants on pre-formed *C. albicans* biofilms (James et al. 2016; Kim and Kang, 2019), but the effect of postbiotics on *C. albicans* biofilm is also a matter of curiosity. Especially considering that EPS is included in the current postbiotic class (Sørensen et al., 2022; Dışhan et al., 2022), EPS contributes to the metabolites in the postbiotic composition (Tiwari et al., 2024). Detecting *C. albicans* biofilm-related genes, especially at the transcriptional level, in strong putative postbiotics also highlights the originality of the study.

This study aims to reveal the effect of postbiotics on the expression level of biofilm-related genes of *C. albicans* in industrial kefir isolates producing EPS by RT-PCR, and to determine biofilm prevention and treatment properties.

MATERIALS and METHODS

Sampling

In this study, a total of 16 industrial kefir samples belonging to eight different brands were collected from supermarkets in Kayseri province. Each sample (10 g) was incubated with 90 mL of Man Ragosa Sharp (MRS) broth overnight (30°C) in anaerobic conditions. The inoculum (100 µL) was spread on MRS agar plates and incubated in anaerobic conditions (30°C) for 2-5 days. Colony morphology was examined under a microscope by Gram staining and suspicious colonies were selected. The obtained colonies were preserved in the culture collection in cryovials at -80°C.

Exopolysaccharide Production

The EPS production of the selected isolates was determined using the ruthenium red staining method. Whether the solid medium prepared by non-fat milk powder, sucrose, yeast extract and ruthenium red produced EPS was expressed on the basis of the occurrence of pink and white colonies on the plate surface. After 48 hours of incubation at 30°C under anaerobic conditions, ruthenium red stained the bacterial cell wall, pink colonies were observed for non-ropy isolates, and white colonies were observed for ropy isolates (Yadav et al. 2024).

Identification of Isolates by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

MALDI-TOF MS to identify isolates was implemented by an Autoflex II TOF/TOF mass spectrometer (Nacef et al. 2017). Detection was performed by comparing the spectra obtained with the instrument's flex control software program (Biotyper 3.0) and library (version 9.0) with the MALDI Biotyper Real-Time Classification (RTC) software. The results obtained were expressed as scores by BioTyper. A score between 2.3 and 3.0 indicates a very high identification at the genus and species level, a score between 0 and 2.3 indicates a high identification at the genus level as well as a possibly correct identification at the species level, a score between 1.7 and 2.0 indicates a possibly correct identification only at the genus level, and a score below 1.7 indicates that the mass spectrum profile of the sample does not match the references in the database.

Standard Strain
Candida albicans ATCC 10231 standard strain was used in the scope of the study.

Preparation of Postbiotics
Selected isolates were activated in an MRS medium at 30°C for 24 hours. After incubation, 5 mL of active culture was transferred to 500 mL of sterile MRS medium and incubated at 37°C for 24 hours. The culture was then exposed to 85°C for 30 minutes to inhibit EPS-producing bacteria and chilled to 20°C to prepare serial dilutions (Song et al. 2020; Sun et al. 2023). The pH of each concentration tested was neutralized. The presence of live bacteria was checked by inoculating a loopful of the obtained postbiotics on an MRS agar medium.

Determination of Minimal Inhibitory and Fungicidal Concentration (MIC; MFC) of Postbiotics and Its Antagonistic Activity
Postbiotics prepared in serial dilution concentrations (100%, 50%, 25%, 12.5%, 6.25%, 3.12%) were added to the wells with *C. albicans* (10^5 CFU/mL) suspension. The plate was then incubated at 37°C for 24 hours. Minimum inhibitory concentration (MIC) values of postbiotics were determined by microplate bioassay using iodonitrotetrazolium chloride solution as an indicator of growth. MIC was determined by diluting microorganisms to a level where no inhibition was observed in their growth (Banakar et al. 2023). In addition, the minimum fungicidal concentration (MFC) level was determined by cultivating each well with a loop.
The agar well diffusion was applied to evaluate the antagonistic activity of postbiotics. The standard pathogen was spread on Dichloran Rose Bengal Chloramphenicol (DRBC) agar plates with a swab stick adjusted to 0.5 McFarland level. Postbiotics (100 µL) were applied to the wells (6 mm) prepared in the medium. The plates were inoculated at 30°C over 24 hours, and the inhibition zone size against the standard pathogen was calculated. When the zone diameter was 0 mm, it was evaluated as no inhibition; when the diameter was between 0 and 3 mm, it was weak inhibition; when the diameter was between 3 and 6 mm, it was good inhibition; and when the diameter was greater than 6 mm, it was strong inhibition (Liu et al. 2022). Determination of MIC, MFC, and antagonistic activity was repeated three times.

Phylogenetic Typing
A dendrogram regarding the phylogenetic tree was obtained by cluster analysis of MALDI-TOF mass spectra. The MALDI Biotyper 3.1 platform (Bruker Daltonics) was used to confirm the distinction among kefir isolates. The phyloproteomic principal component analysis (PCA) method allowed the cluster formation of spectra (Chen et al. 2015).

Coaggregation against *C. albicans* ATCC 10231
The suspensions of selected isolates and pathogens were mixed by adjusting OD600 to 0.5 ± 0.02. The absorbance of the mixture (A1) and then the incubated mixture (A2) at 37°C for 24 hours was measured at OD600. The percentage of coaggregation was calculated by the absorbance value of the mixture at the third and 24th hours as follows (Liu et al. 2022). The tests were performed in two parallels. Measurements were carried out in triplicate. It was calculated according to the following formula:

$$Coaggregation\ (\%) = [(1 - A2/(A1)]\ x\ 100$$

Effect of Postbiotics on *C. albicans* ATCC 10231 Growth Curve
The obtained postbiotics were serially diluted with Sabouraud Dextrose Broth (SDB) to prepare their concentrations (100%, 50%, 25%, 12.5%, 6.25%, 3.12%). *C. albicans* suspension (10^5 CFU/mL) was added to each dilution. Sterile SDB was used as the negative control, while C. albicans suspension (10^5 CFU/mL) was used as the positive control. Optical density was taken at 600 nm for each measurement and recorded at 0, 2, 4, 8, 16, 24, and 48 hours (García-Gamboa et al. 2024). Analyses were performed in two parallels. The measurements were read three times.

Effects on Preventing Biofilm Formation
The effect of preventing biofilm formation was tested in microplates using the Lynch et al. (2021) method with minor modifications. *C. albicans* ATCC 10231 suspension (5 µl) adjusted to 0.5 McFarland was inoculated into the wells of a sterile 96-well microplate containing SDB (195 µl). The adjusted concentrations of each postbiotic (400%, 200%, 100%, 50%, 25%, 12.5%) were added to the microplates in serial dilutions and incubated at 37°C for 24 hours. Wells without postbiotics were considered as positive control, and sterile SDB as negative control. Blank readings without bacteria were generated at each adjusted concentration. At the end of incubation, the well contents were emptied. They were gently rinsed with phosphate buffered saline (PBS), stabilized using methanol, then dyed with 0.05% crystal violet, 33% acetic acid (100 µl) was applied to each well, and optical density (OD) readings were recorded at 600 nm.

Effects on Biofilm Treatment
The effect of postbiotics on biofilm treatment was tested in microplates using the method of Lynch et al. (2021) with minor modifications. *C. albicans* ATCC 10231 suspension (5 µl) adjusted to 0.5 McFarland was added into a 96-well microplate containing SDB (195 µl). After 24 hours of incubation, preformed biofilms were washed once with PBS. Each postbiotic was added to each test well of the microassay at concentrations (400%, 200%, 100%, 50%, 25%, 12.5%, 6.25%). Microplates were incubated at 37°C for

24 hours and then washed once with PBS, fixed with methanol, stained with 0.05% crystal violet, and 100 µl of 33% acetic acid was added to each well to take optical density readings at 600 nm. In the study, the effect of postbiotic application in preventing biofilm and treating the formed biofilm was categorized and evaluated asstated by Stepanovic et al. (2000). The mean absorbance from the negative control (ODc) was measured at 600 nm for biofilm formation. Analyses were performed in two parallels. The measurements were read three times in treatment and prevention assays.

Transcriptional Analysis

For the analysis of *in situ* gene expression of *C. albicans*, RNA isolation and serial dilutions of the postbiotic were treated on *C. albicans* suspension adjusted to 6 log CFU/ml. Total RNA concentration was determined using the Qubit RNA HS Assay Kit (Thermo Fisher Scientific, USA) following RNA extraction by TransZol Up (TransGen Biotech, People's Republic of China). cDNA was synthesized via the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA) from two separate RNA extractions whose concentration was adjusted to 30 ng/µL. The RT-PCR protocol arranged with primers belonging to selected specific biofilm-related genes was determined according to the study of Dishan and Gönülalan (2025) (Table 1). Negative control was used in each analysis. The $2^{-\Delta\Delta CT}$ calculation method of Livak and Schmittgen (2001) was employed to detect transcriptionally fold alterations.

Table1. Biofilm-related genes

Target Gene	Forward	Reverse	Tm
als1	CCTATCTGACTAAGACTGCACC	ACAGTTGGATTTGGCAGTGGA	60.1/57.6
als3	ACCTGACTAAAACTGCACCAA	GCAGTGGAACTTGCACAACG	57.7/60.5
bcr1	GCATTGGTAGTGTGGGAAGTTTGAT	AGAGGCAGAATCACCCACTGTTGTA	57.6/59.9
hwp1	CTCCAGCCACTGAAACACCA	GGTGGAATGGAAGCTTCTGGA	60.1/60
act1	CGTTGTTCCAATTTACGCTGGT	TGTTCGAAATCCAAAGCAACG	60.3/58.1

Field Emission Scanning Electron Microscopy

To demonstrate the biofilm inhibition effect of postbiotics, 500 µl of bacterial suspension in TSB with 2xMIC of postbiotics and only control was added to a sterile glass (1 cm × 1 cm) and kept overnight at 37 °C. Then, the glasses were rinsed with PBS and fixed with 2.5% glutaraldehyde for 4 hours. After washing, dehydration was carried out by adding ethanol (50, 70, 80, 90, and 100%). After gold sputtering, the effects of the obtained postbiotics on biofilm were visualized using a field emission scanning electron microscope (FESEM) (Zeiss, Germany) (Azami et al. 2022).

Statistical Analysis

The statistical significance of the difference between the concentrations in the biofilm prevention and treatment applications where *C. albicans* was exposed to postbiotics belonging to the isolates coded KEF15 and KEF19 was performed with the R statistical software (www.r-project.org/) with the variance analysis. While the significant difference between the coaggregation levels of the selected isolates against the pathogen was determined by the two-way ANOVA, the significant difference between the antagonistic activity levels was determined by Student's t-test. The statistical differences in the biofilm-related gene expression levels of postbiotics at different concentrations for each gene were determined by the two-way ANOVA. The significance level was determined as $p<0.05$.

RESULTS

Identification of Kefir isolates and EPS producing

The selected 20 isolates were identified by MALDI-TOF, and it was revealed that the majority of them were *Lactobacillus acidophilus* (40%) (Table 2). The typing of the isolates is given in the PCA dendrogram within (Figure 1).

Table 2. Identification of selected isolates

Isolate Codes	MALDI-TOF results	EPS producing
KEF1	*Lactococcus lactis*	-
KEF2	*Leuconostoc pseudomesenteroides*	-
KEF4	*Lactobacillus acidophilus*	-
KEF5	*Lactobacillus acidophilus*	-
KEF6	*Leuconostoc pseudomesenteroides*	-
KEF7	*Lactobacillus zeae*	-
KEF8	*Pediococcus acidilactici*	-
KEF9	*Lactobacillus acidophilus*	-
KEF10	*Lactobacillus paracasei*	-
KEF11	*Lactobacillus rhamnosus*	-
KEF12	*Lactobacillus acidophilus*	-
KEF13	*Lactobacillus acidophilus*	-
KEF15	***Lactobacillus rhamnosus***	**+**
KEF16	*Lactococcus lactis*	-
KEF17	*Lactobacillus acidophilus*	-
KEF18	*Lactobacillus acidophilus*	-
KEF19	***Lactobacillus paracasei***	**+**
KEF20	*Lactobacillus acidophilus*	-
KEF21	*Lactobacillus zeae*	-
KEF23	*Leuconostoc pseudomesenteroides*	-

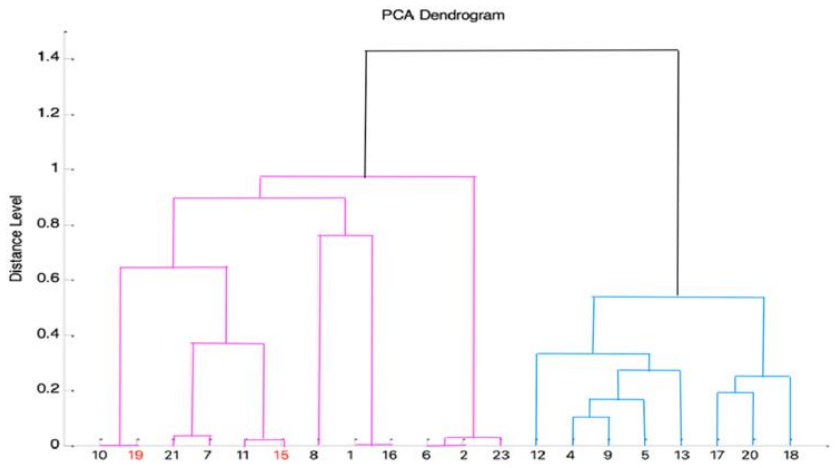

Figure 1: Typing of selected kefir isolates (x-axis: isolate codes, y-axis: distance level)

In the study, the EPS production abilities of the isolates coded KEF15 and KEF19 were phenotypically demonstrated. The isolates coded KEF15 and KEF19 were identified as *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus paracasei*, respectively.

MIC determination, Antagonistic Activity, and Coaggregation

The minimal inhibition and fungicidal concentrations and antagonistic activity of the postbiotics and coaggregation levels of the isolates coded KEF15 and KEF19 against *C. albicans* ATCC 10231 were determined as percentages (Table 3).

When the effect of the obtained postbiotics on the growth curve was examined, it was found that KEF15 postbiotic decreased the growth of *C. albicans* at 25% and 12.5% concentrations between 16 and 48 hours. KEF19 postbiotic reduced the growth of *C. albicans* at 50%, 25%, and 12.5% concentrations between 24 and 48 hours (Figure 2). Based on the measured diameter, the antagonistic activity of postbiotics was found to be strong (Table 3).

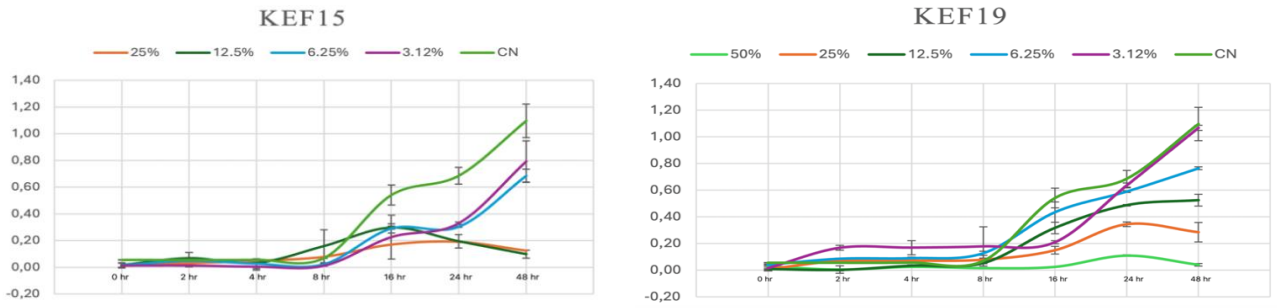

Figure 2: Growth curve of *C. albicans* ATCC 10231 under KEF15 and KEF19 postbiotic exposure (x-axis: time, y-axis: OD600 readings)

Table 3. MIC, MFC and antagonistic activity (mm) of postbiotics and coaggregation levels of isolates

Concentration	MIC	MFC
KEF15	12.5%	25%
KEF19	25%	50%
Coaggregation (%)	4 h	24 h
KEF15	$14.95^{Ab} \pm 0.96$	$27.46^{Ba} \pm 0.53$
KEF19	$11.5^{Bb} \pm 0.45$	$30.45^{Aa} \pm 0.41$
Antagonistic activitiy (mm)		
KEF15	$30.75^{A} \pm 0.63$	
KEF19	$27.46^{B} \pm 0.36$	

A, B: Means shown with different exponential letters in the same row are statistically different ($p<0.05$).
a, b, c: Means shown with different exponential letters in the same column are statistically different ($p<0.05$).

Effects on Growth Curve and Biofilm Forming of Postbiotics

Based on the MIC data obtained, the postbiotic doses applied in biofilm prevention and treatment tests were determined. It was concluded that the 400%-25% (32xMIC-2xMIC) doses of postbiotic from KEF15 prevented biofilm formation compared to the control ($p < 0.05$). The 400%-25% (16xMIC-MIC) doses of postbiotic from KEF19 were effective in preventing biofilm ($p < 0.05$) (Figure 3). In the prevention application, it was observed that the MIC dose application of KEF15 postbiotic was not different from the control ($p > 0.05$), and the biofilm formation remained at a moderate level. In the MIC application of KEF19 postbiotic, unlike the control, biofilm formation was reduced and classified in the weak category ($p < 0.05$) (Figure 4).

It was determined that the postbiotic from KEF15 at doses of 400%-12.5% (32xMIC-MIC) treated the formed biofilm, and the postbiotic KEF19 treated the biofilm at levels of 400%-25% (16xMIC-MIC) ($p < 0.05$) (Figure 3).

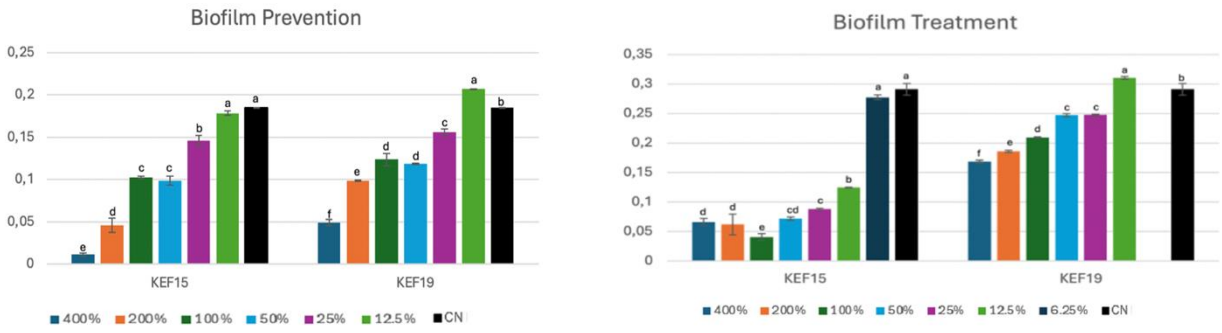

Figure 3: Effect of postbiotics on preventing biofilm formation and postbiotics on the formed biofilm of *C. albicans* ATCC 10231 (x-axis: Postbiotic doses of KEF15 and KEF 19 isolates, y-axis: OD600 readings)

A, B: Means shown with different exponential letters in the same row are statistically different ($p<0.05$).
a, b, c: Means shown with different exponential letters in the same column are statistically different ($p<0.05$).

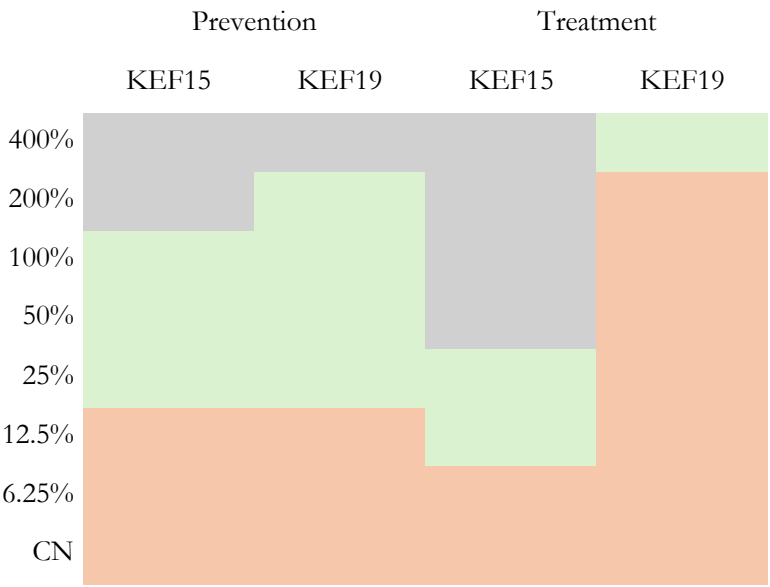

Figure 4: Heat map showing the categorization of biofilm reduction effects of postbiotic applications.
(CN: untreated; gray represents non biofilm, green represents weak biofilm, and salmon orange represents moderate biofilm)

Transcriptional Effects of Postbiotics on Biofilm-Related Genes

It was concluded that *als1*, *als3*, and *bcr* genes were down-regulated at 25% and 12.5% (2xMIC and MIC) concentrations of KEF15 postbiotic, but up-regulation of the hwp gene was observed at all applied doses. At the 50% (2xMIC) dose exposure of KEF19 postbiotic, all genes examined were down-regulated (Table 4). The FESEM image revealed biofilm interaction of 2xMIC levels of postbiotics (Figure 5).

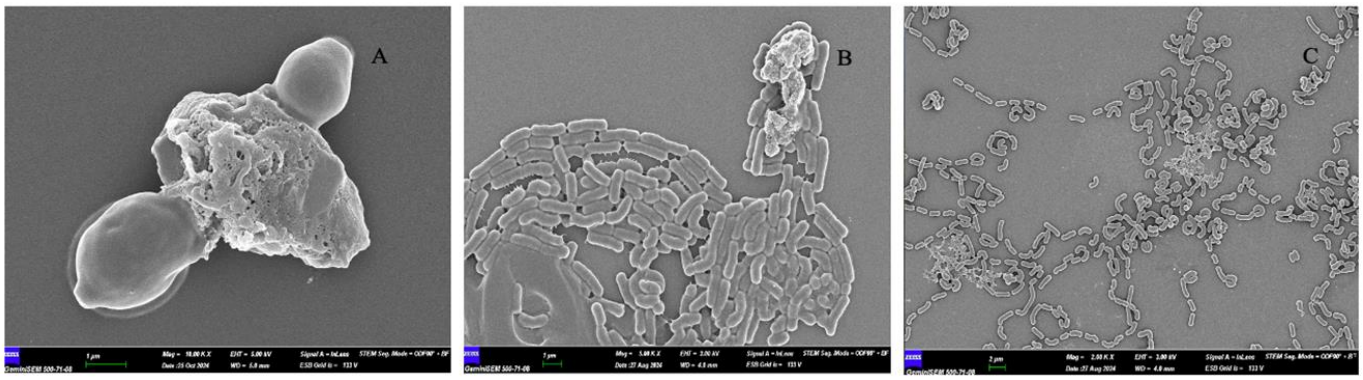

Figure 5: Effect of postbiotic interaction at 2xMIC doses on biofilm formation
(A: control, B: effect of *L. rhamnosus* postbiotic on biofilm formation, C: effect of *L. paracasei* postbiotic on biofilm formation

Table 4. Fold changes in expression levels of biofilm-related genes in postbiotic exposure

			KEF15			KEF19		
Target Gene	Concentration		4 hr	8 hr	24 hr	4 hr	8 hr	24 hr
als1	50%	FC	-	-	-	$0.65^{Bb} \pm 0.04$	$0.74^{Ba} \pm 0.01$	$0.39^{Cc} \pm 0.14$
		log_2(FC)	-	-	-	-1.66	-2.34	-0.75
	25%	FC	$0.91^{Aa} \pm 0.01$	$0.62^{Ab} \pm 0.20$	$0.03^{Bc} \pm 0.02$	$0.58^{C} \pm 0.01$	$0.43^{C} \pm 0.23$	$0.60^{B} \pm 0.02$
		log_2(FC)	-7.35	-1.45	-0.19	-1.31	-0.84	-1.40
	12.5%	FC	$0.34^{B} \pm 0.18$	$0.58^{B} \pm 0.06$	$0.31^{A} \pm 0.22$	$0.13^{Db} \pm 0.04$	$0.88B^{Ca} \pm 0.52$	$1.52^{Aa} \pm 0.50$
		log_2(FC)	-0.64	-1.25	-0.58	-0,34	-5,52	1.64
	6.25%	FC	$0.08^{Cb} \pm 0.06$	$0.07^{Cb} \pm 0.06$	$0.80^{Aa} \pm 0.27$	$0.79^{Ac} \pm 0.01$	$1.76^{Aa} \pm 0.05$	$1.05^{Ab} \pm 0.01$
		log_2(FC)	-0.27	-0.26	-3.11	-3.07	1.22	14.11
als3	50%	FC	-	-	-	$0.69^{Aa} \pm 0.05$	$0.70^{a} \pm 0.01$	$0.05^{Bb} \pm 0.02$
		log_2(FC)				-1.91	-1.98	-0.22
	25%	FC	$0.58^{Bb} \pm 0.01$	$0.63^{Aa} \pm 0.03$	$0.15^{Bc} \pm 0.01$	$0.61^{Ab} \pm 0.17$	$0.74^{b} \pm 0.05$	$2.49^{Aa} \pm 1.51$
		log_2(FC)	-1.27	-1.52	-0.36	-1.39	-2.31	0.76
	12.5%	FC	$0.63^{A} \pm 0.01$	$0.82^{AB} \pm 0.80$	$0.55^{B} \pm 0.51$	$0.07^{Bb} \pm 0.07$	$2.14^{a} \pm 1.43$	$2.38^{Aa} \pm 0.01$
		log_2(FC)	-1.52	-3.40	-1.18	-0.27	0.91	0.80
	6.25%	FC	$0.10^{Cb} \pm 0.09$	$0.25^{Bb} \pm 0.20$	$3.05^{Aa} \pm 0.28$	$1.71^{A} \pm 1.08$	2.03 ± 1.74	$3.08^{A} \pm 1.72$
		log_2(FC)	-0.30	-0.49	0.62	1.29	0.98	0.62
bcr	50%	FC	-	-	-	$0.52^{Bb} \pm 0.03$	$0.89^{Ba} \pm 0.02$	$0.91^{Ba} \pm 0.01$
		log_2(FC)	-	-	-	-1.06	-5.98	-7.12
	25%	FC	$0.52^{A} \pm 0.16$	$0.67^{A} \pm 0.05$	$0.79^{A} \pm 0.22$	$0.36^{Cb} \pm 0.10$	$0.57^{Ca} \pm 0.04$	$0.23^{Cb} \pm 0.19$
		log_2(FC)	-1.06	-1.71	-2.97	-0.68	-1.22	-0.48
	12.5%	FC	$0.55^{Ab} \pm 0.11$	$0.26^{Bb} \pm 0.19$	$0.77^{Aa} \pm 0.01$	$0.17^{D} \pm 0.07$	$0.06^{D} \pm 0.04$	$0.06^{C} \pm 0.04$
		log_2(FC)	-1.15	-0.51	-2.66	-0.39	-0.25	-0.24
	6.25%	FC	$0.13^{Ba} \pm 0.04$	$0.02^{Cb} \pm 0.01$	$0.05^{Bb} \pm 0.02$	$1.78^{Ab} \pm 1.05$	$3.53^{Aab} \pm 1.16$	$4.72^{Aa} \pm 0.07$
		log_2(FC)	-0.34	-0.17	-0.22	1.21	0.55	0.45
hwp	50%	FC	-	-	-	$0.53^{Ba} \pm 0.04$	$0.41^{Bb} \pm 0.01$	$0.04^{Cc} \pm 0.01$
		log_2(FC)	-	-	-	-1.10	-0.77	-0.21
	25%	FC	$0.18^{Bc} \pm 0.05$	$0.25^{Bb} \pm 0.01$	$5.34^{Ba} \pm 0.61$	$0.24^{Cb} \pm 0.10$	$1.09^{Aab} \pm 1.01$	$3.57^{ABa} \pm 2.16$
		log_2(FC)	-0.41	-0.51	0.41	-0.49	7.84	0.54
	12.5%	FC	$0.14^{Bb} \pm 0.02$	$0.39^{Bb} \pm 0.23$	$5.56^{Ba} \pm 3.44$	$0.30^{Cb} \pm 0.13$	$0.28^{Bb} \pm 0.18$	$2.76^{Ba} \pm 0.58$
		log_2(FC)	-0.35	-0.74	0.40	-0.57	-0.54	0.68
	6.25%	FC	$1.52^{Ab} \pm 0.11$	$3.72^{Ab} \pm 2.14$	$16.40^{Aa} \pm 1.74$	$1.28^{Ab} \pm 0.01$	$2.21^{Ab} \pm 0.98$	$7.16^{Aa} \pm 1.75$
		log_2(FC)	1.66	0.53	0.25	2.81	0.87	0.35

FC: Fold Change, log_2(FC): a positive value indicates upregulation, and a negative value indicates downregulation.
Each gene was evaluated separately in KEF15 and KEF19 postbiotic applications.
A, B: Means shown with different exponential letters in the same row are statistically different ($p<0.05$).
a, b, c: Means shown with different exponential letters in the same column are statistically different ($p<0.05$).

DISCUSSION

It was revealed that the LAB dominating the industrial kefir biota from the selected isolates was *Lactobacillus acidophilus* which is known to be a commercially important bacterial probiotic that is frequently used in the dairy industry (Jeon et al. 2021). Notwithstanding the widespread usage of LAB producing EPS in the dairy industry, the phenotypical EPS production level (10%) of industrial kefir isolates was quite low. This situation should be genotypically supported by the fact that the isolates carry the eps gene cluster, and the correlation status should be examined. The health-promoting activities of EPS-producing strains have been previously demonstrated (Bengoa et al. 2018; Bertsch et al. 2019). The anti-biofilm effect of many EPS-producing LAB has also been reported previously (Xu et al. 2020). This study showed that isolates obtained from industrial kefir had anti-candidal biofilm effects, although artisanal products are potentially rich in probiotic strains (Bengoa et al. 2021). Since the potential beneficial use of EPS-producing isolates may be high (Brian-Jaisson et al. 2016; Tiwari et al., 2024), two isolates phenotypically capable of EPS production were selected and identified as *L. rhamnosus* KEF15 and *L. paracasei* KEF19. Rossoni et al. (2020) reported that *L. paracasei* postbiotic was effective against *Candida auris* biofilm. Song and Lee (2017) reported that the prepared spent culture medium of *L. casei* and *L. rhamnosus* showed strong anti-biofilm activity against *Candida* biofilm. The current study showed that two EPS-producing kefir isolates were in the same primary cluster according to phylogenetic typing, and it is known that *L. paracasei* and *L. rhamnosus* constitute the casei group (Huang et al. 2018). However, according to the current study, casei group bacteria may exhibit different profiles.

The *L. paracasei* postbiotics obtained by Rossoni et al. (2020) inhibited the growth of *C. auris* at a dose of 8MIC. García-Gamboa et al. (2024) found that *L. plantarum* postbiotics also reduced the number of *C. albicans* after 24 hours. Rossoni et al. (2024) found that *L. paracasei* postbiotics completely inhibited *C. auris* after 24 hours. In the current study, it was determined that the 2xMIC dose of *L. rhamnosus* KEF15 and *L. paracasei* KEF19 postbiotics were effective in inhibiting *C. albicans*. Pfaller et al. (2004) reported that if the MFC is $\leq 4 \times$ MIC, the agent is considered cidal. The present study showed that postbiotics obtained from *L. rhamnosus* and *L. paracasei* had a cidal effect on *C. albicans*. The integration of postbiotics into hurdle technology presents a promising approach (Khani et al. 2024).

Yocheva et al. (2024) reported that *L. paracasei* and *L. rhamnosus* isolates had low levels of coaggregation activity (0-17%) against *C. albicans*. Lactobacilli coaggregation is also crucial in bacterial colonization, biofilm development, bioadhesion to host tissues, maintenance of microecological stability, and resistance to opportunistic pathogens (Wang et al. 2024). High EPS-forming *Lactobacillus* strains have been demonstrated to correlate with a high coaggregation (Castro-Bravo et al. 2018). The high coaggregation properties of isolates obtained from industrial kefir can prevent biofilm formation.

Postbiotics not only alter the production of biofilms but also disrupt and eliminate them. Zhao et al. (2023) concluded that postbiotics of *L. paracasei* could be candidates with respect to the elimination of oral decays. Rossoni et al. (2018) stated that there was no difference between the *Lactobacillus* species tested in supernatant-inhibited preformed-biofilms. Hossain et al. (2021) revealed the anti-biofilm effect of postbiotics from *L. plantarum* and *L. curvatus* against *L. monocytogenes*. The study findings showed that even if mature biofilms are far more resistant to antifungal therapy (Barros et al. 2016), the effects of postbiotics were consistent with prevention and treatment procedures. The difference was even higher in the postbiotic of *L. rhamnosus* KEF15 treatment compared to the control, which may be due to the higher OD value of the formed biofilm. Behbahani et al. (2024) reported that 1/4xMIC-4xMIC doses of *L. paracasei* B31-2 CFS were effective in treating *L. monocytogenes* biofilm. According to the present study, *C. albicans* ATCC 10231 formed moderate-level biofilm. When biofilm level was categorized, KEF15 postbiotics were more effective in treatment than prevention. Likewise, Behbahani et al. (2024) also reported that the rate of mature biofilm degradation was similar to the rate of prevention of the initial biofilm formation phase. This indicates that the cell-free supernatant derived from *L. paracasei* B31-2 not only hindered the early stage of biofilm formation but also adeptly disintegrated the developed biofilm.

The study suggested that the mechanism of action for the postbiotics' anti-biofilm effect was through damaging targeting genes involved in biofilm formation (Hossain et al. 2021). Transcriptomic analysis of the biofilm-related genes revealed a better understanding of the anticandidal activity of industrial kefir isolates. Similar to the current study, the study of Rossoni et al. (2018) advanced approaches to testing *C. albicans* gene expression to clarify the mechanisms utilized by *Lactobacillus* strains to suppress *C. albicans* biofilms, concentrating on genes known to be essential for biofilm development. Rossoni et al. (2018) reported that the *als3* and *hwp1* genes were respectively downregulated 33- and 20-fold upon treatment with *L. paracasei* 28.4 supernatant. Rossoni et al. (2018) also reported that ywp1 was the solely gene up-regulated positively in Lactobacilli relevant to reference and human strains of *C. albicans*. The *C. albicans* ALS family codifies an adhesin that yields attachment to endothelial cells. The adhesin markers ALS1, ALS3, HWP1, and BCR1 mediate cell-substrate and cell-cell interactions in biofilms (Satala et al. 2022). The ALS

family of adhesins includes eight members (ALS1-ALS9). *als1* and *als3* genes affect adhesion to host epithelial cells and endothelial cells (Richardson et al. 2018). *als1* and *als3* were down-regulated at high doses of postbiotics (50%) but showed differences in regulation among exposures at MIC levels. Nailis et al. (2006) reported that differences in expression between the *als1* and *als3* genes, which contain the same heptapeptide amyloid-forming sequence, may indicate different functions of Als1p and Als3p in *C. albicans* biofilm development. By using different normalization strategies, Nailis et al. (2006) found a significant upregulation of the *als1* gene and downregulation of the *als3* gene in *C. albicans* biofilms grown. *als3* has been declared to be the key gene, as it is considered to actively participate in biofilm formation and is up-regulated throughout in vitro infection of epithelial cells of the oral cavity (Murciano et al. 2012; Zordan and Cormack, 2012). It was observed that the KEF15 postbiotic was insufficient in preventing the formation of hyphal form but was more effective in inhibiting the encoding of glycosylphosphatidylinositol-anchored cell surface glycoproteins. HWP1 is a transglutaminase substrate that can bind *C. albicans* hyphae and host cells via covalent links leading to *C. albicans* infection (Mayer et al. 2013). The overexpression level of the KEF19 postbiotic is high in the distribution of genes, and its effect on prevention and treatment processes is low compared to KEF15. Fanning et al. (2012) reported that among the major functional BCR1 targets, ALS3 is reliant on BCR1 in vivo, whereas HWP1 is non-dependent; however, in general, BCR1 is needed for both ALS3 and HWP1 expression at the time of oropharyngeal candidiasis in vitro. In this study, even if *hwp* is overexpressed, postbiotics prevent biofilm formation at MIC levels and above. Anyway, the simultaneous presence of the *als1*, *als3*, and *hwp1* genes can play a role in enhancing the synergistic effect on the performance of individual genes involved (Mohammadi et al. 2021). Transcription factors often control functionally related target genes.

CONCLUSION

This study demonstrates that heat-killed preparations of EPS-producing industrial kefir isolates are effective in preventing and treating *C. albicans* biofilms, paving the way for the potential use of anti-adhesion transcriptional mechanisms as alternative agents for biocontrol of *C. albicans* biofilms in medical and industrial fields. Thus, these agents will also serve to eliminate many contamination situations that could endanger public health. However, more in-depth analyses are needed to assess the suitability of the prepared postbiotics. Future research should focus on the structural and compositional characterization of EPS-producing *L. rhamnosus* and *L. paracasei* postbiotics, whose effects we have determined in the abiotic environment, for further development of functional foods and on investigating their bioactivity in vivo.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: AD contributed to the project idea, design and execution of the study. AD and EA contributed to the acquisition of data. AD analysed the data. AD drafted and wrote the manuscript. ZG reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

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RESEARCH ARTICLE

Explainable Machine Learning Framework for Milk Quality Grading

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ABSTRACT

This study introduces an explainable machine learning framework for milk quality grading, combining high predictive performance with transparency and practicality. Utilizing Random Forest and HistGradientBoost models, alongside interpretability techniques like Permutation Feature Importance and LIME, the framework achieves robust classification while providing actionable insights. Global explanations identify pH and Temperature as critical factors, highlighting their significance in real-time monitoring and microbial control. Local explanations, based on the two presented examples, demonstrate the practical utility of individual predictions, offering targeted interventions such as optimizing storage conditions or addressing contamination risks. By bridging the gap between predictive accuracy and interpretability, this framework not only enhances trust and usability for stakeholders but also establishes a new perspective for integrating AI-driven quality control systems into the dairy industry.

Keywords: Dairy Industry, Explainable AI (XAI), Machine learning, Milk quality, Veterinary Food Safety

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Süt Kalitesi Derecelendirmesi için Açıklanabilir Makine Öğrenimi Çerçevesi

ÖZ

Bu çalışma, süt kalitesinin değerlendirilmesinde yüksek tahmin doğruluğunu şeffaflık ve kullanışlılık ile birleştiren açıklanabilir bir makine öğrenimi yaklaşımı sunmaktadır. Random Forest ve HistGradientBoost modellerinin yanı sıra Permutasyon Feature Importanee ve LIME gibi yorumlanabilirlik tekniklerini kullanan bu yaklaşım, güçlü bir sınıflandırma performansı sağlarken uygulanabilir içgörüler de sunmaktadır. Global yorumlanabilirlik sonuçları, pH ve Sıcaklık gibi kritik faktörleri belirleyerek gerçek zamanlı izleme ve mikrobiyal kontroldeki önemlerini vurgulamaktadır. Yerel yorumlanabilirlik sonuçları ise, sunulan 2 örnek üzerinden, bireysel tahminlerin pratik faydasını göstererek depolama koşullarının optimize edilmesi veya kontaminasyon risklerinin ele alınması gibi hedefe yönelik müdahalelere olanak tanımaktadır. Tahmin doğruluğu ile yorumlanabilirlik arasındaki boşluğu kapatan bu yaklaşım, yalnızca paydaşlar için güven ve kullanılabilirliği artırmakla kalmayıp, aynı zamanda AI destekli kalite kontrol sistemlerinin süt endüstrisine entegrasyonu için yeni bir perspektif sunmaktadır.

Anahtar kelimeler: Açıklanabilir Yapay Zeka (XAI), Makine öğrenimi, Süt endüstrisi, Süt kalitesi, Veteriner gıda güvenliği

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INTRODUCTION

Milk, a cornerstone of human nutrition, is valued for its high nutritional content, including proteins, fats, lactose, vitamins, and minerals. Despite its importance, milk's perishable nature and susceptibility to adulteration present significant challenges in maintaining its quality throughout the supply chain (Frizzarin et al., 2021; Polat et al., 2021). These challenges necessitate the development of robust and reliable quality assessment methodologies to ensure consumer safety and product consistency.

Fourier-transform mid-infrared spectroscopy (MIRS) has emerged as a promising tool for analyzing milk composition, offering a non-destructive, rapid, and cost-effective method for assessing key parameters like fat, protein, and lactose (Frizzarin et al., 2021). Combined with machine learning techniques, MIRS enables high-precision predictions of technological properties such as coagulation time, paving the way for more efficient dairy processing (Polat et al., 2021). Other advanced methods, such as Raman and near-infrared spectroscopy, coupled with machine learning, have proven effective in detecting anomalies and contaminants in milk, ensuring compliance with safety standards.

Machine learning models, including random forests, support vector machines, and deep learning networks, have demonstrated exceptional capabilities in milk quality classification. These algorithms are employed to analyze parameters like pH, temperature, turbidity, and sensory attributes, achieving high classification accuracy (Bhavsar et al., 2023; Sheng et al., 2022). However, while predictive accuracy is essential, understanding the rationale behind predictions is equally critical.

Explainable artificial intelligence (XAI) addresses the need for transparency in machine learning models, particularly in safety-critical applications like food quality assessment. XAI techniques, such as Local Interpretable Model-agnostic Explanations (LIME) values, provide insights into model behavior, enabling stakeholders to trust and effectively utilize AI-driven decisions (Islam et al., 2022). For instance, XAI has been instrumental in food fraud detection, allowing users to interpret predictions and identify key factors influencing decisions (Buyuktepe et al., 2023). By improving model interpretability, XAI not only enhances trust but also supports error identification, bias reduction, and feature importance analysis (Przybył, 2024).

While machine learning models excel at predictive tasks, their black-box nature often obscures the underlying decision-making processes, which can lead to mistrust among stakeholders (Dang et al., 2022). XAI bridges this gap by offering local and global interpretability, making it possible to understand individual predictions and overall model behavior. This dual perspective ensures that models are not only accurate but also aligned with domain-specific requirements and ethical considerations (Islam et al., 2022; Przybył, 2024).

In this study, we propose a novel framework for milk grading that integrates machine learning and XAI techniques. Our approach classifies milk into three quality categories (Bad, Moderate, and Good) while employing interpretability tools to provide transparent insights into the model's decision-making process. By leveraging features such as pH, temperature, and sensory attributes, this framework aims to enhance the reliability and effectiveness of milk quality assessments.

MATERIAL and METHODS

Study Design and Ethical Statement

This study was designed to assess and interpret milk grading using machine learning and explainable artificial intelligence (XAI) methodologies. The dataset, obtained from Kaggle (https://www.kaggle.com/datasets/prudhvignv/milk-grading), includes key milk quality parameters such as pH, temperature, taste, odor, fat, turbidity, and color (GNV, 2020). The dataset is licensed under the "EU ODP Legal Notice," which allows for its use and redistribution under open data standards, ensuring compliance with ethical data practices. Preprocessing steps, including normalization and feature encoding, were applied to prepare the data for machine learning analysis. Models such as Random Forest and Gradient-Boosted Decision Trees were employed to classify milk quality into three grades: Bad, Moderate, and Good. Explainability was ensured using XAI techniques, including LIME for local interpretations and global feature importance, providing transparency into the decision-making processes of the models (Figure 1). As the dataset is publicly available, ethically sourced, and used in accordance with its licensing terms, no additional ethical approval was required for this study.

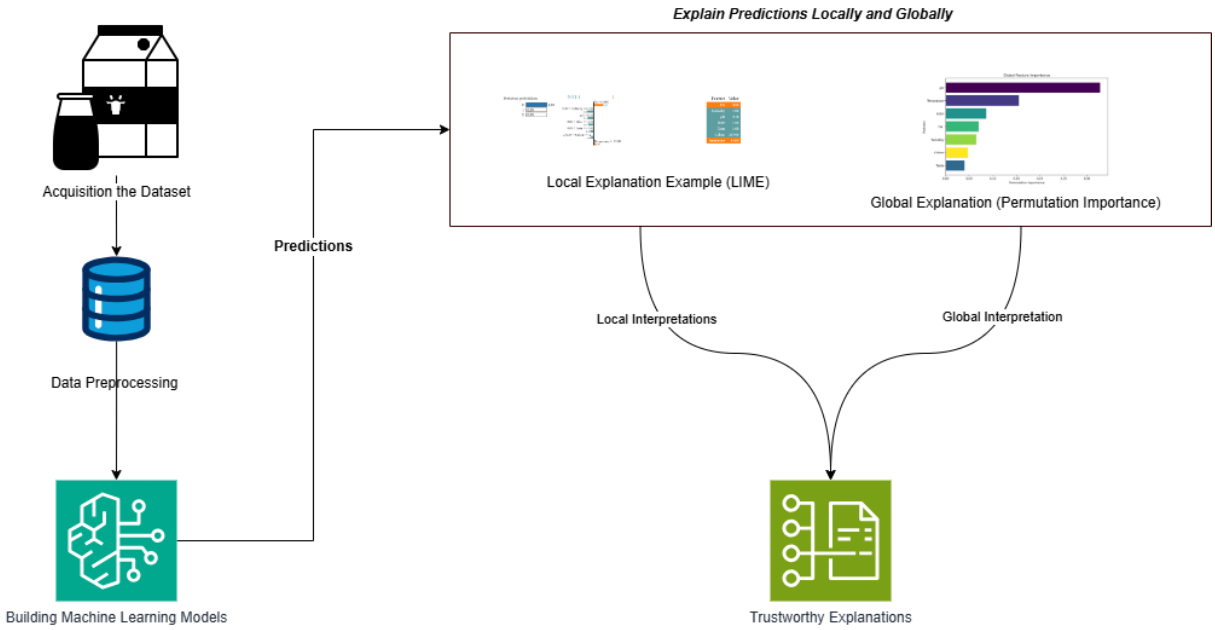

Figure 1: Framework for Generating Predictions and Interpreting Model Explanations

Dataset and Pre-processing

Several important features that are likely to affect the likelihood of a milk quality are included in this dataset (Table 1). Every milk has a distinct set of qualities that enable the development of a prediction model that pinpoints the variables affecting quality results. The dataset includes 1059 data and the following features:

Table 1. Milk Grade Dataset Feature Descriptions

Feature	Description
Taste	Taste of milk (satisfies optimal conditions assign 0 otherwise 1)
Odor	Smell of milk (satisfies optimal conditions assign 0 otherwise 1)
Fat	Fat of milk (satisfies optimal conditions assign 0 otherwise 1)
Turbidity	Turbidity of milk (satisfies optimal conditions assign 0 otherwise 1)
Color	The color of the milk (GreyScale)
pH	pH value of milk
Temperature	Milk temperature Immediately After Milking
Grade	0 (Bad quality), 1 (Moderate quality), 2 (Good quality).

Feature engineering was performed to prepare the dataset for effective machine learning modeling. Since there were no missing values in the dataset, no imputation techniques were required. Continuous variables, such as pH and temperature, were normalized using Min-Max scaling to transform their values into the range [0, 1], ensuring comparability across features. The binary categorical variables (Taste, Odor, Fat, and Turbidity) were retained in their original format (0 or 1) for seamless compatibility with machine learning algorithms. As the dataset was balanced across the target classes, no data augmentation techniques were applied, allowing the analysis to proceed directly with the available data.

Model Development and Evaluation

We examined the effectiveness of various machine learning models on the dataset in order to predict the likelihood of milk grade. We made sure that each of the 6 machine learning models we chose had a distinct mathematical foundation. Additionally, we experimented with decision tree techniques based on various architectures.

Several machine learning models were employed to predict milk grading, leveraging their diverse strengths in handling structured data. Random Forest is an ensemble learning method based on decision trees, known for its robustness against overfitting and its ability to handle non-linear relationships. Sample academic studies using this method (Bovo et al. 2021; Vishnu & Kumar, 2024). Support Vector Machine

(SVM) was utilized for its capacity to find an optimal hyperplane that separates classes, making it particularly effective in high-dimensional feature spaces. Sample academic studies using this method (Mu et al. 2020; Mammadova & Keskin, 2013).K-Nearest Neighbors (KNN), a simple yet powerful algorithm, classified data points based on the majority class of their nearest neighbors, relying on distance metrics. Sample academic studies using this method (Samad et al. 2024; Neware, 2023). XGBoost and LightGBM are gradient boosting algorithms that excel in speed and efficiency, with XGBoost offering regularization techniques to reduce overfitting and LightGBM being optimized for large datasets with high-dimensional features. Sample academic studies using these methods (Mota et al., 2022; Satola & Satola, 2024). HistGradientBoost is another gradient boosting approach that employs histogram-based techniques to accelerate training and enhance performance. Sample academic studies using this method (Ebrahimiet al., 2019; Sheng et al., 2022). By incorporating these diverse models, the study ensured comprehensive exploration of predictive capabilities, selecting the best-performing model based on evaluation metrics such as accuracy, precision, and recall.

To evaluate the performance of each machine learning model, we utilized four key metrics: accuracy, recall, precision, and F1-score. Accuracy (Eq. 1) measures the proportion of correctly classified instances out of the total instances, providing an overall measure of model performance. Recall (Eq. 2) evaluates the model's ability to identify all relevant instances by measuring the proportion of correctly predicted positive cases to all actual positive cases, which is crucial when minimizing false negatives.

$$\text{Accuracy} = \frac{\text{True Positives} + \text{True Negatives}}{\text{Total Instances}} \quad \textbf{(1)}$$

$$\text{Recall} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}} \quad \textbf{(2)}$$

$$\text{Precision} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Positives}} \quad \textbf{(3)}$$

$$\text{F1 Score} = 2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}} \quad \textbf{(4)}$$

Precision (Eq. 3) focuses on the quality of positive predictions, calculating the proportion of true positives to all predicted positives, which helps reduce false positives. **F1-score** (Eq. 4) combines precision and recall into a single metric by taking their harmonic mean, offering a balanced measure when there is a trade-off between these two metrics. Together, these metrics provide a comprehensive assessment of the models' predictive performance and their ability to grade milk quality accurately and reliably.

Explainable Machine Learning

To provide transparency and interpretability to the predictive modeling of milk grading, both local and global explainability techniques were employed. Local explanations were implemented using LIME (Local Interpretable Model-Agnostic Explanations), which interprets individual predictions by approximating the complex model locally with an interpretable surrogate (Ribeiro et al., 2016). This method identifies the contribution of each feature, such as pH or Temperature, to the classification of a specific milk sample as "good," "moderate," or "bad." These insights are particularly valuable for validating predictions and identifying specific factors influencing individual outcomes.

For a broader understanding, global explanations were provided using Permutation Feature Importance. This technique evaluates the overall significance of each feature by quantifying the drop in model performance when the values of a particular feature are permuted. Unlike traditional feature importance methods that can be biased towards features with more categories, permutation importance provides a corrected measure of feature relevance by considering randomized scenarios, thereby improving reliability and interpretability.

The integration of local and global explainability creates a comprehensive framework, addressing both instance-specific and overall model behavior. This approach corrects biases inherent in traditional feature importance metrics, ensuring that variables critical to the prediction process are accurately ranked (Altmann et al., 2010). Such a dual-layered approach is indispensable in high-stakes applications like milk grading, as it ensures not only the transparency of specific decisions but also the identification of the most influential factors shaping the model's predictions. This enhanced interpretability makes the predictive system a trustworthy and actionable tool for stakeholders.

Global Interpretability with Permutation Importance

Permutation Feature Importance is a model-agnostic technique used to assess the global importance of features in a predictive model. The method involves randomly shuffling the values of a single feature and observing the decrease in the model's performance. The rationale is that if a feature is important, shuffling its values will disrupt the model's predictions, leading to a significant drop in performance. This approach is particularly advantageous as it accounts for non-linear interactions and does not assume any specific model structure.

The importance of a feature X_i is calculated as the difference between the baseline performance of the model (without permutation) and the performance after permuting X_i. Mathematically, the permutation feature importance $I(X_i)$ can be expressed as:

$$I(X_i)=E_{\pi\sim Perm(Xi)}\left[M\left(D_{\pi(Xi)}\right)\right]-M(D) \quad \textbf{(5)}$$

where:

- D: Original dataset.
- $D_{\pi(Xi)}$: Dataset where feature X_i is permuted (shuffled).
- $M(D)$:Model performance metric (e.g., accuracy, F1-score) on the original dataset.
- $M(D_{\pi(Xi)})$: Model performance metric on the dataset with X_i permuted.
- E: Expectation operator to average over multiple permutations π.

This formula captures how much the performance metric changes when the feature's information is disrupted, quantifying its importance in the model's predictive power.

Local Interpretability with LIME

LIME is a technique used to explain individual predictions of complex machine learning models by creating an interpretable local surrogate model around the instance being explained. The idea is to approximate the behavior of the black-box model f in the vicinity of a specific instance x by training a simpler, interpretable model g such as a linear regression or decision tree. This enables understanding of how features contribute to the prediction for x.

The core of LIME involves generating perturbed samples around x and observing the corresponding predictions from the black-box model. The surrogate model g is then fitted to this locally weighted dataset, providing a locally interpretable explanation. Mathematically, LIME optimizes the following objective to train the surrogate model g :

$$\text{argmin}_{g\in G}\ L(f,g,\pi_x)+\Omega(g) \quad \textbf{(6)}$$

where:

- f: The black-box model being explained.
- g: The interpretable surrogate model.
- G: The set of all possible interpretable models.
- $L(f,g,\pi_x)$: The loss function measuring how well g approximates f in the locality of x
- π_x: A locality kernel defining the weight of perturbed samples based on their distance to x.
- $\Omega(g)$: A complexity penalty to ensure g remains interpretable.

LIME's power lies in providing interpretable insights (e.g., feature importance weights) for a single prediction while maintaining flexibility across various model types and datasets.

(Equation 5 is taken from Altmann et al., (2010) and Equation 6 is taken from the main study of Ribeiro et al., (2016).)

RESULTS

Model Performance

The data set was first preprocessed to be suitable for machine learning models and divided 80 by 20 for train/test procedures. The performance of the models is summarised in Table 2. Based on the key evaluation metrics, several models showed strong predictive capabilities for the prediction task.

Table 2. Prediction performance of the models

Models	Accuracy	Recall	Precision	F1
Random Forest	0.995	0.993	0.994	0.994
HistGradientBoost	0.993	0.991	0.992	0.991
KNN	0.985	0.986	0.986	0.986
XGBoost	0.973	0.971	0.972	0.972
LGBM	0.970	0.972	0.973	0.972
SVM	0.566	0.564	0.468	0.489

The Random Forest model demonstrated the highest performance across all metrics, achieving an Accuracy of 99.5%, a Recall of 99.3%, a Precision of 99.4%, and an F1-score of 99.4%. This indicates that Random Forest not only accurately predicts the quality of milk but also maintains an excellent balance between correctly identifying positive cases (recall) and minimizing false positives (precision). Similarly, the HistGradientBoost model showed exceptional results with an Accuracy of 99.3% and an F1-score of 99.1%, proving to be a robust alternative with slightly lower metrics than Random Forest. While KNN, XGBoost and LGBM provide reasonable performance, SVM failed to meet the required predictive quality.

Global Interpretability

The global feature importance analysis, as depicted in the permutation importance graph, provides a comprehensive understanding of the factors influencing the milk grading predictions. The Permutation Feature Importance Graph was used to interpret the influence of the features on the prediction results over the entire dataset (Figure 2). This global interpretation provides insight into how each feature influences the model's milk grade probability predictions. The values on the x-axis are permutation importance scores. The y-axis of the graph contains the features. They are ordered from highest influence to lowest influence from top to bottom.

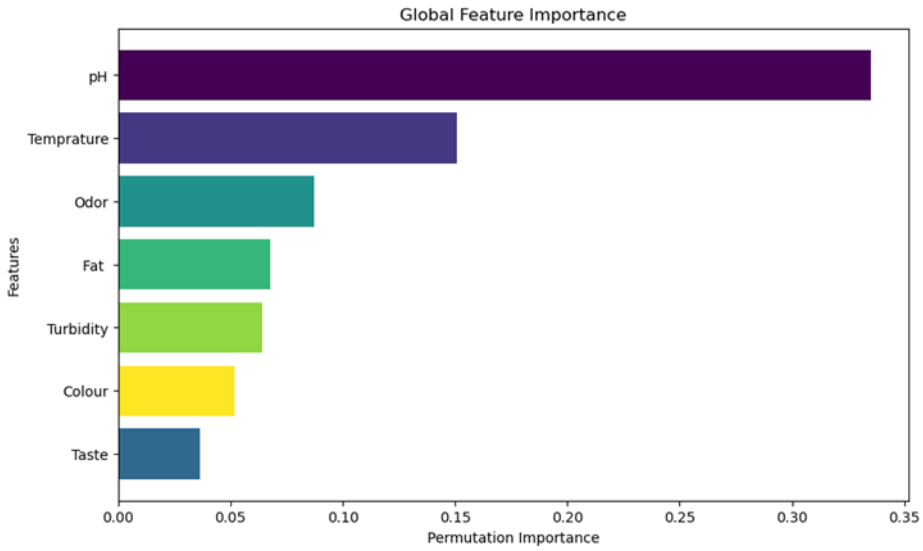

Figure 2: Permutation Importance Graph for Global Explanations

Among the seven independent variables, pH was identified as the most significant feature, showcasing the highest contribution to the model's performance. This result underscores the critical role of pH in determining milk quality, as it directly impacts freshness and spoilage dynamics.

Temperature ranked as the second most important feature, highlighting its pivotal role in maintaining milk quality. The significance of temperature aligns with its known effects on microbial activity and shelf life, making it a key parameter in milk grading.

Sensory attributes such as Odor, Fat, and Turbidity demonstrated moderate importance, reflecting their contributions to both the physical and sensory characteristics of milk. These features play an essential role in assessing milk quality from a consumer and nutritional perspective. Meanwhile, Color and Taste, while contributing to the predictions, were found to have relatively lower importance compared to pH and Temperature, suggesting their secondary role in the milk grading process.

Local Interpretability

Two samples were selected from the data set for local interpretation. Care was taken to ensure that the target variables of the samples had a 'Bad' rating. It will be seen which features of the samples with a 'Bad' rating affect how. In this context, LIME Explanation Graph was used. With this graph, it is possible to obtain information such as Prediction Probabilities, Feature Values, Feature Importance.

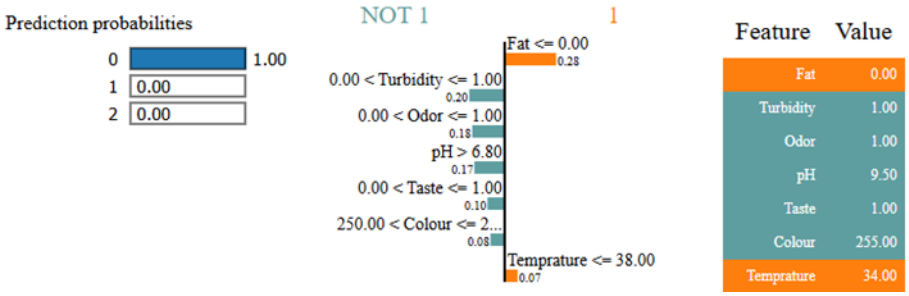

Figure 3: LIME Explanation Graph of Sample 1 (Predicted as "Bad")

The sample in Figure 3 classified as "Bad quality" (Grade 0) was primarily influenced by high turbidity (1.00) and elevated pH (9.50), which deviated significantly from optimal conditions, contributing 20% and 17% to the classification, respectively. Odor (1.00) and taste (1.00) also played significant roles, with contributions of 18% and 11%, indicating suboptimal sensory characteristics. While the fat content (0.00) met optimal conditions, contributing 29% toward the "Not Bad" or "Good" classification, it was insufficient to offset the negative impacts of the other features. The temperature (34.00), though within the normal range immediately after milking (35°C–37°C). Overall, the classification was driven by multiple factors, with turbidity, pH, and sensory attributes playing dominant roles in determining the milk's poor quality.

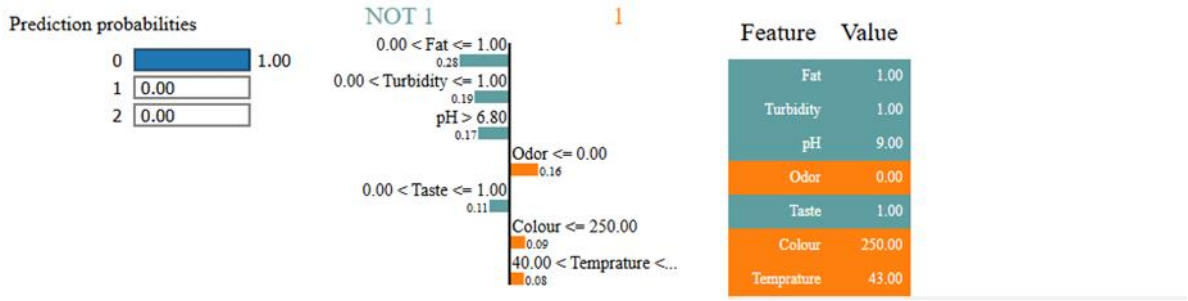

Figure 4: LIME Explanation Graph of Sample 2 (Predicted as "Bad quality")

The second sample (in Figure 4), also classified as "Bad quality" (Grade 0), was primarily influenced by high turbidity (1.00) and elevated pH (9.00), contributing 19% and 17% to the classification, respectively. While the odor (0.00) met optimal conditions, it contributed only 16% toward opposing the "Bad" classification, outweighed by other suboptimal features. Taste (1.00) and fat content (1.00) were suboptimal, with contributions of 11% and 28%, reinforcing the "Bad quality" classification. The temperature (43.00), exceeding but close to the ideal range for milk storage, further contributed 8%, emphasizing the need for rapid cooling after milking. Despite the color (250.00) being close to an acceptable range, it added 9% to the "not bad" classifications. Overall, the combination of turbidity, pH, and temperature, along with sensory attributes, strongly influenced the sample's poor quality classification.

DISCUSSION

This study presents a comprehensive framework for milk quality grading by integrating machine learning models with explainable artificial intelligence (XAI) techniques. The results demonstrate that the Random Forest and HistGradientBoost models outperformed other machine learning algorithms in accuracy, precision, recall, and F1-score. This finding aligns with prior studies, where ensemble learning methods have shown superior predictive capabilities in milk quality assessment (Bhavsar et al., 2023; Sheng et al., 2022). However, the poor performance of SVM highlights the need for algorithm selection tailored to dataset characteristics, such as feature scale and dimensionality (Mu et al., 2020).

The high predictive accuracy ensures reliable milk grading, but accuracy alone is insufficient in safety-critical applications like dairy production. This study addresses the "black-box" nature of machine learning models by integrating XAI techniques, bridging the gap between model performance and interpretability (Islam et al., 2022; Przybył, 2024).

Global explanations using Permutation Feature Importance identified pH and Temperature as the most critical features influencing milk quality. This aligns with scientific evidence highlighting the importance of chemical stability and microbial control in milk (Frizzarin et al., 2021; Polat et al., 2021). For instance, pH values outside the optimal range can indicate spoilage or adulteration, while temperature management is critical for maintaining freshness. These findings underscore the need for real-time monitoring systems that prioritize these parameters.

The moderate contributions of sensory attributes such as Odor, Fat, and Turbidity suggest their complementary role in grading. In practical applications, these features could be integrated into automated quality control systems to supplement chemical and physical measurements. The lower importance of Color and Taste may reflect their subjective nature and the challenge of quantifying these parameters reliably.

The local interpretability results, derived from LIME, provide granular insights into individual predictions, which are invaluable in field settings. For example:

In Case 1, the sample was classified as "Bad" primarily due to high Turbidity and elevated pH. These factors can indicate microbial activity or contamination during storage. For a dairy farm, this insight could prompt a review of milk handling processes or sanitation protocols.

In Case 2, the "Bad" classification was influenced by high temperature and suboptimal Fat content. In this scenario, the results suggest a failure in cooling systems or dietary imbalances in the cattle. These actionable insights enable targeted interventions, such as adjusting cooling equipment or modifying feed compositions.

The integration of global and local interpretability techniques makes this framework highly applicable to field operations. Dairy producers and quality control managers can leverage these insights to address specific issues, such as optimizing storage conditions, detecting contamination, and ensuring compliance with quality standards. Moreover, the transparent nature of these methods fosters trust among consumers and regulators, who require clarity in quality assessment processes (Buyuktepe et al., 2023).

Future studies should explore the scalability of this framework for larger datasets and diverse milk sources. Additionally, combining machine learning with real-time sensors, such as Fourier-transform mid-infrared spectroscopy (Frizzarin et al., 2021) or Raman spectroscopy, could enhance the precision and speed of quality assessments. Expanding the feature set to include advanced biochemical markers may further improve model performance and interpretability.

CONCLUSION

In conclusion, this study highlights the transformative potential of explainable machine learning in milk quality grading. By combining high predictive performance with interpretability, the proposed framework addresses the practical needs of the dairy industry, offering a robust and transparent tool for quality assessment. The insights gained from both global and local explanations not only improve operational efficiency but also pave the way for more trustworthy AI applications in food safety and quality monitoring.

Conflict of interest: The authors have no conflicts of interest to report.

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Ethical approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules. The dataset, obtained from open source database (Kaggle), available at: (https://www.kaggle.com/datasets/prudhvignv/milk-grading). The dataset is licensed under the "EU ODP Legal Notice," which allows for its use and redistribution under open data standards, ensuring compliance with ethical data practices. As the dataset is publicly available, ethically sourced, and used in accordance with its licensing terms, no additional ethical approval was required for this study.

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RESEARCH ARTICLE

Effect of Chemical Castration Using High Osmolarity Solutions on Spermatological Parameters in Rats

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ABSTRACT

In study, the changes in spermatological parameters in rats subjected to chemical castration with high osmolarity solutions were investigated. In study, 8 male rats were used in each group. No chemicals were applied to rats in control group. Rats in second group were sterilized surgically. 0.9% physiological saline was administered to 3rd group, 0.9% physiological saline and 10% calcium chloride to 4th group, 20% mannitol to 5th group, 20% mannitol and 10% calcium chloride to 6th group, 20% dextrose to 7th group, 20% dextrose and 10% calcium chloride solutions were administered intratesticularly to 8th group at dose of 0.1 ml/100gr. Andrological findings compared to control group; it was determined that there was a significant difference in sperm motility and sperm density ($p<0.001$) in groups 3, 5, 7. However, it was observed that there was no difference in rate of abnormal spermatozoon. In groups 2, 4, 6, 8, motility, density, abnormal sperm count and epididymis weights could not be measured because sperm cells could not be collected. In addition, it was determined that the weights of epididymis and right cauda epididymis in groups 3, 5, 7 decreased compared to control ($p<0.05$), while in groups 2, 4, 6, 8, measurements could not be made because tissue samples could not be taken, and weights of testicles, vesicle seminalis and prostate decreased significantly ($p<0.001$) compared to control. In conclusion; it has been concluded that giving high osmolarity solutions in combination with calcium chloride will be much more effective in intratesticular chemical sterilization process.

Key Words: Calcium chloride, Chemical castration, Rat, Spermatological parameters.

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Ratlarda Yüksek Osmolariteli Solüsyonlar Kullanılarak Yapılan Kimyasal Kastrasyonun Spermatolojik Parametrelere Etkisi

ÖZ

Çalışmada, yüksek osmolariteli solüsyonlarla kimyasal kısırlaştırma oluşturulan ratlarda spermatolojik parametrelerin değişimi araştırılmıştır. Çalışmada, her grupta 8 erkek rat kullanıldı. Kontrol grubundaki ratlara herhangi bir kimyasal uygulanmadı. 2. gruptaki ratlar cerrahi olarak kısırlaştırıldı. 3. gruba % 0.9'luk serum fizyolojik, 4. gruba %0.9'luk serum fizyolojik ile %10 kalsiyum klorür, 5. gruba %20 mannitol, 6. gruba %20 mannitol ile %10 kalsiyum klorür, 7. gruba %20 dekstroz, 8. gruba %20 dekstroz ile %10 kalsiyum klorür solüsyonları 0.1ml/100 gr dozda intratestiküler uygulandı. Androlojik bulgular kontrol grubuyla kıyaslandığında; 3, 5, 7. gruplarda sperm motilitesi ve sperma yoğunluğunda ($p<0,001$) önemli derecede farklılık olduğu belirlendi. Ancak anormal spermatozoon oranında fark olmadığı görüldü. 2, 4, 6, 8. gruplarında ise sperm hücresi alınamadığından motilite, yoğunluk, anormal sperm sayısı ve epididimis ağırlıkları ölçümü yapılamadı. Ayrıca 3, 5, 7 gruplarında epididimis ve sağ kauda epididimis ağırlıklarının kontrole göre ($p<0,05$) düştüğü, 2, 4, 6, 8 gruplarında ise doku örneği alınamadığından ölçüm yapılamadığı ve testis, vezikula seminalis, prostat ağırlıklarının ise kontrole göre önemli derecede ($p<0,001$) düştüğü belirlendi. Sonuç olarak; testis içi yapılan kimyasal kısırlaştırma işleminde, yüksek osmolariteli solüsyonların kalsiyum klorür ile kombine olarak verilmesinin çok daha etkili olacağı kanaatine varılmıştır.

Anahtar Kelimeler: Kalsiyum klorür, Kimyasal kastrasyon, Rat, Spermatolojik parametreler.

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INTRODUCTION

In male animals, the termination of reproduction through the partial or complete removal of the testes from the body, or the irreversible or reversible cessation of sexual activity, is referred to as castration, sterilization, orchiectomy, vasectomy, neutering, or emasculation. Castration is commonly performed not only to eliminate reproductive capacity but also to prevent behavioral issues such as urination to mark territory indoors due to pheromone scent marking, or escaping to mate. It is also used to tame working animals, increase productivity in livestock, prevent animals with low fertility from being used as breeders, curb the spread of infectious diseases, and treat deep and complicated testicular injuries, tumors, or various genital disorders (Samsar, 1978; Doğan et al., 2015; Baran et al., 2016).

Animals can be sterilized using open or closed surgical procedures, depending on the species. In veterinary medicine, operative castration is one of the most frequently used methods to prevent reproduction. Although sterilization applies to both male and female animals, castrating males is considered more effective in preventing the fertilization of females. However, surgical castration in male animals may result in various negative effects. Since surgical castration, commonly applied to prevent reproduction and promote fattening in livestock, carries a high risk of complications, there is a growing need to develop alternative methods today (Turk and Ataman, 2016).

From traditional surgical sterilization to present-day practices, there has been a continuous effort to develop new methods to meet the growing need for effective sterilization. In male dogs and cats, non-surgical sterilization techniques such as immunocontraception, suppression of endogenous steroid hormone levels, intratesticular, intraepididymal, and intravas deferens chemical sterilant injections, reproductive toxins, and non-invasive mechanical methods are generally used (Baran et al., 2016).

Commonly used chemicals for chemical sterilization in domestic animals include calcium chloride, lactic acid, sodium chloride, chlorhexidine, formalin, zinc tannate, zinc gluconate, glycerol, glucose, ethanol, and silver nitrate (Başa and Canpolat, 2019). Today, chemical sterilization is increasingly replacing surgical sterilization. In chemical sterilization, drugs are administered directly into the testis and epididymis. These drugs are used intratesticularly to disrupt the structure of testicular tissue and induce atrophy. One of the advantages of intratesticular chemical sterilization is that it results in permanent loss of fertility. It also helps eliminate unwanted sexual behaviors. Chemical castration is considered one of the practical methods that can be performed with a single injection, making it both economical and cost-effective. These solutions can be produced on a large scale and easily applied in the field (Baran et al., 2016).

In recent years, research has continued to seek ideal chemical agents for effective sterilization. This study aims to achieve irreversible chemical sterilization using high-osmolality solutions that are inexpensive and easily accessible. Moreover, due to its ability to be applied to a large number of animals in a short period with minimal financial resources, chemical sterilization presents a strong alternative to surgical methods.

In our study, we aimed to investigate how spermatological parameters change in rats sterilized using low-cost, high-osmolality solutions without the need for surgical intervention. We believe that our research will contribute valuable data to the existing literature.

MATERIALS and METHODS

Research and Publication Ethics

This study was conducted by the decision of the Firat University Animal Experiments Local Ethics Committee dated 23/12/20200 and numbered 202/15, and was deemed appropriate.

Creation of Groups

The study was conducted on 64 Wistar male rats (5-6 months old, 350 g - 450 g) at the Firat University Experimental Animal Center. The rats were kept in plastic cages at 25 ± 2 °C, 55% ± 10% relative humidity, 12 hours of light and 12 hours of darkness, and daily feed (ready pellets) and water were given as ad libitum during the experiment. After a 15-day acclimation period, the animals were divided into 8 groups (8 rats in each group).

The first group was divided into groups as control group, 2nd surgical castration group, 3rd group as intratesticular 0.9% saline injection group, 4th group as intratesticular 0.9% saline and 10% calcium chloride injection group, 5th group as intratesticular 20% mannitol injection group, 6th group as intratesticular 20% mannitol and 10% calcium chloride injection group, 7th group as intratesticular 20% dextrose injection, 8th group as intratesticular 20% dextrose and 10% calcium chloride injection group (Table 1).

Table 1. Table Showing Subject Groups

Groups and Animal Numbers	Applications
Group 1 (8 subjects)	Control group
Group 2 (8 subjects)	Surgical castration
Group 3 (8 subjects)	Intratesticular 0.9% saline injection
Group 4 (8 subjects)	Intratesticular 0.9% saline injection with 10% calcium chloride
Group 5 (8 subjects)	Intratesticular 20% mannitol injection
Group 6 (8 subjects)	Intratesticular 20% mannitol injection with 10% calcium chloride
Group 7 (8 subjects)	Intratesticular 20% dextrose injection
Group 8 (8 subjects)	Intratesticular 20% dextrose injection with 10% calcium chloride

No procedure was applied to the rats in the 1st group used in the study. The rats in the second group of the

study were given general anesthesia with intraperitoneal 5 mg/kg xylazine (Xylazinbio 2%, Ivanovice na Hane, Czech Republic) and 90 mg/kg ketamine (Keta-Control, Istanbul, Turkey). After shaving and disinfecting the testicles, an incision was made in the scrotum and the testicles and spermatic cord were exposed. The spermatic cord was double ligated with 3-0 dexon thread and cut, and the testicles were removed. The scrotum was closed by suturing with 3-0 silk thread. The same procedures were repeated for the other testicle.

After 10 minutes of 5mg/kg xylazine i.m injection to rats in study groups 3, 4, 5, 6, 7, 8, the scrotum was wiped with iodine solution and then 0.1 ml of solutions per 100 g body weight were injected into the testicle in multiple directions with a 27 gauge needle. For study groups 4, 6, 8; 1 gr. calcium chloride (Calcium Chloride Dihyrate 97%, Eschau, Germany) was sterilized at 160 ° C for two hours, then mixed separately with 10 milliliters of sterile 20% mannitol (ready-made medical product, Polifarma, Tekirdağ, Turkey), 20% dextrose (ready-made medical product, Polifarma, Tekirdağ, Turkey) and 0.9% physiological serum (ready-made medical product, Polifarma, Tekirdağ, Turkey) solutions to obtain the desired dose.

The testicles of all rats were evaluated daily for the first week and weekly thereafter for dermatitis, scrotal swelling, discharge, fistula and pain on palpation. In the surgical castration group (group 2), animals were administered 0.1 ml sultamicillin (Sulcid, Istanbul, Turkey) via gavage for 3 days, the operation area was cleaned with antiseptics every day for a week and wound care was performed.

The subjects were euthanized by decapitation at the end of the 60th day. In addition, after euthanasia, testicular tissues were taken from the subjects and spermatological examinations were performed.

Andrological Examinations

At the end of the study, animals in all groups were decapitated under xylazine/ketamine anesthesia. Reproductive organs such as testes, epididymis, seminal vesicles and ventral prostate were removed and cleaned of fatty tissues. Similarly, the right epididymis was used for sperm density determination, and the left epididymis was used for motility and abnormal spermatozoon determination.

Sperm Motility

The slide was placed on the heating table of the microscope and its temperature was allowed to reach 37 °C. After a few drops of Trisbuffer solution [Tris (hydroxymethyl) aminomethane 3.63 g, glucose 0.50 g, citric acid 1.99 g and distilled water 100 ml] were dropped onto the slide on the heating table, 3 microliters of spermatozoon containing spermatozoa taken from the left cauda epididymis were placed on this solution and mixed with the help of a coverslip. Then, motility was determined at 400x magnification. Motility was performed by examining 3 different fields. The average values of these 3 different fields were calculated as the % motility rate (Turk et al. 2008).

Sperm Density

The right cauda epididymis was taken and thoroughly broken into pieces in 1 ml physiological saline (0.9% NaCl) in a petri dish with a scalpel and scissors. Then the particles were thoroughly crushed with a forceps. It was left to incubate for 4 hours at room temperature so that all spermatozoa in the epididymal tissue passed into the liquid. Following the waiting period, the supernatant containing spermatozoa up to the 0.5 line of the red blood cell pipette was drawn from a solution containing 5 g sodium bicarbonate, 1 ml formalin, 25 mg eosin and 100 ml distilled water up to the 101 line. Approximately 10 µl of diluted supernatant was placed on both counting areas (total 400 small squares, 0.1 mm^3 volume) of the Thoma slide to which the coverslip had been previously attached. The Thoma slide was placed under the light microscope and waited for 5 minutes to ensure that the spermatozoa in the solution were distributed homogeneously throughout the area. Spermatozoa falling into all squares in both counting areas were counted at 200x magnification of light microscope. Sperm density in cauda epididymal tissue was calculated (Turk et al. 2008).

Abnormal Sperm Rate

A few drops of Tris buffer solution were dropped onto a clean, dry and pre-warmed (37°C) slide, then a small drop of suspension taken from the left cauda epididymis and a few drops of Eosin-Nigrosin (1.67 g eosin, 10 g nigrosin and 2.9 g sodium citrate for 100 ml distilled water) dye mixture were dropped onto it and mixed with another slide to make it homogeneous. Then, thin smears were taken from this Tris spermatozoon suspension-dye mixture and allowed to dry in air. The dried smears were examined at 400x magnification of light microscope. A total of 200 spermatozoa were examined in a smear and the total abnormal spermatozoon rate was expressed as a percentage (Turk et al. 2008).

d) Weights of Testis and Surrounding Tissues

At the end of the study, the testes and surrounding tissues were removed and separated from each other by removing the fatty tissues. Testis, epididymis, right cauda epididymis, seminal vesicle and prostate tissues were weighed individually on a precision scale and recorded (in grams).

Statistical Analyses

In our study, statistical significance between the groups was evaluated using IBM SPSS Version 22.0 (IBM Corp. Armonk, NY, U.S.A) software for spermatological parameters (sperm motility, sperm density, abnormal spermatozoon rate, testicular and surrounding tissue weights). Shapiro-Wilk normality test was used to determine whether the raw values of all measured parameters showed normal distribution

It was determined that the values of all parameters showed normal distribution.
According to the results of this test, one-way analysis of variance (ANOVA) was used to evaluate group differences and post hoc Duncan test was used to compare binary groups. The findings obtained in the study were expressed as mean and standard error. $P<0.05$ values were accepted as statistically significant.

RESULTS

Clinical Findings

No symptoms of dermatitis, scrotal swelling, discharge, fistula or pain on palpation were observed in the animals. In the animals that underwent intratesticular application, although the testicles were large and hard in volume on the first days, they were not at a level that affected the vital well-being of the animals. No complications occurred in the wounds of the animals in the surgical castration group, and complete recovery was observed at the end of the study.

Andrological Findings

The changes observed in spermatological parameters after the applications are shown in Table 2.

Sperm Motility

Sperm motility rates were found to be significantly lower in the groups that received intratesticular saline, dextrose and mannitol compared to the control group ($p<0.001$) (Table 2).
The decrease in motility was found to be more pronounced in the mannitol group compared to the other groups ($p<0.001$) (Table 2).
Intratesticular physiological saline+$CaCl_2$, dextrose+$CaCl_2$ and mannitol+$CaCl_2$ groups, sperm motility could not be evaluated because there were no cells in the tissue sample (Table 2).

Sperm Density

In intratesticular physiological saline, dextrose and mannitol groups, sperm density was found to be significantly lower compared to the control group ($p<0.001$) (Table 2).
In intratesticular physiological saline+$CaCl_2$, dextrose+$CaCl_2$ and mannitol+$CaCl_2$ groups, sperm density could not be evaluated because there were no cells in the tissue sample (epididymis and ductus deferens that did not contain sperm) (Table 2).

Abnormal Spermatozoa Rate

No statistically significant change was found in abnormal sperm rates in intratesticular physiological saline, dextrose and mannitol groups compared to the control group (Table 2).
Since there were no cells in the tissue samples in the intratesticular physiological serum+$CaCl_2$, dextrose+$CaCl_2$ and mannitol+$CaCl_2$ groups, the abnormal spermatozoon rate could not be evaluated (Table 2).

Weights of Testis and Surrounding Tissues

When the epididymis and right cauda epididymis weights were compared with the control group, they decreased in the groups that were administered intratesticular physiological serum, dextrose and mannitol ($p<0.05$). The decrease in the right cauda epididymis in the group that was administered intratesticular dextrose was found to be insignificant when compared with the control group (Table 2).
When the testis and seminal vesicle weights were compared with the control group, they were found to be significantly lower in all groups ($p<0.001$) (Table 2).
It was determined that the decrease in the testis and seminal vesicle weights was more pronounced in the intratesticular physiological serum+$CaCl_2$, dextrose+$CaCl_2$ and mannitol+$CaCl_2$ groups compared to the other groups (Table 2).
In addition, it was observed that the decrease in testicular weight was more pronounced in the intratesticular dextrose+$CaCl_2$ group ($p<0.001$) (Table 2).
In prostate weight, no difference was observed between the control group and the groups administered intratesticular physiological serum, dextrose, and mannitol, while a significant decrease was observed in the intratesticular physiological serum+$CaCl_2$, dextrose+$CaCl_2$, and mannitol+$CaCl_2$ groups compared to the other groups ($p<0.001$) (Table 2).

Table 2. Table Showing Sperm Motility, Sperm Density, Abnormal Sperm Rate Percentages, Testis, Epididymis, Right Cauda Epididymis, Seminal Vesicle and Prostate Weights in Chemically Castrated Rats.

	Control	SF	Dextrose	Mannitol	SF+$CaCl_2$	Dex+$CaCl_2$	Man+$CaCl_2$	p
Sperm Motility (%)	70,0±5,34^{a}	38,75±7,66^{b}	48,75±4,79^{b}	20,0±5,0^{c}				p<0,001
Sperm Density (million/cauda)	93,87±6,19^{a}	62,12±2,26^{b}	72,75±7,82^{b}	60,37±2,0^{b}				p<0,001
Abnormal Sperm Rate (%)	2,63±0,38	3,25±0,37	3,25±0,25	2,87±0,40				-
Testis (gr)	1,50±0,04^{a}	1,16±0,05^{b}	1,23±0,03^{b}	1,21±0,04^{b}	0,68±0,07^{cd}	0,55±0,04^{d}	0,75±0,08^{c}	p<0,001
Epididymis (gr)	0,63±0,04^{a}	0,49±0,02^{b}	0,52±0,02^{b}	0,56±0,05^{b}				p<0,05
Right Cauda Epididymis (gr)	0,22±0,01^{a}	0,18±0,01^{b}	0,20±0,01^{ab}	0,19±0,01^{b}				p<0,05
Seminal Vesicle (gr)	1,14±0,11^{a}	0,77±0,17^{bc}	1,03±0,15^{ab}	0,58±0,04^{c}	0,18±0,03^{d}	0,20±0,01^{d}	0,23±0,02^{d}	p<0,001
Prostate (gr)	0,46±0,05^{a}	0,38±0,07^{a}	0,45±0,07^{a}	0,43±0,03^{a}	0,08±0,01^{b}	0,08±0,01^{b}	0,08±0,01^{b}	p<0,001

a, b, c, d: Values with different superscripts are statistically different from each other

DISCUSSION

Castration is inevitable for taming work animals, increasing productivity in livestock farming, not using animals with low fertility as breeding stock, preventing the spread of infectious diseases, deep and complicated wounds of the testicles, tumoral cases and various genital organ diseases (Bakır et al. 2006).

There are many methods for sterilization such as surgical castration. In surgical castration; excessive cost, difficulty in postoperative care, use of antibiotics and various postoperative complications create problems in terms of animal welfare (Jana and Samanta 2007; Hassan and Fromsa 2017).

Another alternative method for sterilization is the use of agents that block LH, FSH and GNRH hormones, although it causes testosterone levels and sperm production to decrease to a level that will cause infertility, since the duration of effect is limited and studies show that the ability to fertilize can be restored, the application must be repeated and the suspicion that repeated applications may have side effects make such methods disadvantageous (Bowen 2008; Çevik et al. 2019; Driancourt and Briggs 2020). Intratesticular chemical sterilization has the advantage of creating permanent sterility, having little to no postoperative care, preventing negative sexual behaviors, not causing side effects in target organs, achieving high success when applied by experienced personnel, easy storage and transportation of the chemical agents to be used, and being less costly (Baran et al. 2016). However, it should not be forgotten that animals can be fertile during this period due to the reserve sperm in the epididymis until the 60th day following intratesticular applications and pregnancies can occur after mating (Turk and Ataman, 2016).

In the study conducted by Canpolat et al. (2006a) on intratesticular chemical castration in cattle, no symptoms other than hardness were observed in the testicles after the application of $CaCl_2$. In the study where intratesticular chemical castration was performed on goats, mild swelling was observed in the testicles after intratesticular $CaCl_2$ application. This swelling gradually decreased. The animals continued their lives in good health throughout the study (Jana et al. 2005).

In a study conducted on dogs, swelling was observed in the testicles after intratesticular $CaCl_2$ application and it was observed that the swelling decreased after two days (Canpolat et al. 2006b). In dogs to which $CaCl_2$ was applied, no symptoms were observed except for a 3-4-day testicular hardness (Jana et al. 2005; Leoci et al. 2014a; Leoci et al. 2019). In cats, no undesirable situation was observed except for mild tension in the palpation of the testicles after intratesticular $CaCl_2$ application (Coetzee et al. 2010). In dogs, degeneration was observed in the testicular tubules after intratesticular formalin application (Bakır et al. 2006). Canpolat et al. (2016) showed widespread and significant degenerative changes in the seminiferous tubules of the testicles in the histopathological examination of the testicles on the 60th day after intratesticular application of 20% sodium chloride in dogs.

It was reported that rats tolerated intratesticular $CaCl_2$ well, and there was no restlessness, fever or swelling in the testicles (Jana and Samanta 2006). In a different study, it was stated that no complications were observed after intratesticular $CaCl_2$ application, except fever in the first 3 days. In the studies; it was understood that age, number of applications, and doses of the determined chemicals are the parameters that play an important role in changing testosterone levels (Pařızek 1960; Jana et al. 2005; Canpolat et al. 2006b).

In our preliminary study, a solution was created by dissolving 2g $CaCl_2$ in 10ml physiological serum (%20 $CaCl_2$) and applied intratesticularly. After the application, death was observed in some animals and generally severe necrosis and fistula formation in the testicles.

It was observed that the groups formed with 1g $CaCl_2$ (%10 $CaCl_2$) and other high osmolarity solution applied groups in our study tolerated the solutions well, parallel to the literature. In all groups that received intratesticular application, tension and slight swelling were observed in the testicles in the first days, and the swelling gradually decreased after 48 hours. In the daily checks of the animals, no complications such as restlessness, loss of appetite, fever, inflammation in the testicles, fistula and ulceration were observed. While no undesirable conditions were observed in the surgical castration group, it was observed that wound healing occurred at the end of 2 weeks.

During intratesticular application, the needle tip was directed into the testicle in a cauda-ventral direction, approximately half a centimeter away from the tail of the epididymis, and the injection was made along the determined line. In our study, intratesticular application was made in multiple directions into the testicle, aiming for complete sterilization by completely penetrating the testicular tissue with chemicals. In order to prevent drug leakage into the spermatic cord and surrounding tissues, the appropriate syringe and syringe tip for the animal structure were carefully selected. Care was taken to prevent leakage, and it was thought that it would be more appropriate to wait for a while and terminate the application after the chemical solutions were completely injected into the tissue.

Albino mice were administered cetremide at doses of 5, 10, 15, 20 mg/100 gr. Histopathological examination 30 days after the application revealed degeneration in the testicular tissue tubules and apoptosis in germ cells (Fesseha and Negash 2020).

In another study, it was revealed that intratesticular application of 20% salt solution to Sprague-Dawley rats caused deterioration in the DNA structure of the testis, histopathological examination revealed significant degeneration in the seminiferous tubules, and apoptosis occurred in the testicular cells. It was reported that similar results were obtained between the surgical castration group and the 20% salt solution applied groups (Kwak and Lee 2013).

In another study conducted on rats, 20% salt solution was applied intratesticularly and a significant decrease in testosterone levels was shown after the application. It is thought that 20% salt solution intratesticular application may be an alternative to surgical castration (Dursun 2005). In another study conducted in recent years, 20% mannitol and 20% sodium chloride were applied intratesticularly to rats, and according to spermatological and histopathological results, 20% mannitol application was thought to be an alternative to surgical castration (Maadi et al. 2021).

In our study; 20% dextrose, 20% mannitol solution and 0.9% physiological serum solutions were tried and the aim was to find the best alternative to surgical castration.

Ali et al. (1991) applied a mixture of 25%, 50% and 70% $CaCl_2$ in sterile water intratesticularly to donkeys in their study on donkeys. Considering the measurements of testicular volumes taken on certain days and sperm vitality rates, they predicted that 70% $CaCl_2$ causes infertility in animals. Ibrahim et al. (2016) concluded that although necrosis occurred in the testicular tubules and Sertoli cells were destroyed after intratesticular application of 20% $CaCl_2$ dissolved in pure alcohol in donkeys, since no significant decrease was observed in testosterone levels in donkeys administered 20% $CaCl_2$ dissolved in pure alcohol; this method was ineffective in sterilization in the applied animals.

Our study revealed that there is a connection between the volumetric size of the testicles and the amount of $CaCl_2$ applied, that repeated applications will not be necessary if the correct dose is found, and that the age of the animals, testicular size, weight and the solutions used to dissolve $CaCl_2$ are important criteria for the effectiveness of sterilization in applications with $CaCl_2$. Zeuterin® (Ark Sciences, New York, USA) solution is approved by the FDA in the United States for chemical castration in male dogs. It contains 0.2 M zinc gluconate (13.1 mg zinc/ml) neutralized with 0.2 M zinc arginine (pH 7.0). The same active substance is available for use in both cats and dogs in Latin American countries under the name Esterilsol (Oliveria et al. 2012). Injections were made using calipers for each testicle for dogs of all ages, and the amount of zinc gluconate solution was calculated intratesticularly based on these measurements. Baran et al. applied 0.2 ml of $CaCl_2$ solution into each testicle at different concentrations of 0% (n=1 cat), 5% (n=1 cat), 10% (n=1 cat), and 20% (n=1 cat). Male cats treated with 5% and 10% $CaCl_2$ were oligospermic (<20 million sperm/ml), cats treated with 0% $CaCl_2$ had normal ejaculate (>20 million sperm/ml), while no sperm was found in the ejaculate of a male cat treated with 20% $CaCl_2$, and histological evaluation showed degeneration and calcification in the seminiferous tubules and fibrosis in the interstitial cells on the 60th day after application (Baran et al. 2010).

In a study conducted by Leoci et al. (2014b), they applied $CaCl_2$ solutions prepared with 0.9% physiological saline solution to dogs at doses of 10%, 20%, 30%, and 60% intratesticularly. They showed that sperm motility was close to zero and testosterone levels decreased significantly in the treatment groups. It was concluded that the 20% dose did not cause any complications, but different solvents should be used to increase the sterilization power and duration of effectiveness.

In our study, physiological saline, high osmolarity dextrose, and mannitol solutions were used, and a significant decrease in sperm motility was observed compared to the control groups. It was understood that the decrease was most pronounced in the mannitol group. Again, when sperm density was evaluated, it was determined that there was a significant decrease in the physiological saline, dextrose, and mannitol groups compared to the control groups. In the combined groups with $CaCl_2$, since sperm samples could not be taken (i.e. epididymis and ductus deferens that do not contain sperm), sperm motility and sperm density could not be evaluated. Again, when the groups were evaluated in terms of abnormal spermatozoon rate, no significant difference was found in the physiological serum, dextrose and mannitol groups compared to the control group. Since not even a single sperm cell could be found in the combined groups with $CaCl_2$, an evaluation could not be made in terms of abnormal spermatozoon rate.

Since the testis and surrounding tissues atrophied in the $CaCl_2$+dextrose, $CaCl_2$+mannitol and $CaCl_2$+physiological serum combined groups, even weights could not be taken for andrological examination. In fact, it was found that the testis and surrounding tissues in the $CaCl_2$+dextrose group were the most significant group in terms of shrinkage among the groups. In another study, after intratesticular application of $CaCl_2$ in various doses (ranging from 2.5mg/100g to 20mg/100g) to albino rats, it was determined that the valid and ideal dose was 10mg/100g and 20mg/100g (Jana et al. 2002).

In high doses of $CaCl_2$, fistula structures may form on the testis due to destruction of surrounding tissues and scrotum. In the presented study, it is thought that the combination of 10% $CaCl_2$ with suitable solvents, especially in the $CaCl_2$+dextrose group of our study, can increase the significant decrease in testicular weight, the effectiveness of $CaCl_2$ at low doses, and that sterilization can be achieved without damaging the surrounding tissues of the testis.

In recent studies; the search for an ideal sterilization with chemical drugs continues. In our study, high osmolarity mannitol and dextrose were used to find a chemical that could solve this problem. As a result; In this study, it was tried to show that an ideal chemical castration can be done with easily available and low-cost high osmolarity solutions.

In addition, in the $CaCl_2$ combined groups, the testicles and surrounding tissues atrophied, therefore sperm production completely stopped. It was observed that the group with the most positive results among the combined groups was the $CaCl_2$+Dextrose group, and it is predicted that effective castration can be done by using small amounts of $CaCl_2$.

CONCLUSION

The most important aspect of our study; It was concluded that cats and dogs, whose populations are rapidly increasing in our country, can be castrated with easily accessible chemicals without wasting time with a simple injection, complications can be minimized with appropriate doses, and it is possible to perform irreversible castration with a single dose application in terms of animal welfare. We believe that the results of our study will provide a new perspective and create a resource while chemical castration research is being conducted.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: ŞÖK, İC and HA contributed to the project idea, design and execution of the study. ŞÖK, İC and HA contributed to the acquisition of data. ŞÖK, İC and HA analysed the data. ŞÖK, İC and HA drafted and wrote the manuscript. ŞÖK, İC and HA reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This study was carried out at Firat University Research Animals Application Center. This research was approved by Fırat University Animal Experiments Local Ethics Committee, (With the decision of the ethics committee dated 23/12/2020 and numbered 2020/15).

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RESEARCH ARTICLE

Detection of The Presence of Leptospirosis in Horses by ELISA Method

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ABSTRACT

Leptospirosis is one of the common zoonotic diseases worldwide. It significantly affects humans and animals, causing socio-economic losses. Equine leptospirosis often manifests itself with recurrent genital tract infections, reproductive disorders, abortion, embryonic death, and birth of weak foals. Studies on Leptospirosis, especially in horses, are quite limited. For this reason, our study was planned to investigate the transmission cycle in terms of farm animals and public health, and also to determine the presence of Leptospirosis in horses. For our research, 187 horse blood serum samples were collected from different settlements in Turkey. In the collected samples, *Leptospira* spp. ELISA test was used to investigate the presence of antibodies. A total of 8 samples (4.27%) were detected as seropositive in horse serum samples. The agent, a significant pathogen for both human and animal health, was identified as seropositive. In addition, the high rate of transmission of leptospirosis through contact with infected horses and the potential for spread through different animals should be taken into consideration. Leptospirosis was determined as seropositivity by ELISA in the horses sampled. Therefore, it is important to diagnose and control the infection rapidly.

Keywords: ELISA, Horse, Leptosipirosis, Serologic Test, Zoonoses

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Atlarda Leptospirosis Varlığının ELISA Yöntemi ile Tespiti

ÖZ

Leptospirosis dünya çapında yaygın zoonotik hastalıklardan biridir. İnsanları ve hayvanları önemli ölçüde etkileyerek sosyo-ekonomik kayıplara neden olur. At leptospirozu sıklıkla tekrarlayan genital sistem enfeksiyonları, üreme bozuklukları, abort, embriyonik ölüm ve zayıf tayların doğumu ile kendini gösterir. Özellikle atlarda Leptospirozis üzerine yapılan çalışmalar oldukça kısıtlıdır. Bu sebeple, çiftlik hayvanları ve halk sağlığı açısından bulaşma döngüsünü araştırmak, aynı zamanda atlarda Leptospirozis'in varlığını belirlemek amacıyla çalışmamız planlanmıştır. Araştırmamız için Türkiye'nin farklı yerleşim yerlerinden 187 adet at kan serumu örneği toplandı. Toplanan örneklerde, *Leptospira* spp. antikorlarının varlığını araştırmak için ELISA testi kullanıldı. At serum örneklerinde toplam 8 örnek (%4,27) seropozitif olarak tespit edildi. İnsan ve hayvan sağlığı için önemli bir patojen olan etken seropozitif olarak teşhis edilmiştir. Ayrıca, leptospirozis hastalığının enfekte atlarla temas yoluyla bulaşma oranının yüksek olması ve farklı hayvanlar ile yayılma potansiyeli göz önüne alınmalıdır. Örnek alınan atlarda Leptospirosis ELISA ile seropoziti olarak belirlenmiştir. Bu nedenle enfeksiyonun hızla teşhis edilerek kontrol altına alınması önem taşımaktadır.

Anahtar Kelimeler: At, ELISA, Leptosipirosis, Serolojik Test, Zoonozlar

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INTRODUCTION

The epidemiology of leptospirosis is complex as it occurs in humans, domestic and wild animals. Leptospirosis exhibits a complex epidemiological cycle involving humans, domestic animals, and wildlife populations. There are a wide variety of animal species that can be seen as potential reservoirs for Leptospirosis disease, which is a common zoonosis, as in Brucellosis infections. (Andersen-Ranberg et al., 2016; Kaya et al., 2017; Kepenek et al., 2021). Leptospiral infection can have fatal consequences and the disease can cause abort and other reproductive problems in horse, cattle, goats and pigs. In humans, symptoms range from subclinical infection to acute febrile illness, pulmonary hemorrhage, and renal failure (Yamaguchi et al., 2018).

Most animals are either reservoir hosts that chronically retain leptospira in their kidneys or are incidental hosts. (Levett et al., 2001). According to epidemiological information to date, rodents are identified as the primary reservoir host for Leptospirosis and other different zoonotic infections (Levett et al., 2001; Ayçiçek, 2004; Temel et al., 2020). Rodents are considered the primary reservoirs of Leptospira and play a key role in the transmission of leptospirosis to other hosts such as horses, domestic animals, dogs, and humans (Levett et al., 2001). Climate changes also contribute to the transmission cycle. Especially floods, disproportionately increasing temperatures, poor hygiene conditions, housing problems due to inadequate economic conditions, large numbers of mice and other reservoir animals cause Leptospirosis. Many of the areas known to have high incidence of leptospirosis have been affected by these factors (Lau et al., 2010). Leptospirosis can spread to the environment, especially through the urine of animals. Seropositive animals in the population are also thought to contribute to the transmission cycle through genital and urinary secretions. (Yamaguchi et al., 2018).

In the diagnosis of Leptospirosis, ELISA tests and microagglutination tests (MAT) are used to test for the presence of Leptospirosis in individual animals and herds. It can be used to confirm the diagnosis, especially in samples. (Mulla et al., 2006). In particular, screening herds and detecting antibodies with these serological tests is a very useful method for animal and human health. Leptospirosis serovars are classified into 28 serogroups based on serological similarity. These serovars were identified using cross agglutination absorption testing (CAAT). According to previous reports, different methods are used for serological diagnosis of Leptospirosis. It has been reported that specificity issues between these serological tests can be overcome by treating an antigen produced by acetic acid extraction with various enzymes or by using Mab-based competitive ELISA (Costa et al., 2012; Saito et al., 2013; Bourhy et al., 2014).

The objective of this study is to ascertain the presence of leptospirosis in equine blood sera by employing the ELISA method, a serological test.

MATERIAL and METHODS

Blood Sera Sampling

Horse blood samples were collected from Konya (n=35), Mersin/Tarsus (n=50), Adana (n=23), Mersin/Mezitli (n=17)Niğde/Kemerhisar(n=62). A total of 187 horse blood samples were collected in February 2024. The horses included in the study were 187 horses aged 24 months and over between. Horses were kept on different farms. According to the information received from farm owners from time to time, cases of fever, genital system infections were seen (Figure 1).

Figure 1: Locations of farms where horse blood serum is collected

Method

Serological analysis

Blood serum samples were collected from farms in different settlements across Türkiye. The samples were then subjected to a centrifugal process at 1000g for a duration of 20 minutes. Thereafter, the samples were stored at a temperature of -20°C until utilisation. Blood samples brought to the laboratory were analyzed after separating their serums. For the qualitative detection of IgG class antibodies against Leptospira spp., the Horse Leptospira spp. Antibody ELISA Kit (Abbexa, United Kingdom) was used. The test was performed according to the manufacturer's recommendations.

All samples were run in 96-well microtiter plates for ELISA testing. Optimal working concentrations/dilutions of antigens/antibodies or reagents were determined by titration. Standards/controls and diluted samples (diluted 5-fold) were dispensed into microplate wells in a volume of 50 µl and plates were covered with plate sealer. Incubated for 30 minutes at 37°C. Cover were removed and washed the plate 5 times with 1X Wash Buffer. After washing, 50 µl of Detection Reagent to each well were added. Plate were covered with a plate

sealer and incubated for 30 minutes at 37°C. Then cover were removed, liquid were discarded and repeated the wash process as described, 5 times. 50 µl of TMB Substrate A and 50 µl of TMB Substrate B were added into each well. The plate were covered with the plate sealer and gently tapped the plate to mix thoroughly. Incubated at 37°C for 10-15 minutes, avoided exposure to light. 50 µl of Stop Solution were added for ending process. The results were read at 450 nm on the ELISA reader. ELISA optical density (OD) reading were transformed to serum / positive percentage (S / P) according to a specific equation cited by manufacturer (Abbexa, 2024).

Interpretation of ELISA Results

Mean OD of the Positive Control should be ≥ 1.0.
Mean OD of the Negative Control should be ≤ 0.2.
CUT OFF value = Negative Control + 0.15

Ethics statement

This research was performed with the permission of SÜVDAMEK dated 01.02.2024 and numbered 2024/006.

RESULTS

Eight (4.27%) of 187 horse blood sera samples were seropositive for Leptospirosis. These findings suggest that leptospirosis is present in horses in these provinces (Table 1).

Table 1. Seropositivity results of horse blood sera tested by ELISA

Location	Number of Horses	Number of Seropositive Horses	Seropositive Percentages
Adana	23	1	0.53%
Tarsus/Mersin	50	3	1.60%
Mezitli/Mersin	17	-	-
Ereğli/Konya	35	1	0.53%
Kemerhisar/Niğde	62	3	1.60%
Totaly	187	8	4.27%

DISCUSSION

Leptospirosis can be observed in many domestic and wild mammals, primarily cattle, sheep, dogs, and horses. The disease may appear in various clinical forms in animals. In general, it manifests with clinical signs such as fever, anorexia, jaundice, abortion, and organ involvement. In horses, leptospiral infections are particularly characterized by abortion, infertility, genital tract infections, renal disorders, and uveitis in the eyes. It can cause significant economic losses and serious animal health problems. Therefore, leptospirosis in horses is an infection that should be carefully considered in terms of both herd health and its zoonotic potential (Ellis, 2023; Šimpraga et al., 2024)

Leptospirosis often causes a mild and self-limiting febrile disease in humans, but it can reach lifethreatening severity in rare cases. Due to its systemic course, various organs may be affected during the disease. Jaundice and renal failure, called Weil's disease, are the most clinically significant manifestations of leptospirosis.

Leptospirosis poses a worldwide problem for both animal and public health, affecting humans, domestic animals, wildlife and even reptiles, spanning many species and serovars. Seropositivity rates for leptospirosis vary widely depending on the species, geographic region, and the population studied. Generally, seropositivity rates in humans range between 5% and 20%, while these rates tend to be higher in animals, reaching up to 30% in some species. (Levett et al., 2001; Ellis 2014; Pissawong et al., 2020).

Cases of leptospirosis are also commonly reported in coastal countries of the Mediterranean and in Africa. Leptospirosis Seropositivity was observed in 1.3% of human samples in Morocco. Epidemiological assessments have highlighted that there is a significant increase in seropositivity rates in areas with high rodent numbers and poor hygiene conditions. (Bourhy et al., 2014).

The clinical presentation of Leptospira infection in horses varies, often making definitive diagnosis difficult. The majority of equine cases are asymptomatic. Clinical signs are mostly related to the mare's reproductive system and kidneys. Studies on this have been reported (Ellis et al., 2014; Divers et al., 2019). In addition, Leptospira infection contributes to the development of recurrent uveitis in horses, which appears to be the most important clinical outcome (Lowe, 2010; Spiess, 2010).

In 2021, Wasiński et al. demonstrated the serological presence of leptospirosis in Arabian horses. They found a very high rate of 33.2% seropositivity. In this study conducted on randomly selected horse populations, despite high Leptospira seropositivity, it

was clinically reported that the number of cases in the herd was low. For this reason, they thought that the disease was exposed to different ways. Additionally, when this study is compared to ours, there are large differences in climate in selected regions. Although seropositivity has been detected in horses on many farms, especially in a tropical region such as the Mediterranean region, seropositivity values do not show parallelism with this study. The areas we took as a sample have a much milder climate than Poland. We know that leptospira is actually a tropical climate bacteria. Here the different result emerges. The authors supported idea that very high seropositivity has been found in the region due to the increase in Leptospira carrier populations. Moreover, many horses may have only been exposed to this infectious disease for a short period of time. Therefore, it has been reported that horses may have merely seroconverted without any clinical consequences. Another point awaiting clarification is the occurrence and duration of infections in horses that do not show clinical signs. In our study, Eight (4.27%) of 187 horse blood sera samples were seropositive for Leptospira. Although regionally low seropositivity was shown, the presence of the disease was revealed.

In our study, the samples were taken on February in a cold climate also affected the results. In addition, previously reported genital tract infections and fever were not observed at the time the sample was taken. These symptoms have been seen from time to time on the mentioned farms. Therefore, even if horses do not have any clinical symptoms, previous exposure to the infection is evident. In parallel with this study, seropositivity has serologically demonstrated the presence of the disease, even though no clinical symptoms are observed. It should not be ignored that horses with leptospirosis seropositivity may pose a risk, especially for people involved in care and nutrition.

Microscopic Agglutination Test (MAT) is considered as the reference test for the diagnosis of leptospirosis as it allows the detection of serovar-specific antibodies with high sensitivity and specificity. However, it has some difficulties due to its reliance on live Leptospira cultures and technical complexity.

In 2014, Ye et al. compared the Microagglutination test and ELISA test in horse blood serum. They emphasized that the use of some antigens in the ELISA test is more sensitive and specific. They reported that the ELISA test was easier to apply, especially among serological tests, and the results were compatible with the standard. Our aim for our study was to quickly detect the disease. Although there is no comparison between serological tests such as this study in terms of specificity, we can say that the ELISA test gives very easy and fast results.

ELISA tests offer many advantages over MAT (Microagglutination Test). It uses non-hazardous reagents, relatively sensitive and specific. In addition, the stringent quality control criteria established for this test ensure the reproducibility of objectively interpreted results. The use of frozen antigen-coated plates is relatively long-lasting. Because of these advantages described, ELISA could potentially be used as a screening test for leptospirosis and other spesific diseases. Serum that scores positive in this screening ELISA can then be tested with serovar-specific ELISAs or MAT to identify the infecting serovar. Today, this method has been applied to both leptospira and other reported infections (Niloofa et al., 2015 ;Behera et al ., 2022).

Complementary serological tests are very important, especially when considering cases where MAT gives negative results when clinical symptoms indicate the disease (Haake et al., 2015; Day, 2021). ELISA tests are reported as complementary serological tests, especially with MAT (Levett et al., 2001, Haake et al., 2015; Day, 2021). For this reason, ELISA tests are important for the early diagnosis of acute Leptospirosis infection and detect immunoglobulin M antibodies, which can be detected before MAT produces a positive result (Goris et al., 2012; Tan et al., 2014, Kaya, et al., 2017). Previous studies have reported that MAT is less reliable than ELISA tests in chronic leptospiral infections (Who, 2003; Day, 2021).

The ELISA test offers numerous advantages in the serological diagnosis of leptospirosis. Its rapid turnaround time, high sample throughput, and compatibility with automation provide practicality in field studies and large-scale population screenings. ELISA tests, based on antigen-antibody interactions, enable reliable detection of the specific immune response elicited by the infection. Additionally, ELISA requires less technical expertise and does not depend on live Leptospira cultures, thereby enhancing laboratory safety and ease of implementation. The ELISA test offers several advantages in the serological diagnosis of leptospirosis in animals. A study conducted in Egypt analyzed 600 bovine serum samples using an ELISA kit. The results demonstrated that ELISA is a reliable method for detecting leptospiral antibodies and provides a practical alternative to traditional diagnostic techniques (İbrahim et al., 2022). Furthermore, research carried out in South Sudan highlighted the importance of serological methods such as ELISA for epidemiological surveillance of Leptospira spp. in cattle (Rosa et al., 2017). Collectively, these studies underscore the role of ELISA in enhancing the accuracy and efficiency of leptospirosis diagnosis in veterinary practice. While planning our research, we took these studies as a reference, with the primary aim of investigating the presence of infection in the region using the ELISA test. Due to its practicality, safety, ability to rapidly detect specific antibodies, and compatibility with automation, ELISA proved to be a particularly suitable method, especially under field conditions. In this respect, our findings are consistent with the

aforementioned studies and confirm that ELISA is an advantageous test for the serological diagnosis of leptospirosis in animals.

Leptospirosis, which is endemic in many tropical regions, can cause major epidemics, especially after heavy rains and floods, and is more common in individuals who have contact with contaminated water or soil (Saito et al., 2013). Professional groups such as veterinarians, slaughterhouse workers, farmers, hunters and laboratory workers who have direct contact with infected animals in daily life are at risk for leptospirosis found leptospirosis positivity in only two of 102 slaughterhouse workers in Ankara (Türkiye) (Babür et al., 2003). These results emphasize the need to protect people, especially those in the risk group, from Leptospirosis disease.

CONCLUSION

In our study, the presence of Leptospirosis seropositivity in horses were revealed by ELISA method. Especially for leptospirosis, environment, transmission routes and climatic factors are very effective. To prevent leptospirosis in horses, good hygiene practices, minimizing contact with rodents, and vaccinating other species and pets are important.

The incidence and significance of leptospirosis in horses remains unclear. The incidence reported in almost all epidemiological studies varies greatly depending on the geographical region examined. Large-scale studies are needed to evaluate the disease from a general perspective and offer solution suggestions.

Conflict of interest: The authors declared that there is no conflict of interest.

Authors' Contributions: Conceptualization, DKY. writing-original draft preparation, DKY. and YP. writing-review and editing, DKY. YP. DA. and AB. All authors reviewed and approved the final version of the manuscript.

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RESEARCH ARTICLE

The Effects of Thyme Oil and Thymol on Hepatic Gene Expression Levels in Rabbits with High Cholesterol Diet-Induced Hepatic Lipidosis

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ABSTRACT

Rabbits fed a high cholesterol diet (HCD) exhibit various physiopathologic features of hepatic lipidosis. This study aimed to investigate the effects of thyme oil (TO) and thymol (T) on inflammation-related hepatic gene expression in a rabbit model of high cholesterol diet–induced hepatic lipidosis. Male New Zealand rabbits were divided into six groups. The groups were: Standard rabbit diet (SD, n= 8), Standard rabbit diet + thymol (SD+T, n= 8), Standard rabbit diet + thyme oil (SD+TO, n= 8), High cholesterol diet (HCD, n=8), High cholesterol diet + thymol (HCD+T, n= 8), and High cholesterol diet + thyme oil (HCD+TO, n= 8). Blood samples were collected at weeks 0, 4, 8, and 11 of the study. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels were analyzed. The mRNA expression levels of inflammation-related genes from liver tissue were analyzed by the real-time polymerase chain reaction (RT-PCR) method. The expression levels of *interleukin 4* (*IL4*), *IL17* and *interferon gamma* (*IFNγ*) genes were lower, whereas the expression levels of *IL9*, *IL13*, *IL18* and *RAR-related orphan receptor gamma* (*RORγ*) genes were higher in rabbits fed with HCD compared to the normal diet group ($p < 0.05$). Thymol increased *T-box transcription factor* (*Tbet*), *IL4*, *IL17A,* and *IL18* gene expression. Thyme oil increased *GATA-binding protein 3* (*Gata3*)and *IL18* gene expression. In conclusion, an HCD successfully established a non-obese rabbit model of hepatic lipidosis characterized by microvesicular steatosis, liver injury, and immune gene alterations. Thyme oil and thymol modulated immune responses by affecting key cytokine expressions but did not improve lipid profiles or liver histopathology. These results suggest that the immunomodulatory effects of thyme compounds are complex and their therapeutic potential may depend on specific disease contexts and treatment parameters. Further research is needed to clarify their role in hepatic lipidosis management.

Keywords: Hepatic lipidosis, High cholesterol diet, Hypercholesterolemia, Thyme oil, Thymol

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Yüksek Kolesterol Diyetiyle İndüklenmiş Hepatik Lipidozisli Tavşanlarda Kekik Yağı ve Timolün Karaciğer Gen Ekspresyonu Düzeylerine Etkisi

ÖZ

Yüksek kolesterol diyetiyle beslenen tavşanlar hepatik lipidozisin çeşitli fizyopatolojik özelliklerini gösterirler. Bu çalışmada, yüksek kolestrol diyeti ile indüklenmiş non-obez hepatik lipidozis tavşan modelinde, kekik yağı (TO) ve timol'ün (T) inflamasyonla ilişkili hepatik gen ekspresyon seviyeleri üzerine etkisinin araştırılması amaçlandı. Erkek Yeni Zelanda ırkı tavşanlar altı gruba ayrıldı: Standart tavşan yemi (SD, n= 8), Standart tavşan yemi + timol (SD+T, n= 8), Standart tavşan yemi + kekik yağı (SD+TO, n=8), Yüksek kolesterol diyeti (HCD, n= 8), Yüksek kolesterol diyeti + timol (HCD+T, n= 8) ve Yüksek kolesterol diyeti + kekik yağı (HCD+TO, n= 8). Çalışma süresince 0., 4., 8. ve 11. haftalarda kan örnekleri alındı. Total kolesterol (TC), yüksek yoğunluklu lipoprotein kolesterol (HDL-C), düşük yoğunluklu lipoprotein kolesterol (LDL-C) ve trigliserit (TG) düzeyleri analiz edildi. Karaciğer dokusundan inflamasyonla ilişkili genlerin mRNA ekspresyon seviyeleri gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) yöntemiyle analiz edildi. Yüksek kolesterol diyetiyle beslenen tavşanlarda, normal diyet grubuna kıyasla *interlökin 4* (*IL4*), *IL17A* ve *interferon gamma* (*IFNγ*) geninin ekspresyon seviyesi düşük, *IL9*, *IL13*, *IL18* ve *RAR-related orphan receptor gamma* (*RORγ*) genlerinin ekspresyon seviyesinin yüksek olduğu belirlendi ($p< 0.05$). Timol *T-box transkripsiyon faktörü* (*Tbet*), *IL4*, *IL17A* ve *IL18* gen ekspresyonunu artırmıştır. Kekik yağı *GATA bağlayıcı protein 3* (*Gata3*) ve *IL18* gen ekspresyonunu artırmıştır. Sonuç olarak, yüksek kolesterol içeren diyet, mikrovakuoler steatoz, karaciğer hasarı ve immün gen ifadelerinde değişikliklerle karakterize edilen, obez olmayan bir tavşan hepatik lipidozis modeli başarıyla oluşturmuştur. Kekik yağı ve timol, önemli sitokin ekspresyonlarını etkileyerek bağışıklık yanıtlarını modüle etmiş, ancak lipid profilleri veya karaciğer histopatolojisini iyileştirememiştir. Bu sonuçlar, kekik bileşiklerinin immün modülatör etkilerinin karmaşık olduğunu ve terapötik potansiyellerinin hastalığın özel bağlamı ve tedavi parametrelerine bağlı olabileceğini göstermektedir. Hepatik lipidozis yönetimindeki rollerini netleştirmek için daha fazla araştırmaya ihtiyaç vardır.

Keywords: Hepatik lipidozis, Hiperkolesterolemi, Kekik yağı, Timol, Yüksek kolesterol diyeti

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INTRODUCTION

Epidemiological studies show that a high cholesterol diet (HCD) is linked to hepatic lipidosis (Kainuma et al. 2006). Rabbits fed an HCD show various physiopathological features of hepatic lipidosis (Kim et al. 2014; Kim et al. 2017). The hypercholesterolaemic rabbit model is also widely used in atherosclerosis studies due to their high sensitivity to dietary cholesterol and rapid development of atherosclerosis (Lozano et al. 2019). Since this model does not show insulin resistance or obesity, it is mainly used in studies of non-alcoholic fatty liver disease (NAFLD) related to hyperlipidaemia (Kainuma et al. 2006). In rabbits, fatty liver is induced by feeding a HCD (1% w/w) for two weeks or a low cholesterol diet (LCD) (0.3% w/w) for 16 weeks, and the livers of these rabbits show typical pathological features of hepatosteatosis (Kainuma et al. 2006; Kim et al. 2017). Cholesterol overload stimulates de novo lipogenesis by increasing liver X receptor-α (LXRα) expression and activates fatty acid synthesis by increasing the level of oxysterol, a metabolite in the sterol regulatory element binding protein-1c (SREBP-1c) pathway (Kainuma et al. 2006; Lozano et al. 2019). Inflammatory stress also exacerbates hepatic cholesterol accumulation by impairing cellular cholesterol export (Kainuma et al. 2006; Kim et al. 2017; Lozano et al. 2019).

Thyme (*Thymus vulgaris* [*T. vulgaris*]) is widely recognized as a therapeutic plant due to its biological and pharmacological qualities (Prasanth Reddy et al. 2014). In traditional medicine, leaves and flowering portions of Thymus species are commonly used as a tonic and herbal tea, antiseptic, antitussive, and carminative, as well as for treating colds (Prasanth Reddy et al. 2014). Thyme oils (TO) and extracts are widely utilized in the medicinal, cosmetic, and perfume industries, as well as for flavoring and preserving a variety of foods (Prasanth Reddy et al. 2014). Thymol (isopropyl-5-methylphenol) is a natural monoterpene phenol obtained from thyme species (Marchese et al. 2016). Thyme and thymol, one of its active components, have antioxidant, anti-inflammatory, anti-bacterial, anti-fungal, anti-cancer activity, hepatoprotective properties, immunomodulatory and platelet aggregation inhibitor properties (Rašković et al. 2015; Marchese et al. 2016; El Boshy et al. 2019; Lee et al. 2023; Sheng et al. 2024). It has been stated that thyme may be a promising natural therapeutic drug in improving intestinal conditions associated with obesity and high-fat diet (Lee et al. 2023). Thyme polyphenol-rich extract substantially reduces high-fat diet-induced NAFLD via regulating the gut-liver axis, focusing on gut microbiota and bile acid metabolism (Sheng et al. 2024). Thyme extract reduced lead-induced stress in hepatic and renal tissues and showed promise as an immunomodulator, antioxidant, and protective agent against lead toxicity (El Boshy et al. 2019). Thyme preparations exerted antioxidant effects in the liver by preventing carbon tetrachloride-induced increase of lipid peroxidation (Rašković et al. 2015).

Although the aetiology of NAFLD is multifactorial and remains largely enigmatic, it is well established that inflammation is a central component of NAFLD pathogenesis. Inflammation disrupted PPAR-LXR-CYP7A1/ABCA1-mediated bile acid production and cholesterol efflux, leading to increased cholesterol buildup in the livers and HepG2 cells (Chen et al. 2012). In addition, excessive cholesterol accumulation in cells may exacerbate NAFLD by inducing endoplasmic reticulum (ER) stress, oxidative stress, and apoptosis (Senokuchi et al. 2008). Thyme extract may suppress TNF-α, IL-6, and other inflammatory cytokines. It has the potential to protect against disease-related consequences and be used as a therapeutic agent (Nadi et al. 2023). Thyme extract significantly suppressed the synthesis and gene expression of inflammatory mediators, whereas it upregulated both synthesis and gene expression of the anti-inflammatory cytokine IL-10 (Ocaña et al. 2012). Thyme extract acts as an anti-inflammatory drug by scavenging nitric oxide radicals that contribute to the beginning of inflammatory conditions and greatly inhibiting the production of inducible nitric oxide synthase mRNA (Vigo et al. 2004). Thyme polyphenol-rich extract improves intestinal barrier function and reduces inflammation by increasing tight junction protein expression (ZO-1 and occluding) and inhibiting the TLR4/NF-κB pathway in high-fat diet-fed rats (Sheng et al. 2023). The roles of critical immune mediators, particularly inflammation-related genes, as well as loci of immune activation, immune signalling pathways, and mechanisms underlying disease progression, are still poorly understood. Knowledge of genes associated with inflammation in the liver, particularly in a high-cholesterol diet–induced hepatic lipidosis model, may help to identify and develop new therapeutic targets. Therefore, this study aimed to investigate the effects of thyme oil and thymol on inflammation-related hepatic gene expression in a rabbit model of high cholesterol diet-induced hepatic lipidosis.

MATERIAL and METHOD

Ethics Committee

Ethics committee approval was received for this study from the Animal Experiments Local Ethics Committee of Erciyes University (No: 20/069).

Animals

A total of 48 healthy male New Zealand rabbits with an average weight of 3.41±0.56 kg and 10-12 weeks of age were used in the study. The rabbits were housed in single 60x60x30 cm rabbit cages at Erciyes

University Experimental Research and Application Center (DEKAM) during the 15-day adaptation period and throughout the study. During the study, the daily diet of each animal was restricted to 100 g to maintain consistent final body weights across all groups. Water was provided ad libitum. Rabbits were kept in single cages with appropriate ventilation, standardized light (12 hours light/12 hours darkness daily), and temperature (22±1 ºC) conditions throughout the experiment.

After a 2-week adaptation period, the rabbits were randomly divided into 2 groups: standard diet (SD) and high cholesterol diet (HCD). The rabbits in the SD groups were fed a standard rabbit diet (Optima Besin Maddeleri San. ve Tic. A.Ş, Lüleburgaz/Kırklareli). Rabbits in the high cholesterol diet (HCD) groups were fed a standard rabbit dietsupplemented with 1.0% (wt/wt) cholesterol. These groups (SD, HCD) were then divided into 3 subgroups (8 rabbits/group). The standard diet (SD) group was fed only standard rabbit chow for 11 weeks; standard diet (SD) + thymol (SD+T) group, standard rabbit diet + thymol (6 mg/kg, oral) for 11 weeks; standard diet + thyme oil (SD+TO) group, standard rabbit diet + thyme oil (0.1mL, oral) for 11 weeks); high cholesterol diet group (HCD), fed standard rabbit chow containing 1% (wt/wt) cholesterol [≥92. 5% (GC), Sigma-Aldrich]; high cholesterol diet + thymol group (HCD+T), fed standard rabbit chow containing 1% cholesterol + thymol (6 mg/kg, oral) for 11 weeks; high cholesterol diet + thyme oil group (HCD+TO), fed standard rabbit chow containing 1% cholesterol + thyme oil (0.1mL, oral) for 11 weeks.

Measuring Body Weights

The body weights of all rabbits were recorded using a digital weighing scale (PLUSMED pM-BS01) at weeks 0, 4, 8, and 11 during the study.

Blood Sample Collection

A 3.5 mL blood sample was collected from the marginal ear vein of rabbits into blue-capped vacuum tubes containing 3.2% (0.109 M) sodium citrate (BD Vacutainer®, Becton Dickinson, USA). Additionally, 2 mL of blood was drawn from the same vein into yellow-capped serum separator tubes (BD Vacutainer®, Becton Dickinson, USA). Blood samples were centrifuged at 3000 × g for 15 minutes (ROTOFIX 32A, Hettich, Germany), and the resulting serum and plasma were aliquoted into 0.5 mL portions and stored at -20°C under appropriate conditions.

Analyses of Lipid Parameters

Analyses of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were performed by the Gülser-Dr. Mustafa Gündoğdu Central Laboratory at Erciyes University, through service procurement.

Gene Expression Analyses

At the end of the experiment (week 11), following overnight fasting, all rabbits were euthanized under anesthesia with pentobarbital sodium (50 mg/kg). Liver tissue samples of euthanized rabbits were stored at -80°C in Eppendorf tubes containing 300 µL TRIzol reagent (Qiagen, Germany) for mRNA isolation. Total RNA isolation, real-time PCR, and gene expression analysis of liver tissue samples were performed at Erciyes University, Betül-Ziya Eren Genome and Stem Cell Research Center.

Total RNA Isolation and Determination of RNA Concentration

mRNA isolation was performed according to the total RNA extraction procedure with Qiazol (Qiagen, Germany). After thawing, the tissues were disintegrated with a homogenizer (Scilogex D-160, Malaysia). 200 µL of chloroform (Merck, Germany) was added. Centrifuged at 12.000 g for 15 min at +4 ºC. The supernatant was transferred to a new tube. 200 µL isopropanol (Merck, Germany) (1:1 ratio) was added. Incubated for 10 min at room temperature. Centrifuged at 12.000 g for 10 min at +4 ºC. The supernatant was removed. 1000 µL of ethanol was added and centrifuged at 7500 g for 5 min at +4 ºC. The supernatant was removed and allowed to dry for 10 min. 50 µL of nuclease-free water was added and dissolved. RNA measurements were performed with a nanodrop spectrophotometer.

Complementary-DNA Synthesis

Complementary DNA (cDNA) synthesis was performed according to the protocol of the high-Capacity cDNA reverse transcription kit (Thermo Fisher Scientific, USA). Mixture preparation: At this stage, the following amounts of mixture were prepared for one sample in a 0.2 µl Eppendorf tube. To this mixture, 500 ng/µl of isolated RNA was added and incubated in a thermal cycler (Qiagen, Germany). cDNA reverse transcription reaction composition: Total reaction (20 µL) = 10× RT Buffer (2.0 µL) + 25× dNTP Mix (100 mM) (0.8 µL) + 10× RT Random Primers (2.0 µL) + MultiScribe™ Reverse Transcriptase (1.0 µL) + Nuclease-free H2O (4.2 µL) + RNA (10 µL). Thermal Cycler Program: Step 1 (Temp.: 25 °C, Time: 10 sec), Step 2 (Temp.: 37 °C, Time:120 sec), Step 3 (Temp.: 85 °C, Time: 5 sec), Step 4 (Temp.: 4 °C, Time: ∞).

Real Time PCR Analysis

The Real-Time expression phase of the study was performed using a Light Cycler Nano (Roche Ltd., Mannheim, Germany) Real-Time PCR device and SYBR Green PCR Master Mix (Thermo Fisher Scientific). DNA synthesized samples were placed on a block at +4 °C 10 minutes before the study and

kept for a while. The *Hprt1* gene was used as a housekeeping gene in the study. Two separate working mixtures were prepared for the target and control genes to be studied. SYBR Green Master Mix, Primer F, Primer R, PCR Grade H_2O, and c-DNA were taken in 96-well plates in the following amounts for one sample. Samples with Light Cycler Nano Real-Time PCR device (Roche, Switzerland): 10 minutes at 95 °C, 10 seconds at 95 °C, 10 seconds at 60 °C, 10 seconds at 72 °C, 45 cycles of 10 seconds at 95 °C and 30 seconds at 95 °C. The melting temperature was analyzed by increasing the amount of heat by 0.1 °C per second at temperatures between 65 °C and 95 °C, and a melting graph analysis of the amount of fluorescence generated over time. Gene data for each sample were normalized to the *Hprt1* gene.

The Ct averages of *signal transducer and activator of transcription 4* (*STAT4*), *interferon gamma* (*IFNγ*), *Foxp3* (*Forkhead box P3*), *T-box transcription factor* (*Tbet*), *Gata3* (*GATA binding protein 3*), *granulocyte-macrophage colony-stimulating factor* (*GMCSF*), *RORγ* (*RAR-related orphan receptor gamma*), *interleukin 4* (*IL4*), *IL5*, *IL8*, *IL9*, *IL10*, *IL13*, *IL17A*, *IL18* genes were subtracted from the Ct averages of Hprt1 gene (Delta Ct: Ct [Control Gene]-Ct [Target Gene]) and the data were normalized. Power was calculated with the formula [Power (2ΔCt)], and the Log10 of the power values was taken and made suitable for statistical study. Arbitrary relative expression units were calculated by the division of the expression of the gene of interest by *hypoxanthine phosphoribosyltransferase 1* (*Hprt1*) mRNA expression. Primer sequences for each gen are given in Table 1.

Table 1. Primer sequences(http://www.ncbi.nlm.nih.gov)

Gene	***5′ to 3'***
STAT4	F: 5'-CAGATCATACAGCCAATGTGC-3' R: 5'-GGTTGAGGTTTTTGCGGAGT-3'
IFNγ	F: 5'-TGCCAGGACACACTAACCAGAG-3' R: 5'-TGTCACTCTCCTCTTTCCAATTCC-3'
Tbet	F: 5'-CCTTCCAAGAGACGCAGTTC-3' R: 5'-AGGAAGCTCGGGGTAGAAAC-3'
IL4	F: 5'-CGACATCATCCTACCCGAAGTC-3' R: 5'-CCTCTCTCTCGGTTGTGTTCTTG-3'
IL5	F: 5'-AGACCCTGACACTGCTCTCA-3' R: 5'-AGGTGATGATTTTTATGGACCGGA-3'
IL13	F: 5'-TCATCGAGGAGCTGGTCAAC-3' R: 5'-AGCCTTGTCTGTGCAGAGTC-3'
Gata3	F: 5'-AGGCAGGGAGTGTGTGAACT-3' R: 5'-CGTCGTGGTCTGACAGTTTG-3'
IL17A	F: 5'-CCAGCAAGAGATCCTGGTCCTA-3' R: 5'-ATGGATGATGGGGGTTACACAG-3'
GMCSF	F: 5'-TTCCTCCTAGGCAGTGTGGT-3' R: 5'-TCTACCATTTCGCCCAGCAC-3'
***RORγ* (*RORC*)**	F:5'-GGGCTTCATACCACCTTGAA-3' R: 5'-GTGCTCTGGGCCTATCTCTG-3'
IL9	F: 5'-ATCCCGTCTGACAACTGCAC3' R: 5'-GGCTTCCACCGTTCTTCTCA3'
IL10	F: 5'-CTTTGGCAGGGTGAAGACTTTC-3' R: 5'-AACTGGATCATCTCCGACAAGG-3'
IL18	F: 5'-ACCAAGGACAGCAACCTGTGTT-3' R: 5'-ACAGAGAGGCTTACAGCCATGC-3'
Foxp3	F: 5'-CACAGTGCCCCTAGTCATGG-3' R: 5'-CTGAGAGCTGGTGCATGAAGT-3'
IL8	F: 5'-CCACACCTTTCCATCCCAAAT-3' R: 5'-CTTCTGCACCCACTTTTCCTTG-3'
Hprt1	F:5'-GCAGACCTTGCTTTCCTTGGT-3' R: 5'-GCAGGCTTGCGACCTTGAC-3'

IFNγ: *Interferon gamma*, ***IL8***: *Interleukin 8*, ***Foxp3***: *Forkhead box P3*, ***IL18***: *Interleukin 18*, ***IL10***: *Interleukin 10*, **IL9**: *Interleukin 9*, ***RORγ*(*RORC*)**: *RAR-related orphan receptor gamma*, ***GMCSF***: *Granulocyte-macrophage colony-stimulating factor*, ***IL17A***: *Interleukin 17A*, ***Gata3***: *GATA binding protein 3*, ***IL-13***: *Interleukin 13*, ***IL4***: *Interleukin 4*, ***Tbet***: *T-box transcription factor*, ***STAT4***: *Signal transducer and activator of transcription 4*, ***Hprt*1**: *Hypoxanthine phosphoribosyltransferase*, **F**: Forward, **R**: Reverse

Histopathological Analyses

Histopathologic examinations were performed at the Department of Pathology, Erciyes University Faculty of Medicine Health Application and Research Center, Kayseri, Türkiye. The liver was dissected directly under physiologic saline perfusion, and the right lobe was fixed in 10% formalin solution for 1 hour. After the tissues were processed using the routine method, 3-5 µm thick sections were taken and stained with hematoxylin-eosin (H&E) and examined under a light microscope.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows Release 25.0 (SPSS Inc., Chicago, IL, USA). All data were analysed graphically and tested for normality using the Shapiro-Wilk test and the Q-Q plot. All data that passed the normality test were expressed as mean and standard deviation (SD). One-way ANOVA (alternative: Kruskal-Wallis Test) was used for comparisons between groups. Repeated Measures ANOVA was used for comparisons between times. Bonferroni and Tukey tests were used for multiple comparisons. Graphs were drawn with the free version of GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, CA, USA). A p-value of < 0.05 was considered statistically significant.

RESULTS

Body Weight Findings

Body weight of rabbits in the SD groups (SD, SD+T, and SD+TO) increased. On the other hand, the body weights of rabbits in the HCD groups (HCD, HCD+T, HCD+TO) decreased slightly, and this phenomenon persisted until the end of the experiment. A similar effect was observed in the SD+TO group, but this effect disappeared after the 4th week. In the 11th week, rabbits in the HCD groups had a lower mean body weight compared to those fed an SD group ($p< 0.05$) (Figure 1).

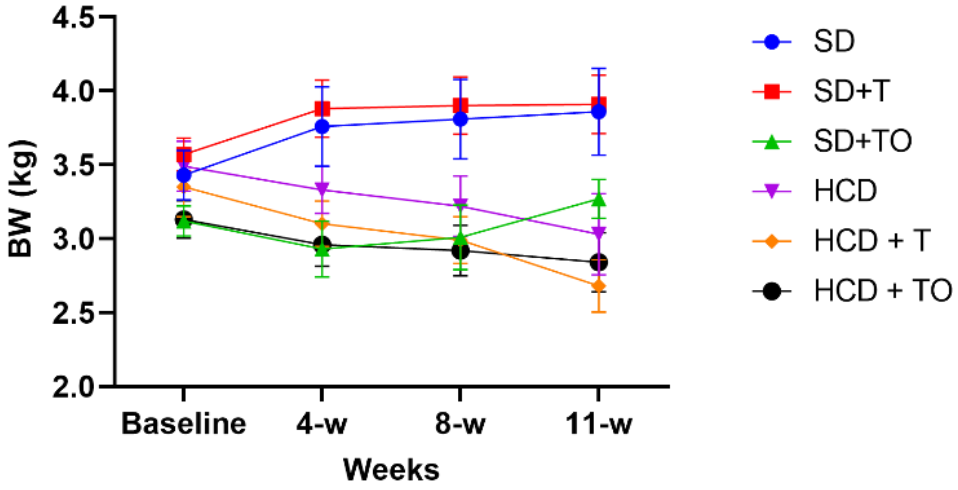

Figure 1: Changes in body weight according to time in SD and HCD groups, BW; body weight, SD (n = 8); standard diet group, SD+T (n = 8); standard diet + thymol group, SD+TO (n = 8); standard diet + thyme oil group, HCD (n = 8); high cholesterol diet group, HCD+T (n = 8); high cholesterol diet + thymol group, HCD+TO (n = 8); high cholesterol diet + thyme oil group.

At week 4, the mean body weight of rabbits in the ND-TO group (2.93 ± 0.50) was significantly lower than in the ND (3.76 ± 0.66, p = 0.048) and ND+T (3.89 ± 0.55, p = 0.008) groups. The HCD-T (3.10 ± 0.41, p = 0.043) and HCD-TO (2.96 ± 0.36, p = 0.016) groups also had lower body weights than the ND+T group (3.89 ± 0.55) at week 4.

At week 8, body weight in the ND-T group (3.90 ± 0.55) was higher than in the ND-TO (3.01 ± 0.58, p= 0.031), HCD+T (2.99 ± 0.42, p= 0.027), and HCD+TO (2.92 ± 0.42, p= 0.019) groups.

At week 11, rabbits in the ND (3.86 ± 0.72) and ND+T (3.91 ± 0.56) groups had higher body weights than those in the HCD+T (2.68 ± 0.47, p= 0.008 and p= 0.002, respectively) and HCD+TO (2.84 ± 0.49, p= 0.039 and p= 0.016, respectively) groups.

No significant changes over time were observed in ND, ND+T, ND-TO, and HCD groups. However, in the HCD+T group, body weight at weeks 0 (3.35 ± 0.20) and 4 (3.10 ± 0.16) was higher than at week 11 (2.68 ± 0.18) (p= 0.004 and p= 0.049). Similarly, in the HCD+TO group, body weight at week 0 (3.13 ± 0.31) was higher than at weeks 4 (2.96 ± 0.36), 8 (2.92 ± 0.42), and 11 (2.84 ± 0.49) (p= 0.011, p= 0.013, p = 0.022).

Lipid Profile Findings

The high cholesterol diet groups (HCD, HCD+T, and HCD+TO) had significantly higher TC, TG, LDL-C, and HDL-C. The mean triglyceride concentration of rabbits in the HCD (210.86 ± 13.63 mg/dl) and HCD + TO (151.50 ± 34.57 mg/dl) groups at week 4 was higher than that of the SD + TO (67.29 ± 9.62 mg/dl) group ($p< 0.05$). The mean triglyceride concentration of rabbits in HCD + T (216.00 ± 20.13 mg/dl) and HCD + TO (294.33 ± 92.27 mg/dl) groups at week 8 was higher than SD (139.67 ± 22.21 mg/dl), SD+T (102.0 ± 17.10 mg/dl), SD-TO (124.71 ± 10.14 mg/dl) and HCD (142.86 ± 73.66 mg/dl) groups ($p< 0.05$). The mean triglyceride concentration of rabbits in HCD (344.43 ± 39.58 mg/dl), HCD + T (389.71 ± 21.20 mg/dl) and HCD + TO (405.33 ± 50.92 mg/dl) groups at week 11 was higher than SD (228.0 ± 35.63 mg/dl), SD+T (178.50 ± 35.81 mg/dl) and SD-TO (248.29 ± 28.33 mg/dl) groups ($p<0.05$) (Figure 2).

The mean triglyceride concentration of the SD group at week 11 (228.0 ± 35.63 mg/dl) was higher than at week 0 (114.50 ± 32.15 mg/dl) ($p= 0.001$). The mean triglyceride concentration of the SD+T group at week 11 (178.50 ± 35.81 mg/dl) was higher than that at week 0 (145.50 ± 19.78 mg/dl), week 4 (129.0 ± 5.35 mg/dl) and week 8 (102.0 ± 17.10 mg/dl) ($p<0.05$) (Figure 2).

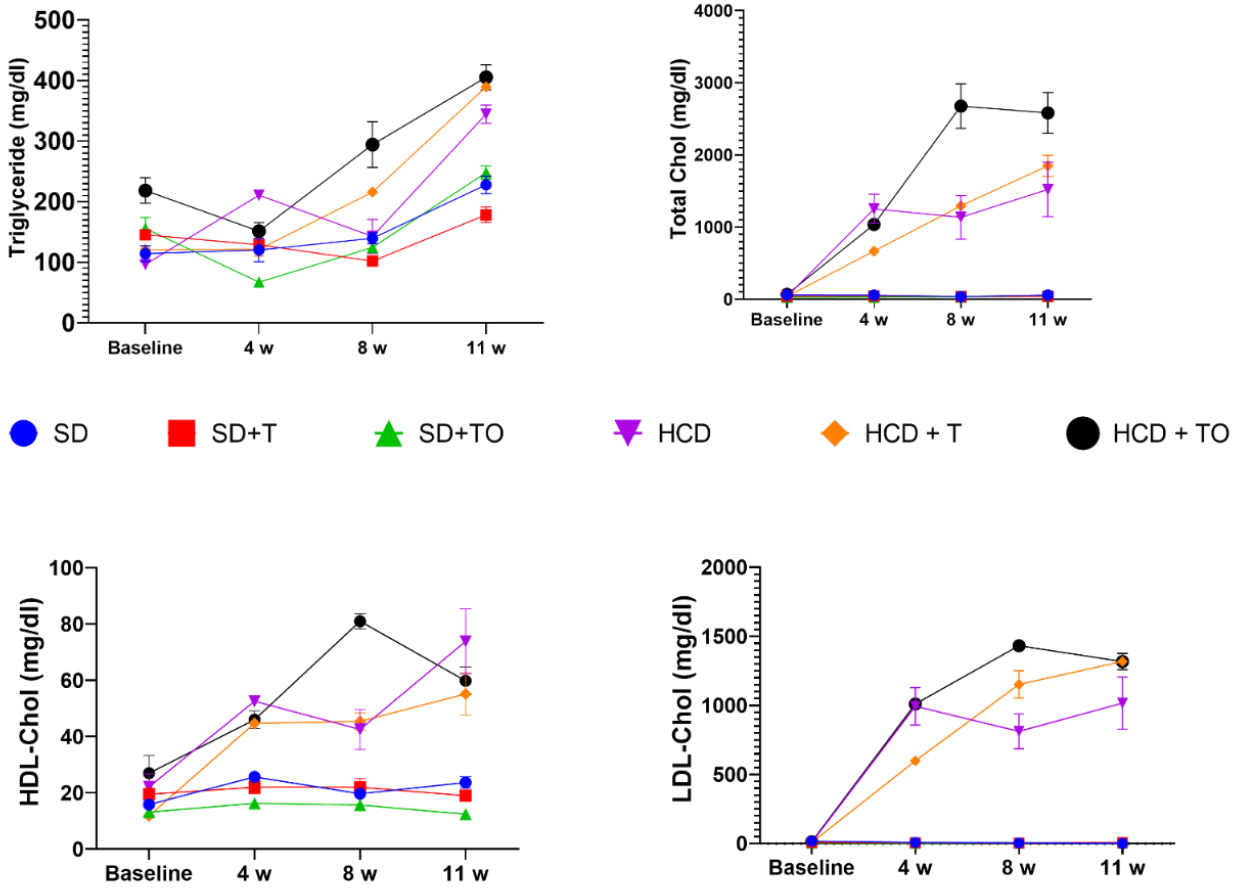

Figure 2: Changes in lipid concentrations of rabbits in SD and HCD groups according to time, BW; body weight, SD; standard diet group, SD+T; standard diet + thymol group, SD+TO; standard diet + thyme oil, HCD; high cholesterol diet, HCD+T; high cholesterol diet + thymol, HCD+TO; high cholesterol diet + thyme oil.

Hepatic Gene Expression Findings

The mRNA expression levels of inflammation-related genes in liver tissue samples are shown in Figure 3-4. There was a statistical difference between the groups in terms of the expression level of *IFNγ* ($p < 0.001$). The relative gene expression levels of *IFNγ* in SD + T, SD + TO, HCD, HCD + T, and HCD + TO groups were significantly higher than those of the SD group. There was a statistical difference between the groups in terms of the expression level of *IL4* ($p= 0.006$). The relative gene expression level of *IL4* in the SD + T group was significantly higher than that of the HCD and HCD + TO groups. There was a statistical difference between the groups in terms of the expression level of *Tbet* ($p < 0.001$). The relative gene expression level of *Tbet* in SD and SD+T groups

was significantly lower than that in SD+TO group, while the relative gene expression level in SD+TO group was significantly higher than that in HCD and HCD+TO groups. There was a statistical difference between the groups in terms of the expression level of *Gata3* ($p < 0.001$). The relative gene expression level of *Gata3* in the SD group was significantly lower than that of the SD + T and SD + TO groups. The relative gene expression level of *Gata3* in the SD+T group was significantly lower than in the SD+TO group and significantly higher than in the HCD and HCD+T groups. The relative gene expression level of *Gata3* in the SD+TO group was significantly lower than that of the SD+TO group and significantly higher than that of the HCD, HCD+T, and HCD+TO groups. There was a statistical difference between the groups in terms of the expression level of *IL17A* ($p= 0.002$). The relative gene expression level of *IL17A* in the SD group was significantly lower than that in the SD+T group, while the relative gene expression level of IL17A in the SD+T group was significantly higher than that in the HCD, HCD+T, and HCD+TO groups. There was a statistical difference between the groups in terms of the expression level of *IL18* ($p= 0.041$). The relative gene expression level of *IL18* in the HCD + T group was significantly higher than that in the SD group. There was a statistical difference between the groups in terms of the expression level of *IL8* ($p= 0.016$). The relative gene expression level of *IL8* in the HCD+T group was significantly higher than that of the HCD and HCD+TO groups.

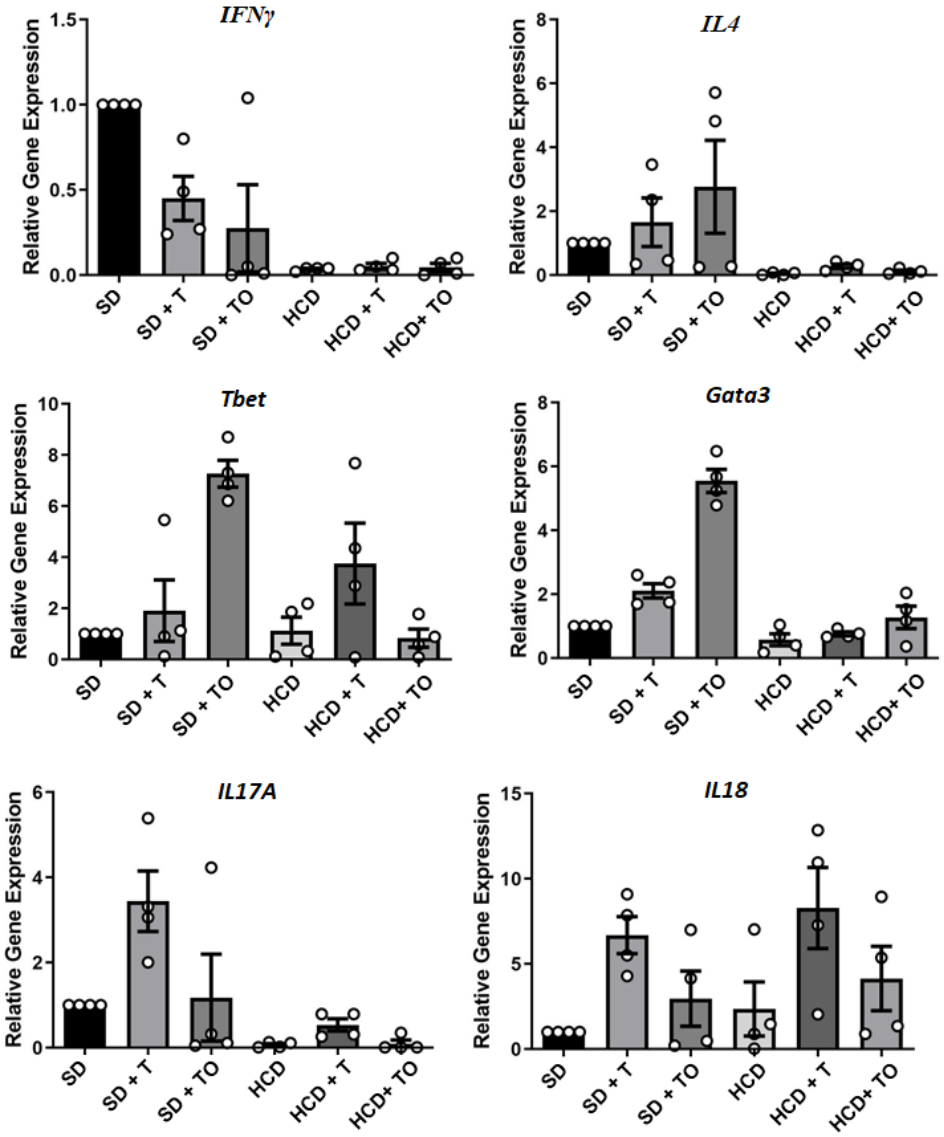

Figure 3: Comparison of hepatic gene expression levels of *IFNγ*, *Tbet*, *Gata3*, *IL4*, *IL18* and *IL17A*. *IFNγ*: *Interferon gamma*, *IL18*: *Interleukin 18*, *IL17A*: *Interleukin 17A*, *Gata3*: *GATA Binding Protein 3*, *IL4*: *Interleukin 4*, *Tbet*: *T-box transcription factor*.

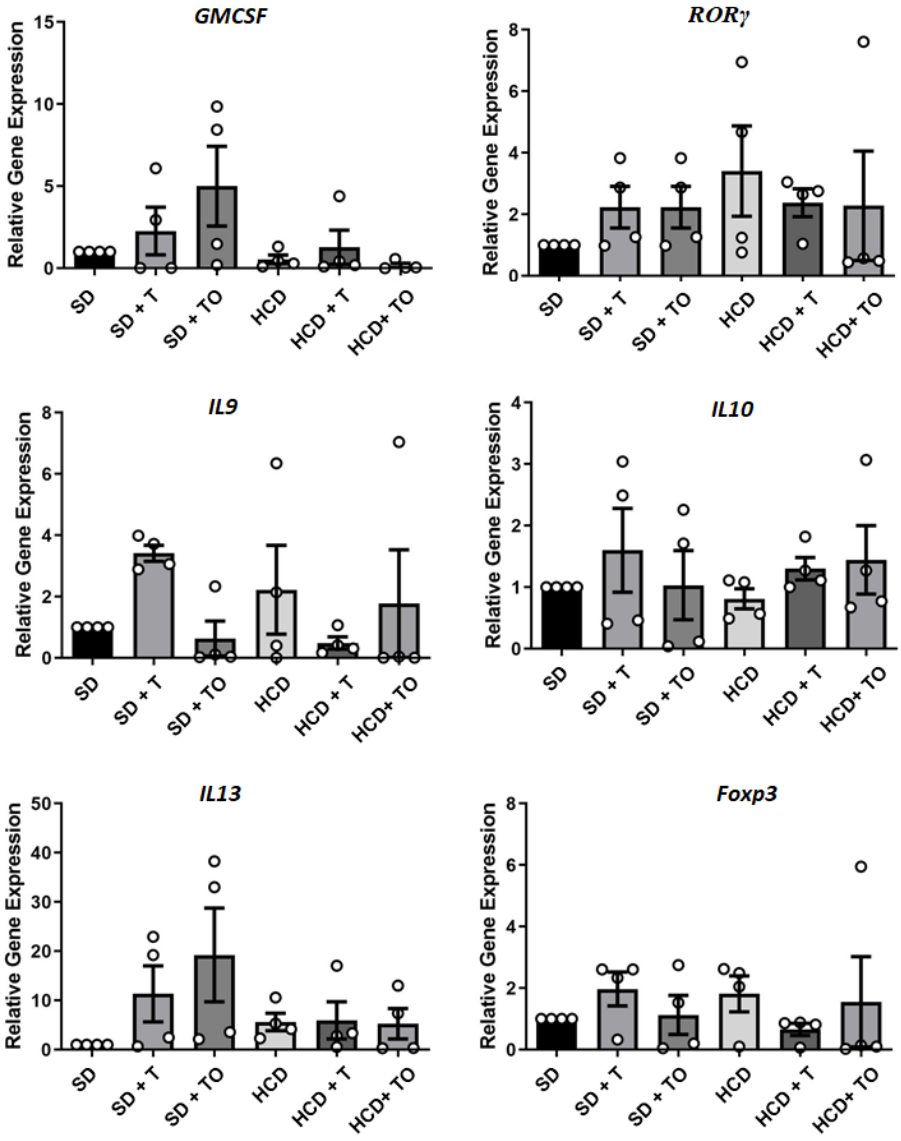

Figure 4: Comparison of hepatic gene expression levels of *Foxp3*, *IL9*, *IL10*, *RORγ*, *GMCSF,* and *IL13*. *Foxp3*: *Forkhead box P3*, *IL9*: *Interleukin 9*, *IL10*: *Interleukin 10*, *RORγ* (*RORC*): *RAR-related orphan receptor gamma*, *GMCSF*: *Granulocyte-macrophage colony-stimulating factor*, *IL13*: *Interleukin 13*.

Macroscopic and Histopathologic Findings

The livers of the rabbits in the SD-fed groups were macroscopically normal, and no lesions were observed. On the other hand, the livers of the rabbits fed with HCD were macroscopically observed to have yellowish discoloration, increased volume, blunted edges, shiny, slippery, and protruding cross-sectional surfaces, friable consistency, and some of them adhered to the diaphragm. In addition, ascites was observed in 1 of the rabbits in the HCD + T group. Histopathologic examination of the livers of the rabbits in the SD group showed normal liver structure. The histopathologic appearance of the livers of the rabbits in SD + T and SD + TO groups was the same with the histopathologic appearance of the livers of the rabbits in the SD group, no pathologic lesion was observed and they were found to have normal structure. Histopathologic appearance of the livers of HCD group animals showed balloon-like degeneration, fat vacuoles, hemosiderin pigment in hepatocytes, lymphocyte-rich mononuclear cell infiltrations in the portal areas, and an increase in the number of Kupffer cells. In the liver sections of rabbits in the HCD + T and HCD + TO groups, microscopically balloon-like degeneration and partially reduced fat vacuoles were observed. There was no change in the severity of hepatic findings in HCD + T and HCD + TO groups compared to the HCD group (Figures 5 and 6).

Figure 5: Macroscopic appearance of the livers of rabbits fed SD and HCD. SD; standard diet group, SD + T; standard diet + thymol group, SD + TO; standard diet + thyme oil, HCD; high cholesterol diet, HCD + T; high cholesterol diet + thymol, HCD + TO; high cholesterol diet + thyme oil.

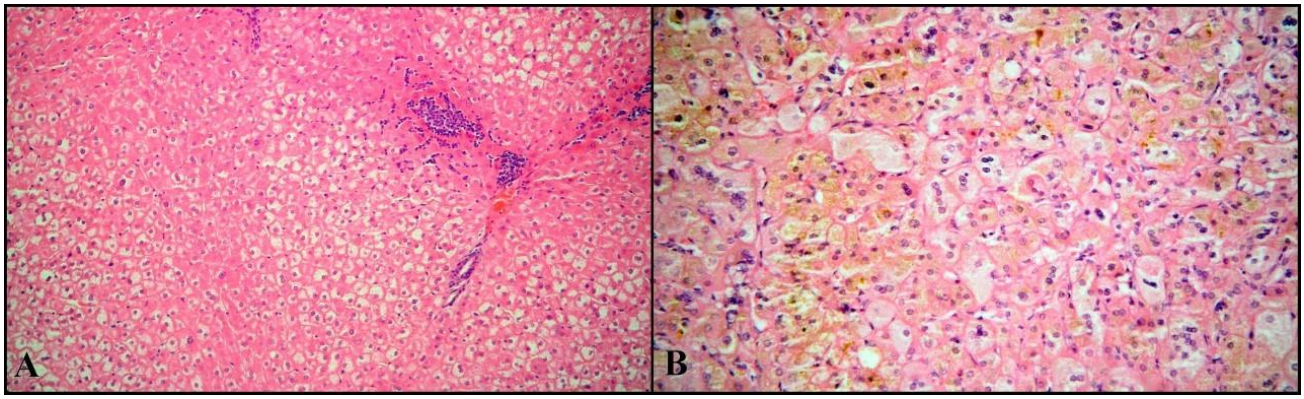

Figure 6: Histopathological appearance of the livers of the high cholesterol diet (HCD) group animals. (A) In the liver tissue of the high cholesterol diet (HCD) group, distinct morphological changes, intercellular irregularities, enlarged sinusoid areas, and local inflammatory foci were observed in hepatocytes. Disruption of the liver lobular structure and ballooning (swelling) of the hepatic cells are noted. (B) At higher magnification, dense fat accumulation (vacuole-like spaces), degeneration, areas of necrosis, and inflammatory cell infiltration are clearly observed in the hepatocyte cytoplasm. There is also marked steatosis in the liver and histopathological changes due to cell damage.

DISCUSSION

Cholesterol-fed rabbits have been widely used as an experimental model to investigate cardiovascular disorders associated with hypercholesterolemia and atherosclerosis (Kainuma et al., 2006; Kim et al., 2014; Kim and Kim, 2017). In addition to cardiovascular effects, a HCD has been shown to induce non-alcoholic fatty liver disease (NAFLD) in various studies (Kainuma et al., 2006; Kim et al., 2014). Notably, this diet appears to contribute more significantly to the development of non-obese NAFLD compared to the obese form of the disease (Kim et al., 2014; Kim and Kim, 2017). Consistent with these findings, in the present study, a non-obese hepatic lipidosis model characterized by hypertriglyceridemia, hypercholesterolemia, and fatty liver was successfully established in rabbits fed a high-cholesterol diet.

In the present study, consistent with the findings of Kim et al. (2014), blood levels of TC, TG, LDL-C, and HDL-C were significantly elevated in rabbits fed a high-cholesterol (1%) diet. Similarly, El Sebaei et al. (2019) reported increased serum levels of TC, triacylglycerol (TAG), and LDL-C in rabbits on a high-cholesterol diet compared to those fed a normal diet; however, no significant difference was observed in serum HDL-C levels. Cholesterol overload leads to

accumulation in the liver, and hepatic cholesterol accumulation is likely to contribute to the progression of NAFLD, analogous to the effects of hepatic triglyceride overload (Tous et al., 2005; Lozano et al., 2019). Our results demonstrated that treatment with thyme oil and thymol did not significantly affect TC, HDL-C, LDL-C, or TG levels.

In the present study, consistent with the findings of Kainuma et al. (2006), the livers of rabbits fed a high-cholesterol diet exhibited notable hepatocellular fat deposition, cellular ballooning, and mild infiltration of neutrophils. Although macrovesicular fatty changes were not clearly distinguishable, significant microvesicular fat accumulation and aggregation of enlarged hepatic stellate cells were observed, particularly in the perivenular regions. This pattern of microvesicular fat deposition has been emphasized by Fromenty and Pessayre (1995) as indicative of a more severe hepatic injury compared to macrovesicular fat, reflecting mitochondrial dysfunction and impaired β-oxidation pathways. Previous studies have demonstrated the hepatoprotective potential of thyme-derived compounds. For instance, Sheng et al. (2023) reported that supplementation with thyme polyphenol-rich extract alleviated high-fat diet-induced liver injury in C57 mice in a dose-dependent manner, resulting in improved liver histology and reduced oxidative stress markers. Similarly, Yan et al. (2021) showed that thyme ethanolic extract exerted protective effects in a laboratory model of alcoholic liver disease by decreasing inflammatory cytokines and enhancing antioxidant enzyme activity. Furthermore, Lahmi et al. (2023) observed that administration of thymol at a dose of 50 mg/kg in rats with fatty liver disease led to decreased serum liver enzyme levels, reduced histopathological degenerative changes, and restoration of normal liver architecture. Contrary to these reports, our study found that neither thyme oil nor thymol treatment significantly reduced the severity of fatty liver disease in rabbits fed a high-cholesterol diet when compared to control groups. This discrepancy may be attributed to differences in animal species, dosage, duration of treatment, or the specific hepatic insult model used. It is also possible that the pathophysiological mechanisms underlying cholesterol-induced hepatic lipidosis differ from those in alcoholic or other diet-induced liver injuries, which might influence the efficacy of thyme compounds. Further research is warranted to clarify the potential therapeutic roles of thyme oil and thymol in diverse models of hepatic steatosis and to determine optimal treatment parameters.

In the present study, both food intake and body weight of rabbits fed a high-cholesterol diet were reduced, and this trend persisted until the end of the experiment. At the conclusion of the study, rabbits in the high-cholesterol diet groups exhibited significantly lower mean body weights compared to those fed a normal diet ($p < 0.05$). Similarly, El Sebaei et al. (2019) reported that rabbits fed a hypercholesterolemic diet showed decreased body weight, daily feed intake, total weight gain, and feed conversion ratio at the end of the eighth week compared to rabbits on a normal diet. This reduction may be attributed to changes in the palatability of the pellet feed caused by the high cholesterol content. Another possible explanation involves endoplasmic reticulum (ER) stress and oxidative stress resulting from excessive cholesterol accumulation in hepatocytes (Senokuchi et al., 2008; Zhang et al., 2010).

In this study, the expression level of the *IFNγ* gene in rabbits fed an HCD was significantly lower than in the standard diet group ($p< 0.05$). Contrary to our findings, Inzaugarat et al. (2017) reported that the relative expression of the *IFNγ* gene in liver biopsy samples from patients with NAFLD was significantly higher compared to controls. The suppression of *IFNγ* gene expression observed in our HCD groups may be related to the non-obese nature of our model and the stress induced by elevated plasma cholesterol levels. Supporting this, Luo et al. (2013) demonstrated in a high-fat diet-induced steatohepatitis mouse model that fibrosis-related gene expressions (including *a-smooth muscle actin*, *type I collagen*, *matrix metalloproteinase-1 tissue inhibitor*, and *matrix metalloproteinase-2*) were significantly increased in wild-type mice but markedly suppressed in IFNγ-deficient knockout mice. Moreover, a human study by Ghaedi et al. (2021) found no significant effect of thyme supplementation on serum IFN-γ and TNF-α levels in individuals with NAFLD. In the present study, administration of thymol and thyme oil downregulated *IFNγ* gene expression in SD groups, which is thought to result from their anti-inflammatory properties, potentially exerting a protective effect on liver cells.

In the present study, the expression level of the *IL4* gene was significantly higher in the normal diet group compared to the HCD groups ($p < 0.05$), with strong gene suppression observed in the HCD groups. In patients with NAFLD, pro-inflammatory cytokines such as TNF-α and IL-6 are typically elevated, while levels of the anti-inflammatory cytokine IL-4 are decreased, and IL-10 levels remain unchanged (Das and Balakrishnan, 2011). Zhou et al. (2014) reported that thymol reduced ovalbumin-specific IgE levels, inhibited recruitment of inflammatory cells into the airways, and decreased IL-4, IL-5, and IL-13 levels in bronchoalveolar lavage fluid in a mouse model of asthma. In our study, treatment with thymol and thyme oil increased *IL4* gene expression in liver tissue. This upregulation is likely attributable to the anti-inflammatory properties of thymol and thyme oil. Although *IL4* gene expression was strongly suppressed in the high-cholesterol groups, thymol and thyme oil treatments effectively upregulated *IL4* expression, potentially contributing to the reduction of pathological inflammation.

In this study, thymol increased *T-bet* gene expression in rabbits fed both normal and HCD. Thyme oil upregulated *T-bet* expression in normal diet rabbits ($p< 0.05$) but downregulated it in the high-cholesterol group. While previous studies reported higher *T-bet* expression in NAFLD patients and HCD rabbits (Inzaugarat et al., 2017; Sheng et al., 2011), our study found no difference between the high-cholesterol and control groups. Notably, thymol treatment caused a 7-fold increase in *T-bet* expression, supporting its immunomodulatory role by promoting Th1 responses and inhibiting Th17-mediated inflammation. This is consistent with our IL-17 results, showing reduced IL-17 expression after thymol administration.

Zeyda et al. (2011) reported increased *Gata3* gene expression in the subcutaneous adipose tissue of obese patients compared to controls. In our study, an HCD suppressed hepatic *Gata3* gene expression. This suppression may be attributed to the non-obese nature of our model, as rabbits in the HCD group had lower body weights than those in the control groups. Treatment with thyme oil and thymol increased *Gata3* expression in rabbits fed both a normal diet and an HCD, with a greater increase observed in the thyme oil group. However, in the HCD groups, where gene suppression was pronounced, these treatments only slightly elevated *Gata3* expression. Notably, in parallel with the increase in *Gata3* expression, *IL4* and *IL13* genes were also upregulated in this study. Given that *Gata3* is known to promote the secretion of IL-4, IL-5, and IL-13 from Th2 cells (Yagi et al., 2011), these findings suggest that thyme oil and thymol may exert their anti-inflammatory effects, at least in part, through the activation of Th2-associated pathways.

Obese humans and animals exhibit elevated *IL17A* expression, associated with increased adipose tissue Th17 cell infiltration (Sumarac-Dumanovic et al., 2009). Anti-IL-17 antibodies have been shown to improve liver function, suppress Kupffer cell activation, and reduce pro-inflammatory cytokines via NF-$\varkappa$B inhibition (Xu et al., 2013). In our study, *IL17A* expression was significantly higher in the SD+T group than in controls. HCD suppressed *IL17* expression, whereas thymol increased it. Previous studies report that obesity, NAFLD, and NASH increase *IL17* expression (Tang et al., 2011; Chackelevicius et al., 2016). As our model represents a non-obese condition, suppression of *IL17* in HCD groups may relate to reduced body weight and adipose tissue. Given IL-17's role in NAFLD progression, the lack of *IL17* increase with thyme oil may indicate a more controlled inflammatory response, potentially limiting progression to NASH.

Flisiak-Jackiewicz et al. (2018) reported elevated serum IL-18 concentrations in patients with NAFLD compared to healthy controls. In the present study, *IL18* expression was higher in the HCD group than in the normal diet group. Furthermore, thyme oil and thymol treatments increased *IL18* gene expression, with a greater increase observed in rabbits receiving thymol. Given that IL-18 is a pro-inflammatory cytokine involved in activating both innate and adaptive immune responses, its upregulation may contribute to hepatic inflammation, fibrosis, and the progression from simple steatosis to NASH. Elevated IL-18 has also been linked to endothelial dysfunction and increased cardiovascular risk, suggesting that its rise in our model may reflect both hepatic and systemic inflammatory activation. Interestingly, our findings on *IL18* expression align with the observed effects on *Tbet* and *IL17*. Thymol markedly upregulated T-bet expression while simultaneously reducing *IL17* expression, suggesting a shift towards a Th1-dominant immune profile and suppression of Th17-mediated responses. Since IL18 is known to synergize with T-bet–driven pathways to enhance Th1 responses, the concurrent increase in IL-18 and T-bet with thymol treatment may represent a coordinated immunomodulatory mechanism. However, while this shift may help restrain IL-17–driven chronic inflammation, it could also potentiate Th1-associated inflammation, which warrants careful consideration in the context of NAFLD progression and cardiovascular risk.

CONCLUSION

In this study, a non-obese rabbit model of hepatic lipidosis, hypercholesterolemia, and hypertriglyceridemia was successfully established using a high-cholesterol diet. This model effectively replicated the hallmark histopathological and immunological changes of non-obese hepatic lipidosis, including extensive microvesicular steatosis, liver cell injury, and pronounced modulation of immune-related gene expression. Thyme oil and thymol did not improve lipid profiles or reduce the severity of fatty liver histopathology; however, both compounds modulated immune gene expression, notably increasing *IL4*, *Gata3*, and in the case of thymol, *Tbet*, while influencing *IL17* and *IL18* expression in a manner suggestive of a shift toward Th1- and Th2-associated responses with reduced Th17 activity. These findings highlight the complex immunomodulatory effects of thyme-derived compounds, which may confer both anti-inflammatory and pro-inflammatory influences depending on the immune pathway involved. While thymol and thyme oil showed potential to modulate hepatic immune responses, their lack of efficacy in improving lipid metabolism or histopathology in this cholesterol-induced model suggests that their therapeutic utility for hepatic lipidosis may be context- and model-dependent. Further studies in diverse hepatic lipidosis models, with varying obesity status, treatment durations, and dosages, are warranted to clarify their role in disease prevention and management.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: VG and GE contributed to the project idea, design and execution of the study. VG and GE contributed to the acquisition of data. VG and GE analysed the data. VG and GE drafted and wrote the manuscript. VG and GE reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval:"Ethics committee approval was received for this study from the Animal Experiments Local Ethics Committee of Erciyes University (Date: 04.03.2020, Number: 20/069). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules."

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RESEARCH ARTICLE

Investigation of the Prevalence of Irresponsible Antibiotic Use in Large Ruminants

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ABSTRACT

This study aimed to investigate the prevalence of irresponsible antibiotic usage in large ruminants within Kayseri province. A face-to-face survey was conducted with 120 animal owners who brought their animals to the Ruminant Clinic of the Internal Medicine Department of the Faculty of Veterinary Medicine at Erciyes University for diagnosis and treatment, and who had previously administered antibiotic therapy. The study categorized participants into three groups: Group 1 (n=23) consisted of informed animal owners who adhered to the veterinarian's prescribed antibiotics post-examination; Group 2 (n=56) included those who administered antibiotics based on the veterinarian's recommendations derived from the anamnesis; and Group 3 (n=41) comprised animal owners who randomly selected and administered leftover antibiotics from prior treatments. The beta-lactam group was identified as the most often utilized (26.8%) and recommended (37.0%) antibiotic group by veterinarians. Likewise, the most common antibiotic category utilized by animal owners was beta-lactam antibiotics (30.8%). It was observed that various antibiotic classes were supplied either alone or in combination to animals receiving antibiotics. Single antibiotic usage was mostly utilized and recommended by veterinarians (52.17%), whereas dual combination antibiotic usage was primarily utilized by participants in Group 3 (48.78%). The usage of combinations of more than two antibiotics was mostly used by veterinarians (21.74%). This study revealed extensive and unregulated antibiotic usage on certain scale. Thus, it is crucial at both national and international levels to safeguard animal production from potential food crises and to implement strategies against resistant strains emerging from irresponsibly antibiotic usage, which might negatively affect human, animal, and environmental health. Additionally, it is essential to educate veterinarians and breeders on responsible use of antibiotics.

Keywords: Antibiotics, Antimicrobial resistance, Irresponsible antibiotic use

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Büyük Ruminantlarda Akılcı Olmayan Antibiyotik Kullanımının Yaygınlığının Araştırılması

ÖZ

Bu çalışmada Kayseri ilindeki büyük ruminantlarda akılcı olmayan antibiyotik kullanımının yaygınlığının araştırılması amaçlandı. Bu amaçla Erciyes Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı Ruminant Kliniği'ne teşhis ve tedavi amacıyla hayvanını getiren, daha önce antibiyotik tedavisi uygulamış 120 hayvan sahibine yüz-yüze anket çalışması yapıldı. Çalışmada, veteriner hekim tarafından muayene sonrası reçete edilen antibiyotik uygulamasına devam eden bilinçli hayvan sahipleri (Grup 1, n=23), veteriner hekim tarafından muayene edilmeden, verilen anamnez sonucu veteriner hekim tavsiyesi ile antibiyotik uygulaması yapan hayvan sahipleri (Grup 2, n=56) ve daha önceki uygulamalardan artan ve elinde bulunan antibiyotikleri rastgele seçerek uygulayan hayvan sahipleri (Grup 3, n=41) olarak gruplar oluşturuldu. Veteriner hekimlerin en çok kullandığı (%26,8) ve tavsiye ettiği (%37,0) antibiyotik grubunun beta-laktam grubu olduğu tespit edildi. Benzer şekilde hayvan sahiplerinin de en çok kullandığı antibiyotik grubu beta-laktam grubu antibiyotiklerdi (%30,8). Antibiyotik kullanılan hayvanlara farklı antibiyotik gruplarının tek veya kombine olarak uygulandığı tespit edildi. Tek bir antibiyotik kullanımının en çok veteriner hekim tarafından uygulandığı (%52,17) ve tavsiye edildiği (%60,71), ikili kombine antibiyotik kullanımının ise en çok Grup 3'teki katılımcılar tarafından uygulandığı görüldü (%48,78). İkiden daha fazla kombine antibiyotik kullanımının ise veteriner hekim tarafından uygulandığı (%21,74) belirlendi. Bu çalışma ile belirli bir ölçekte yaygın ve kontrol dışı antibiyotik kullanımı ortaya konuldu. Sonuç olarak gelecekte ortaya çıkması muhtemel bir gıda krizine karşı hayvansal üretimi korumak ve insan, hayvan ve çevre sağlığını olumsuz etkileyecek bilinçsiz antibiyotik kullanımı sonucu ortaya çıkabilecek dirençli suşlara karşı önlem almak, veteriner hekimlerin ve yetiştiricilerin akılcı antibiyotik kullanımı hakkında bilgilendirmek, ulusal ve uluslararası boyutta önem arz etmektedir.

Anahtar Kelimeler: Akılcı olmayan antibiyotik kullanımı, Antibiyotik, Antimikrobiyal direnç

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GİRİŞ

Antibiyotikler, hayvan ve insan sağlığında bakteriyel enfeksiyonların tedavisinde kullanılır. Tüm dünyada artan antibiyotik kullanımı ile beraber akılcı olmayan antibiyotik kullanımı da hızla artmaktadır. COVID-19 salgınından önce, Dünya Sağlık Örgütü tarafından belirlenen en önemli 10 riskten biri, "sessiz pandemi" olarak da bilinen antimikrobiyal dirençtir (AMD). Ancak hem insanlarda hem de hayvanlarda gereksiz, yanlış ve akılcı olmayan antibiyotik kullanımı dirençli bakterilerin oluşmasına yol açmıştır (Paneri ve Sevta, 2023; Sungur ve ark., 2025). AMD, bakterilerin, virüslerin, mantarların ve parazitlerin zamanla evrimleşip ilaçlara yanıt vermediği, salgınların tedavisini son derece zorlaştıran ve bulaşıcı, yaşamı tehdit eden hastalık ve ölüm riskini artıran bir durumdur. Her yıl 700,000'den fazla kişi AMD nedeniyle hayatını kaybetmektedir. 2050 yılana kadar AMD nedeni ile 10 milyon insanın öleceği tahmin edilmektedir (Paneri ve Sevta, 2023).

Veteriner hekimliğinde kullanılan çeşitli antibiyotikler, insanlarda bakteriyel enfeksiyonlarla mücadelede kullanılanlarla aynı etki mekanizmasına sahiptir (Jauregi ve ark., 2021). Hayvancılıkta, antimikrobiyal ajanlar çeşitli hastalıkları tedavi etmek ve büyüme hızlandırıcılar olarak kullanılır (Mann ve ark., 2021). Dünyada ve Türkiye'de artan insan nüfusunun gıda ihtiyacını karşılamak amacıyla, insan nüfusu ile orantılı olarak gıda değeri olan hayvanların da sayısı artmaktadır (Ergün ve Bayram, 2021). Büyük ruminantlar, et ve süt üretiminde temel kaynaklar arasında yer almaktadır. Gelişmekte olan ülkelerde, hayvansal proteinlere olan yüksek talep nedeniyle yoğun çiftçilik uygulamaları bulunmaktadır (Herrero ve Thornton, 2013). Türkiye'de özellikle kırsal bölgelerde yürütülen hayvancılık faaliyetlerinde, üreticilerin bilgi eksiklikleri, veteriner hizmetlerine erişimdeki sınırlılıklar ve ekonomik kaygılar, antibiyotiklerin yanlış ve/veya bilinçsiz şekilde kullanılmasına neden olabilmektedir. Bu hayvanlarda yapılan bilinçsiz antibiyotik uygulamaları, sadece hayvan sağlığını değil, aynı zamanda gıda güvenliğini ve çevresel sürdürülebilirliğini de riske atmaktadır (Yavuz ve ark., 2020).Hayvanlarla ilişkili dirençli patojenler, gıda zinciri aracılığıyla hayvanlardan insanlara kolayca bulaşabildikleri ve çevrede yaygın olarak dağıldıkları için insanlar için büyük bir tehdittir (Manyi-Loh ve ark., 2018; Kaur ve ark., 2024). Fakat yetersiz politikalar ve enfeksiyon kontrol stratejilerinin benimsenmesi noktasındaki eksiklikler, hayvancılıkta çeşitli antimikrobiyallerin kullanılmaya devam edilmesinenedenolmaktadır (Manyi-Loh ve ark., 2018). AMD, insan ve hayvan sağlığı için bir tehditoluşturmaktadır ve ciddiye alınması gereken bir durumdur.

Bu çalışmanın amacı, büyük ruminantlarda antibiyotik kullanımına yönelik hayvan sahiplerinin davranışlarını değerlendirmek ve veteriner hekimler ile iletişim düzeylerine göre antibiyotik kullanım şekillerinisınıflandırarak akılcı olmayan antibiyotik kullanımının yaygınlığının incelenmesidir.

MATERYAL ve METOT

Etik kurul

Bu çalışma için Erciyes Üniversitesi Sosyal ve Beşeri Bilimler Etik Kurulu'ndan (28.06.2022 tarihli, 279 No'lu) etik kurul izni alınmıştır.

Çalışma Sahası

Erciyes Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı Ruminant Kliniği'ne Kayseri ilindeki büyükbaş hayvancılık işletmelerinden teşhis ve tedavi amacıyla hayvanlarını getiren, daha önce antibiyotik tedavisi uygulamış 120 hayvan sahibi/yetiştirici oluşturdu.

Çalışma Zamanı

Çalışma, 1 Temmuz 2022 ile 1 Temmuz 2023 tarihleri arasında yapıldı.

Evren ve Örneklem

Çalışmaya, hayvanlarının antibiyotik tedavi sürecine doğrudan dahil olan ve bu süreç hakkında bilgi sahibi olan 120 hasta sahibi katılmıştır. Katılımcılar, Kayseri ilinde aktif olarak hayvancılıkla uğraşan ve 18 yaş üzeri bireyler arasından, bilgilendirilmiş onam formunu imzalayarak gönüllü katılım sağlamış kişilerden seçilmiştir. Örneklem büyüklüğü, kliniğe başvuran hasta sahipleri arasından uygunluk esasına göre belirlenmiş olup, örnekleme yöntemi olarak kolayda örnekleme (non-olasılıklı rastgele olmayan örnekleme) yöntemi tercih edilmiştir.

Anketin Hazırlanması

Anket soruları, saha deneyimleri ve klinik tecrübeler doğrultusunda oluşturulmuş olup, Oğur ve Tekbaş'ın (2003) çalışması temel alınarak yapılandırılmıştır. Anket formu toplam 9 sorudan oluşmakta olup, sorular hasta sahiplerinin antibiyotik kullanım alışkanlıklarını ve veteriner hekimler ile olan iletişimlerini değerlendirmeye yönelik olarak hazırlanmıştır (Tablo 1; Anket Soruları).

Tablo 1. Katılımcıların anket sorularına verdikleri yanıtlar
Table 1. Participants' responses to survey questions

	Hayvan Sahiplerine Antibiyotik Kullanımına İlişkin Sorulan Anket Soruları	Cevaplar	% (n)
1	İşletmede aşı protokolü uyguluyor musunuz?	Evet	27,5 (33/120)
		Hayır	72,5 (87/120)
2	İşletmeye dışarıdan alınan her hayvana tedbir olarak antibiyotik uyguluyor musunuz?	Evet	30 (36/120)
		Hayır	70 (84/120)
3	Her yeni doğan buzağıya tedbir olarak antibiyotik uyguluyor musunuz?	Evet	22,5 (27/120)
		Hayır	77,5 (93/120)
4	Antibiyotik uygulanan hayvanın sütünü tüketmemeye, sütünü satmamaya dikkat ediyor musunuz?	Evet	80,8 (97/120)
		Hayır	19,2 (23/120)
5	Antibiyotik uygulanan hayvanın etinin tüketilmesine dikkat ediyor musunuz?	Evet	80,8 (97/120)
		Hayır	19,2 (23/120)
6	Kullandığınız/kullanacağınız antibiyotik seçiminde ilaç fiyatına dikkat ediyor musunuz?	Evet	42 (51/120)
		Hayır	58 (69/120)
7	Hastaya reçete edilen antibiyotiği kullanımı tavsiye edilen günde önce hayvan iyileşse bile, tavsiye edilen son güne kadar uyguluyor musunuz?	Evet	69,2 (83/120)
		Hayır	30,8 (37/120)
8	Kullandığınız antibiyotiği ilaç prospektüsünde yazan ticari markanın tavsiye doza uyarak mı yapıyorsunuz?	Evet	79,2 (95/120)
		Hayır	20,8 (25/120)
9	Genellikle tek doz (uzun etkili) uygulanan antibiyotikleri mi yoksa uzun süreli kullanım gerektiren antibiyotikleri mi tercih edersiniz?	Tek doz	24,8 (31/120)
		Çoklu doz	71,2 (89/120)

Veri Toplama Yöntemi

Anketler, yüz-yüze görüşme yöntemiyle uygulanmış olup, hasta sahipleriyle etkili iletişim kurulabilmesi amacıyla teknik terimlerin kullanımından kaçınılmıştır. Soruların daha iyi anlaşılabilmesi için bazı sorular öncesinde kısa açıklamalar yapılmıştır. Ayrıca, antibiyotik uygulaması yapılan hayvanların tedavi sürecinin izlenebilmesi amacıyla, anketin uygulanmasından 30 gün sonra hasta sahipleriyle tekrar iletişime geçilmiş ve hayvanların sağkalım durumları hakkında bilgi alınmıştır.

Gruplandırma

Çalışmada, veteriner hekim tarafından muayene sonrası, reçete edilen antibiyotik uygulamasına devam eden bilinçli hayvan sahipleri (Grup 1, n=23), veteriner hekim tarafından muayene edilmeden, verilen anamnez sonucu veteriner hekim tavsiyesi ile antibiyotik uygulaması yapan hayvan sahipleri (Grup 2, n=56) ve daha önceki uygulamalardan artan ve elinde bulunan antibiyotikleri rastgele seçerek uygulayan hayvan sahipleri (Grup 3, n=41) olarak gruplar oluşturuldu.

Veri Analizi

Çalışma sonunda elde edilen anket verileri değerlendirilerek, istatistiksel analizleri (IBM SPSS 25.0) gerçekleştirildi. Kategorik değişkenler için isefrekans ve yüzde değerleri hesaplandı. Çalışmada kategorik değişkenler arasındaki ilişkiyi değerlendirmek amacıyla Ki-kare (Chi-square) analizi uygulandı. Anlamlılık düzeyi $p<0,05$ olarak kabul edildi. Venn diyagramı için UGent, Genomics, & 927 üzerinden https://bioinformatics.psb.ugent.be/webtools/Venn / adresi kullanıldı.

BULGULAR

Çalışmaya dahil edilen hayvanların %70'inin erkek (n=84), %30'unun dişi (n=36) olduğu belirlendi. Hastaların 47 tanesinin yaşadığı, 67 tanesinin de öldüğü öğrenildi. 6 hasta sahibine ulaşılamadı. 6 hayvanın yaşayıp yaşamadığı ile ilgili bir bilgi alınamadı. Hayvanların ırkları, %80'i simental (n=100), %8,3'ü montofon (n=10), %6,7'si holstein (n=8), %0,8'si şarole (n=1) ve %0,8'si yerli kara (n=1) olduğu belirlendi. Hayvanların %52,5'inin 0-30 günlük neonatal dönemde (n=63), %23,3'ü 1-6 aylık (n=28), %10,8'i 6-12 aylık (n=13), %3,3'ü 12-24 aylık (n=4), %10'unun 24 aylıktan büyük (n=12) yaşta olduğu belirlendi.

Hayvanların %65,8'i sindirim (n=79), %26,7'si solunum (n=32), %5'i sistemik (n=6) ve %2,5'i dolaşım (n=3) sistemi kaynaklı hastalık teşhisi konuldu. Sindirim sistemi hastalıklarının %30,4'ü bakteriyel (n=24), %27,8'i nonenfeksiyöz (n=22), %16,5'i viral (n=13), %15,2'si paraziter (n=12), %6,3'ü paraziter+viral (n=5), %2,5'i paraziter+bakteriyel (n=2), %1,3'ü viral+bakteriyel (n=1) kaynaklı olduğu tespit edildi. Sindirim sistemi hastalığı teşhisi konulan hayvanların %47,3'ünün canlı (n=35), %52,7'sinin (n=39) ise öldüğü öğrenildi. 5 hayvanın yaşayıp yaşamadığı ile ilgili bir bilgi alınamadı. Solunum sistemi hastalıklarının %90,6'sı bakteriyel (n=29), %6,3'ü viral (n=2), %3,1'i nonenfeksiyöz (n=1) nedenlere bağlı olduğu belirlendi. Solunum sistemi hastalığı teşhisi konulan

hayvanların %32,3'i canlı (n=10), %67,7'sinin (n=21) öldüğü öğrenildi. 1 hayvanın yaşayıp yaşamadığı ile ilgili bir bilgi alınamadı. Sistemik hastalıkların %83,3'ünün nonenfeksiyöz (n=5), %16,7'sinin ise bakteriyel (n=21) nedenlere bağlı olduğu belirlendi. Solunum sistemi hastalığı teşhisi konulan hayvanların %33,3'ü canlı (n=2), %66,7'sinin (n=21) ise öldüğü bilgisi alındı. Dolaşım sistemi hastalıklarının %100'ü protozooner (paraziter) (n=3), nedenlere bağlı olduğu belirlendi. Dolaşım sistemi hastalığı teşhisi konulan hayvanların %100'ünün (n=3) öldüğü öğrenildi.

Antibiyotik kullanılan hayvanlara farklı antibiyotik grupları tek ya da kombine edilerek uygulandığı tespit edildi. En çok kullanılan antibiyotik grubu %32,62 (n=61) oranında beta-laktamlardır. Kullanılan diğer antibiyotik grupları ise %21,93 (n=41) kinolonlar, %9,63 (n=18) sulfanomidler, %9,09 (n=17) makrolitler, %8,02 (n=15) aminoglikozitler, %8,02 (n=15) tetrasiklinler, %7,49 (n=14) amfenikoller ve %3,20 (n=6) linkozamidlerdir. Beta-laktamlar grubunda (n=61) ise %63,93 (n=39) oranında penisilinler ve %36,07 (n=22) oranında sefalosporinler kullanıldığı belirlendi (Tablo 2).

Tablo 2. Kullanılan antibiyotiklerin alt grupları / etken maddeleri
Table 2. Subgroups/agent substances of antibiotics used

Antibiyotikler % (n)		Alt grupları / Etken maddeleri	% (n)
Beta-Laktam İnhibitörleri % 32,62 (n=61)	Penisilinler	Amoksisilin	7,48 (14)
		Amoksisilin +Klavulanat	2,67 (5)
		Prokain Penisilin - Dihidrostreptomisin sülfat	10,69 (20)
	Sefalosporinler	Seftiofur	5,88 (11)
		Sefkuinom	3,74 (7)
		Sefaleksin	1,06 (2)
		Sefazolin	1,06 (2)
Kinolonlar %21,93 (n=41)		Enrofloksasin	20,85 (39)
		Marbofloksasin	1,06 (2)
Makrolidler %9,09 (n=17)		Tulatromisin	6,95 (13)
		Spiramisin	2,13 (4)
Aminoglikozidler %8,2 (n=15)		Gentamisin	5,34 (10)
		Neomisin	1,06 (2)
		Aminosidin sülfat (Paromomisin)	1,60 (3)
Amfenikoller %7,49 (n=14)		Florfenikol	6,41 (12)
		Kloramfenikol	1,06 (2)
Sülfanomidler %9,63 (n=18)		Sülfametazin	2,13 (4)
		Sülfadoksin	2,67 (5)
		Sülfametoksazol	2,13 (4)
		Sülfadimidin	2,67 (5)
Tetrasiklinler %8,2 (n=15)		Tetrasiklin	1,06 (2)
		Klortetrasiklin	1,60 (3)
		Oksitetrasiklin	5,34 (10)
Linkozamidler %3,20 (n=6)		Linkomisin	3,21 (6)

120 ayrı işletmeden, sadece 33 işletmenin (%27,5) hayvanlarına solunum sistemi, sindirim sistemi ve klostridial enfeksiyonlar yönünden aşı protokolü uyguladığı öğrenildi. Anket uygulaması yapılan işletme sahiplerine hayvanlarına hastalıklardan koruma amacı ile tedbir olarak antibiyotik ilaç uygulayıp uygulamadığı sorulduğunda 36 hayvan sahibinin (%30) dışarıdan satın aldığı her hayvana ve 27 hayvan sahibinin (%22,5) ise her yeni doğan buzağıya bilinçsizce ve rastgele antibiyotik uyguladığını belirtti. Hasta sahiplerinin %40'ının (n=48) profilaksi amacı ile antibiyotik kullandığı belirlendi. Bununla birlikte 97 işletme sahibinin antibiyotik uygulamalarının ette ve sütte kalıntılara sebep olduğunu bildiği ve bu ürünlerin kalıntı süresince tüketiminden uzak durduğu öğrenildi. Aynı etken maddeye sahip farklı ticari markalara ait ilaçların hasta sahiplerince kullanıldığı daha önceden bilindiğinden, antibiyotik ilaç

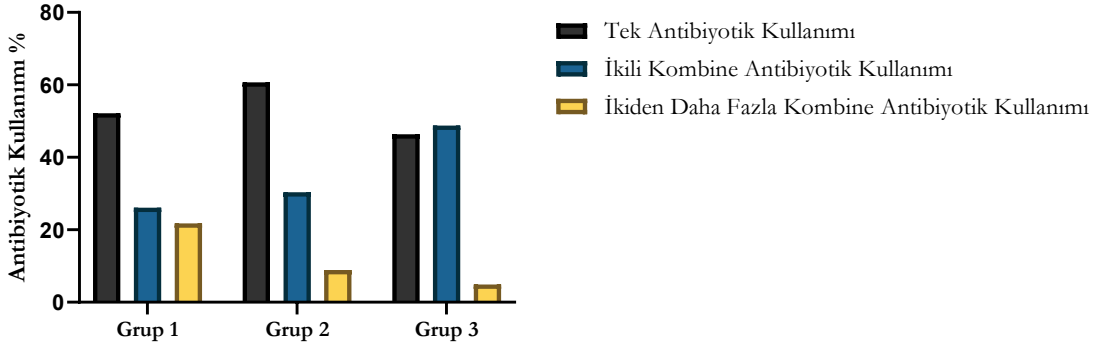
seçiminde, ilaç fiyatlarının göz önünde bulundurulup bulundurulmadığını anlamak amacı ile hasta sahiplerine ilaç fiyatlarının tercih nedeni olup olmadığı ile ilgili bir soru yöneltildi. 51 hasta sahibi (%42) antibiyotik ilaç seçiminde daha ucuz olan antibiyotik ilaçları tercih ettiklerini belirtti. Hasta sahiplerine antibiyotik uygulamalarında doz ve kullanım sürelerinin doğru olarak uygulanıp uygulamadığı anlamak amacı ile de anket soruları soruldu. İşletme sahiplerinden 83 tanesi (%69,2) veteriner hekim tarafından reçete edilen antibiyotiği tavsiye edilen gün süresince uyguladığını, 95 tanesi (%79,2) de antibiyotik ilaçları prospektüsünde yazan tavsiye doza uygun olarak uyguladığını belirtti (Tablo 1).

Kliniğimize getirilmeden önce hasta hayvanlarına tek bir antibiyotiği uygulayan hasta sahiplerinin oranının %54,17 (n=65), iki antibiyotiği kombine olarak kullananların oranının %35,83 (n=43), ikiden fazla antibiyotiği kombine ederek uygulayanların oranının ise %10 (n=12) olduğu tespit edildi (Tablo 3).

Tablo 3. Tek ve kombine antibiyotik kullanımı
Table 3. Single and combined antibiotic use

Antibiyotik Kullanımı	**% (n)**
Tek Antibiyotik Kullanımı	54,17 (65/120)
İkili Kombine Antibiyotik Kullanımı	35,83 (43/120)
İkiden Daha Dazla Kombine Antibiyotik Kullanımı	10 (12/120)

Antibiyotik kullanım kategorileri ile gruplar arasında istatistiksel olarak anlamlı düzeyde bir bağlantı görülmedi ($\chi2=7,337$; $p=0,119$). Gruplara göre antibiyotik kullanımı değerlendirdiğinde tek bir antibiyotik kullanımının en çok veteriner hekimin bizzat kendisi tarafından uyguladığı (%52,17) ve tavsiye ettiği (%60,71), ikili kombine antibiyotik kullanımının ise en çok Grup 3'teki katılımcılar tarafından uygulandığı görüldü (%48,78). İkiden daha fazla kombine antibiyotik kullanımının ise veteriner hekimin bizzat kendisi tarafından uyguladığı (%21,74) uygulandığı belirlendi (Şekil 1).



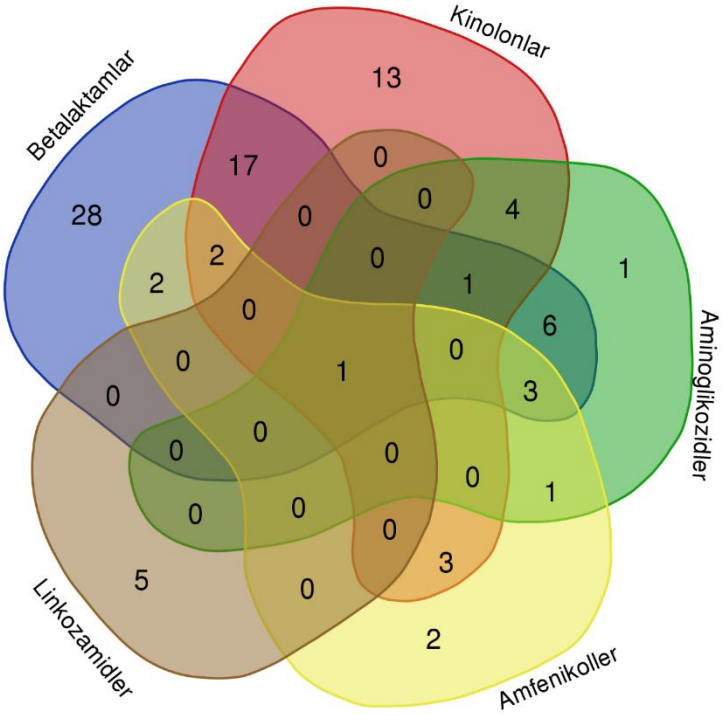
Şekil 1: Gruplara göre tek ve kombine antibiyotik kullanımı
Figure 1: Single and combined antibiotic use by groups

Çalışmamızda beta-laktamların en çok kullanılan antibiyotik grubu olduğu belirlendi. Antibiyotik kategorileri ile gruplar arasında istatistiksel olarak anlamlı düzeyde bir bağlantı görülmedi ($\chi2=15,441$,$p=0,349$). Veteriner hekimlerin en çok kullandığı(%26,8) ve tavsiye ettiği (%37) antibiyotik beta-laktam grubuydu. Benzer şekilde hayvan sahiplerinin de en çok kullandığı antibiyotik grubu beta-laktam grubuantibiyotiklerdi (%30,8). Kullanılan makrolit ve amfenikol grubu antibiyotiklerin çoğunluğu (sırasıyla; %52,9, %57,1) veteriner hekimlerin tavsiyesi üzerine kullanılmıştır. Tetrasiklin ve linkozamid grubu antibiyotiklerin ise çoğunluğu hayvan sahiplerinin kendileri tarafından, veteriner hekimin tavsiyesi ve reçetesi olmaksızın kullanılmıştır (sırasıyla; %40,%66,7). Veteriner hekimlerin en az kullandığı antibiyotik grubu ise kinolonlardır (%14,6) (Tablo 4).

Tablo 4. Gruplara göre uygulanan antibiyotikler
Table 4. Antibiotics administered by groups

Antibiyotikler	Grup 1 (n=23)		Grup 2 (n=56)		Grup 3 (n=41)	
	n	%	n	%	n	%
Beta-laktamlar (n=61)	11	26,83	30	37,04	20	30,77
Kinolonlar (n=41)	6	14,63	18	22,22	17	26,15
Makrolidler (n=17)	4	9,76	9	11,11	4	6,15
Aminoglikozidler (n=15)	5	12,19	4	4,94	6	9,24
Amfenikoller (n=14)	2	4,88	8	9,88	4	6,15
Sülfanomidler (n=18)	8	19,51	6	7,41	4	6,15
Tetrasiklinler (n=15)	4	9,76	5	6,17	6	9,24
Linkozamidler (n=6)	1	2,44	1	1,23	4	6,15
Toplam	41	100	81	100	65	100

Çalışmamızda, linkozamid, kinolon, beta-laktamaz, aminoglikozid ve amfenikol grubu antibiyotiklerin hepsini kombine olarak kullanan sadece bir hasta sahibi olduğu tespit edildi (Şekil 2.).



Şekil 2: Bir katılımcının hayvanına kullandığı beş antibiyotik grubu
Figure 2: Five antibiotic groups used by a participant on his animal

Antibiyotik kategorileri ile hastalıklar arasında istatistiksel olarak anlamlı düzeyde bir bağlantı görülmedi ($\chi 2=29,625$, $p=0.100$). Beta-laktam (%65,6), kinolon (%58,5), aminoglikozid (%66,7), amfenikol (%50), sülfanamid (%89,9), tetrasiklin (%86,7) ve linkozamid (%100) grubu antibiyotikler en çok sindirim sistemi hastalıklarında kullanıldığı belirlendi. Makrolit grubu (%64,7) antibiyotikler ise en

çok solunum sisteminde kullanıldığı tespit edildi (Tablo 5).

Tablo 5. Çeşitli sistem hastalıklarında kullanılan antibiyotikler
Table 5. Antibiotics used in various system diseases

Antibiyotikler	Sindirim Sistemi Hastalıkları (n=79)		Solunum Sistemi Hastalıkları (n=32)		Sistemik Hastalıklar (n=6)		Dolaşım Sistemi Hastalıkları (n=3)	
	n	%	n	%	n	%	n	%
Beta-laktamlar (n=61)	40	33,33	15	27,27	4	50	2	50
Kinolonlar (n=41)	24	20	14	25,45	2	25	1	25
Makrolidler (n=17)	4	3,33	11	20	1	12,5	1	25
Aminoglikozidler (n=15)	10	8,33	5	9,09	-	-	-	-
Amfenikoller (n=14)	7	5,83	6	10,91	1	12,5	-	-
Sülfanomidler (n=18)	16	13,33	2	3,64	-	-	-	-
Tetrasiklinler (n=15)	13	10,83	2	3,64	-	-	-	-
Linkozamidler (n=6)	6	5	-	-	-	-	-	-
Toplam	120	100	55	100	8	100	4	100

TARTIŞMA

Gelişmekte olan ülkelerde antimikrobiyaller, kötü hijyen koşullarını ve düzensiz hayvan yönetim sistemlerini dengelemek için hastalıkları önlemek ve büyümeyi teşvik etmek amacıyla rutin olarak alt-terapötik dozlarda kullanılmaktadır (Silbergeld ve ark., 2008; Elliott ve ark., 2017; Hosain ve ark., 2021; Sungur ve ark., 2025). Antibiyotiklerin aşırı ve uygunsuz kullanımına ek olarak dirençli bakterilerin gelişmesi nedeniyle çeşitli ülkelerde farklı yıllarda ve Türkiye'de 2005 yılında hayvan yetiştiriciliğinde antibiyotik türevi büyüme faktörlerinin kullanımı yasaklanmıştır (Şahal, 2012; Küçükbüğrü ve Acaröz, 2020). Ayrıca hayvansal üretimde antimikrobiyallerin kullanımına ilişkin tek tip düzenlemelerin bulunmaması, bu ilaçların hayvanlarda akılcı kullanımını tehdit etmektedir (Thakur and Panda, 2017). Antimikrobiyallerin terapötik konsantrasyonların altında kullanımı bakteriler üzerinde seçici baskı oluşturur ve bir veya daha fazla antimikrobiyale dirençli bakteri suşlarının ortaya çıkmasına neden olur. AMD dünya çapında bir "Tek Sağlık" sorunudur. Antimikrobiyallerin uygunsuz kullanımı, AMD'nin gelişmesinde ve yayılmasında önemli bir etkendir (Thakur and Panda, 2017).

Çalışmamızda katılımcıların %40'ının profilaksi amacı ile antibiyotik kullandığı tespit edildi. ABD'de sığır yetiştiricilerinin %20'sinin besi çiftliklerinde profilaktikamaçlı klortetrasiklin uygulamalarınınsığırlarda solunum sistemi hastalıklarının önlenmesinde kullanıldığı belirtilmektedir. (Miller ve ark., 2018). Araştırmacılar antibiyotik ilaçların profilaktik amaçlı kullanımının AMD gelişimdeki etkisi hakkında farklı sonuçlara ulaşmışlardır. Dobrzanska ve ark., (2020) yaptıkları bir çalışmada sağlıklı buzağılarda koruyucu amaçlı kullandıkları bir antibiyotiğin (florfenikol) buzağılarda disbiyoza neden olduğunu ve kullanılan antibiyotiğe yanıt olarak yüksek bir antibiyotik direnci geliştiğini belirlemişlerdir. Okada ve ark., (2023) kullanılan klortetrasiklin preparatlarının buzağılarda büyüme gelişmesinde hiçbir etkisinin olmadığını, aksine buzağıların bağırsak fonksiyonu ve performansı üzerinde olumsuz bir etkiye sahip olabileceğini belirtmişlerdir. Miller ve ark., (2018) ise yaptıkları çalışmada sığırların solunum sistemi hastalıkların önlenmesinde klortetrasiklin uygulamalarının AMD gelişiminde bir etkisinin bulunmadığını belirtmektedir. Çeşitli çalışmalarda profilaksi amaçlı antibiyotiklerin kullanımının AMD gelişiminde etkili olduğunun ortaya konulması, sığır işletmelerinde immunoprofilaksi ve biyogüvenlik önlemlerinin artırılması gibi alternatif ve etkili yöntemlerin gerekliliğini ortaya koymaktadır. Çalışmamızda katılımcıların sadece %27,5'inin hayvanlarına solunum, sindirim sistemi ve klostridial enfeksiyonlar yönünden aşı protokolü uyguladığı belirlenmiştir. Bu durum Kayseri'de bulunan işletmelerde aşı uygulamalarının düşük düzeyde yapıldığını göstermektedir. Yetiştiriciler enfeksiyöz hastalıklara karşı korunmada antibiyotik ilaç uygulamaları yerine immunoprofilaksi uygulamaları ile koruyucu önlemler alınması yönünde teşvik edilmelidir.

Hayvanlarda kullanılan antibiyotikler, et, süt ve yumurta gibi hayvansal ürünlerin tüketimi yoluyla düşük düzeyde ilaç kalıntı maruziyetine yol açabilir (Öztürk ve ark., 2019). Çalışmamızda hayvan sahiplerinin %80,8'inin antibiyotik uygulamalarının ette ve sütte kalıntılara sebep olduğunu bildiği ve bu ürünlerin kalıntı süresince tüketiminden uzak durduğu öğrenildi. Hindistan'da yapılan bir anket çalışmasında yetiştiricilerin %64'ünün, Nijerya'da %52,7'sinin ve Etiyopya'da ise %28,26'sının antibiyotik kalıntıları hakkında bilgi sahibi olmadıkları belirlenmiştir (Olasoju ve ark., 2021; Dhayal ve ark., 2023; Ragassa ve ark., 2023). Bu veriler dikkate alındığında, bölgemizde bulunan yetiştiricilerin ilaç kalıntıları hakkında daha fazla farkındalık sahibi oldukları söylenebilir. Sungur ve ark., (2025), Hatay bölgesinde satışa sunulan özellikle markasız olan kırmızı et, beyaz et, süt ve yumurta gibi tüm hayvansal ürünlerde çeşitli antibiyotik kalıntılarının bulunduğunu belirtmişlerdir. Bu durum, Hatay bölgedeki hayvan sahiplerinin akılcı antibiyotik kullanımı konusunda yeterli bilinç düzeyine sahip olmadığını göstermektedir. Türkiye'nin birbirine yakın farklı bölgelerinde elde edilen akılcı antibiyotik kullanımı oranlarındaki farklılığın, büyük ölçüde veri toplama yöntemlerindeki ayrılıklar ile çalışma popülasyonlarının farklılık göstermesinden kaynaklandığı düşünüldü.

Yapılan bir anket çalışmasında, antibiyotik seçiminde ekonomik faktörleri dikkate almayan yetiştiricilerin daha az antibiyotik ilaç kullandıkları belirlenmiştir (Borelli ve ark., 2023). Çalışmamızda 51 hayvan sahibi daha ucuz olan antibiyotik ilaçları tercih ettiklerini belirtmişlerdir. Jones ve ark., (2015) İngiltere ve Galler'deki 118 katılımcının dahil olduğu bir anket çalışmasında süt işletmesi sahiplerinin %70'inin antibiyotik kullanımı azaltmak yönünde eğilimleri olduğunu fakat bu düşüncenin asıl sebebinin AMD değil, işletme ilaç maliyetlerinden tasarruf etmek olduğunu bildirmişlerdir. Ayrıca katılımcıların %60'ının maliyetleri düşürmek için daha az antibiyotik kullandıklarını belirtmişlerdir. Bununla birlikte Jones ve ark., (2015) çalışmaya katılan yetiştiricilerin %55'inin ineklerinde antibiyotik kullanmadan önce veteriner hekimlerine danışmamış olabileceğini belirtmişlerdir. Çiftçilerin zaman zaman, önceki reçetelerden artan antibiyotikleri kullandığını bildirmişlerdir. Bu veri ile uyumlu olarak çalışmamızda grup 3 de yer alan katılımcılar (n=41) veteriner hekime danışmadan ellerinde kalan ilaçları rastgele kullanmışlardır. Yetiştiricilerin ilaç fiyatlarına ve maliyetlere olan yaklaşımları dikkate alındığında antibiyotik ilaç fiyatlarındaki artışın tek başına antibiyotik ilaçların kullanımının azaltılmasında etkili olmayacağı anlamına gelebilir. Antibiyotik ilaçların fiyat artışına ek olarak antibiyotiklerin uygulama dozlarında ve sınırlı miktarlarda yetiştiricilere reçete edilmesi yetiştiricilerin kontrolsüz antibiyotik kullanımının önüne geçilmesinde daha etkili olabilir.

Çalışmamızda katılımcıların %69,22'si veteriner hekim tarafından reçete edilen antibiyotiği tavsiye edilen gün süresince uyguladığını, %79,2'si ise antibiyotik ilaçları prospektüsünde yazan tavsiye doza uygun olarak uyguladığını belirtti. 360 katılımcı ile yapılan bir anket çalışmasında, katılımcıların %45'inin hayvanlar herhangi bir iyileşme belirtisi göstermediği sürece antibiyotik dozunu ve uygulama sıklığını artırdıkları ve %59'unun antibiyotik kullanımından bir gün sonra bir iyileşme gözlemlediğinde antibiyotik uygulamasını durdurdukları belirlenmiştir (Öztürk ve ark., 2019). Bu durum yetiştiricilerin antibiyotik kullanımında tavsiye doz ve tavsiye gün sayısına dikkat etmediklerini ve antibiyotik kullanımı konusunda inisiyatif aldıkları şeklinde yorumlanabilir. Tedavi süresi ve tedavi dozuna uyulmadan gerçekleştirilen antibiyotik uygulamalarının AMD yol açma ihtimalinin yüksek olduğu bilinmektedir (Öztürk ve ark., 2019).

Veteriner hekimliği ve insan hekimliğinde kombine antibiyotik kullanımı tedavi başarısını artırmak için kullanılan bir yöntemdir (Akkan ve Karaca, 2003; Giguere, 2013). Çalışmamızda ikili kombine antibiyotik kullanımının (%48,78) en çok Grup 3'teki katılımcılar tarafından uygulandığı, ikiden daha fazla kombine antibiyotik kullanımının ise veteriner hekimin bizzat kendisi tarafından (%21,74) uygulandığı belirlendi. Antibiyotik ilaç kombinasyonlarının başarısız tedavi durumlarda sinerjistik etkilere sahip olduğu bilinmektedir (Pillai ve ark., 2005). Antimikrobiyal kombinasyonlarının antimikrobiyal sinerjizm, polimikrobiyal terapi, dirençli izolatların ortaya çıkmasını azaltmak ve doz ilişkili toksisitenin azaltılması gibi endikasyonları bulunmaktadır. Bununla birlikte kombinasyon hataları sonucu antibiyotiklerin nötralizasyonu, toksisite, normal mikrobiyal floranın tahrip edilmesi gibi olumsuz farmakokinetik etkileşimlere neden olabilmektedirler (Giguere, 2013). Antibiyotiklerin akılcı kullanımın yanında kombine antibiyotik kullanımına da dikkat edilmelidir. Kombinasyonlar yalnızca etkinliklerinin kanıtlandığı yerlerde kullanılmalı ve sinerjistik etkiyi en üst düzeye çıkaracak şekilde yapılmalıdır (Giguere, 2013). Çalışmamızda bir hasta sahibinin kullandığı antibiyotiklerden cevap alamaması nedeni ile kısa aralıklarla bir hafta içerisinde toplam beş farklı antibiyotiği (Beta-laktam, kinolon, aminoglikozit, linkozamid ve amfenikol grubu antibiyotikler) hasta hayvanına kullandığı belirlenmiştir. Kullanılan bu antibiyotikler arasında sinerjik etkinin yanında antagonistik etki de bulunmaktadır. Bu durum yetiştiricilerin antibiyotik ilaçlara ulaşım kolaylığı sayesinde kontrolsüz antibiyotik kullanımından kaçınmadıklarına örnek olarak verilebilir.

Çalışmamızda araştırma süreci ve yönteme bağlı olarak bazı sınırlılıklar söz konusudur. İlk olarak, araştırma belirli bir coğrafi bölgede ve sınırlı sayıda katılımcı ile gerçekleştirilmiştir. Bu durum, elde edilen bulguların tüm ruminant hayvan sahiplerine genellenebilirliğini yani çalışmanın istatistiksel gücünüsınırlandırmaktadır. İkinci olarak

araştırmamızın bir anket uygulaması olması nedeniyle hasta sahiplerinin verdikleri yanıtlar toplumsal beklenti etkisi nedeni ile yanıltıcı olabilir. Benzer şekilde, hatırlama yanlılığı da katılımcıların geçmişteki antibiyotik kullanım pratiklerini doğru şekilde aktaramamalarına neden olmuş olabilir. Üçüncü olarak, katılımcıların eğitim düzeyi ve teknik bilgi birikimleri farklılık göstermektedir. Çalışmamızda yetiştiricilerin eğitim düzeyleri, kaç yıldır hayvancılık yaptıkları, antibiyotik uygulamaları hakkında almış oldukları eğitimler, işletme tipleri (gelişmiş veya eski tip) gibi demografik ve sosyoekonomik bilgilerin kaydedilmemiş olması da çalışmamızın bir diğer kısıtlayıcı yönüdür.

SONUÇ

Sonuçta, antibakteriyel ilaçların, özellikle de beta-laktam grubu antibiyotiklerin hem tek hem de kombine antibiyotikler olarak en sık tercih edilen ilaçlar olduğu, ancak bu ilaçların yalnızca veteriner hekimler tarafından değil, hasta sahipleri tarafından da kontrolsüz bir şekilde uygulandığı belirlenmiş; bu durum, hayvan sağlığı ve antibiyotik direnci açısından ciddi riskler oluşturduğu için dikkatle ele alınması gereken bir sorun olarak ortaya konulmuştur. Antimikrobiyallerin bilinçsizce kullanımı hem hayvan sağlığını hem de toplum sağlığını tehdit eden antibiyotik direncine zemin hazırlamaktadır. Bu nedenle, veteriner hekim kontrolü dışında antibiyotik kullanımının engellenmesi, reçetesiz antibiyotik satışının yasaklanması ve ilaçların son kullanım takibinin sağlanması büyük önem taşımaktadır. Türkiye gibi gelişmekte olan ülkelerde, çiftçilere antibiyotik kullanımını azaltacak ekonomik teşviklerin sunulması ve profilaktik yaklaşımların benimsenmesi önemlidir. Gelecekte yaşanması muhtemel bir gıda krizlerine ve akılcı olmayan antibiyotik uygulamalarına bağlı ortaya çıkabilecek daha çok sayıda dirençli suşlara karşı hayvan, insan ve çevre sağlığının korunması amacıyla ulusal ve uluslararası düzeyde sürveyans çalışmaları, eğitim programları, antibiyotik kullanım rehberleri ve antibiyogram temelli tedavi yaklaşımlarının hayata geçirilmesi büyük önem arz etmektedir**.**

Çıkar Çatışması: Yazarların bildirecekleri herhangi bir çıkar çatışması bulunmamaktadır.

Yazarların Katkıları: ET, projenin fikrine, tasarımına ve yürütülmesine katkıda bulunmuştur. MOU, MK ve OGŞ verilerin toplanmasına katkıda bulunmuştur. ET ve MOU verileri analiz etmiştir. ET ve MOU makalenin taslağını hazırlamış ve yazmıştır. ET,makaleyi eleştirel bir şekilde incelemiştir. Tüm yazarlar, nihai halini okuyup onaylamıştır.

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RESEARCH ARTICLE

Determination of Systemic Inflammation and Diagnostic Markers Using Iron Parameters in Foals Infected with *Rhodococcus equi*

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ABSTRACT

Rhodococcus equi is a significant respiratory pathogen in foals, particularly those under six months of age. This study aimed to evaluate the effects of *R. equi* infection on serum iron (Fe), unsaturated iron-binding capacity (UIBC), and fibrinogen (Fbg) concentrations. Fifteen foals with confirmed *R. equi* pneumonia and ten healthy control foals were included. Infected foals were treated with oral azithromycin and rifampicin for 15 days. Samples were collected on days 0, 7, and 14, and Fe, UIBC, and Fbg levels were analyzed. Statistical analysis showed significantly higher UIBC and Fbg levels in the infected group at the start of treatment compared to controls ($p=0.004$ and $p<0.001$, respectively). Serum Fe levels did not differ significantly between groups ($p=0.138$), though a trend toward lower levels in infected foals was noted, reflecting the inflammatory response. Changes in UIBC and Fbg levels reflect the progression of infection and response to treatment. Among the parameters evaluated, fibrinogen appears to be the most reliable biomarker for monitoring systemic inflammation during *R. equi* pneumonia in foals. However, the diagnostic value of iron parameters warrants further investigation with larger sample sizes and time-point-specific analyses.

Keywords: Horse, Iron, Unsaturated iron-binding capacity

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Rhodococcus equi ile Enfekte Olmuş Taylarda Demir Parametreleri Kullanılarak Sistemik İnflamasyon ve Tanı Belirteçlerinin Belirlenmesi

ÖZ

Rhodococcus equi, özellikle altı aylıktan küçük taylarda önemli bir solunum sistemi patojenidir. Bu çalışmada, R. equi enfeksiyonunun serum demiri (Fe), doymamış demir bağlama kapasitesi (UIBC) ve fibrinojen (Fbg) düzeylerine etkisi değerlendirilmiştir. Çalışmaya, tanısı doğrulanmış ve tedavi edilen 15 tay ile sağlıklı 10 tay dahil edilmiştir. Enfekte gruptaki taylara 15 gün boyunca azitromisin ve rifampisin uygulanmıştır. Örnekler tedavi öncesi (0. gün), 7. ve 14. günlerde alınmış ve Fe, UIBC, Fbg düzeyleri analiz edilmiştir. İstatistiksel analizler sonucunda, tedavi başlangıcında enfekte taylarda UIBC ve Fbg düzeylerinin kontrol grubuna göre anlamlı derecede yüksek olduğu bulunmuştur (sırasıyla $p=0,004$ ve $p<0,001$). Serum Fe düzeyleri açısından gruplar arasında anlamlı fark gözlenmemiştir ($p=0,138$), ancak enfeksiyonun inflamatuvar etkisine bağlı bir azalma eğilimi izlenmiştir. ROC analizine göre Fbg, tanı açısından yüksek duyarlılık ve özgüllüğe sahip bulunmuştur. UIBC ve Fbg düzeylerindeki değişiklikler enfeksiyonun seyrini yansıtabilir. Fbg, özellikle tedavi sürecinin takibinde güvenilir bir inflamasyon belirteci olarak öne çıkmaktadır. Demir parametrelerinin tanısal değeri ise daha ileri çalışmalarla desteklenmelidir.

Anahtar kelimeler: At, Demir, Doymamış demir bağlama kapasitesi

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INTRODUCTION

Rhodococcus equi (R.equi) is classified within the *Actinobacteria phylum*, encompassing mycobacteria. *R. equi* is a prevalent Gram-positive coccobacillus that infects macrophages, leading to pulmonary and extrapulmonary pyogranulomatous infections in several animal species including humans (Vázquez-Boland and Meijer 2019). *R. equi* is a notable pathogen in foals (Giguere et al. 2011). It exerts a considerable financial burden on the equine sector due to treatment costs and labor, compounded by the absence of particular immunizations for prophylaxis. In humans, *R.equi* mostly impacts immunocompromised persons, particularly those infected with HIV. The infection often resembles pulmonary tuberculosis (Yamshchikov et al. 2010). Numerous analogous operational modes exist for *R. equi* and *Mycobacterium tuberculosis* infections (Deniz et al. 2024).

Most iron (Fe) is sequestered in hepatocytes and macrophages of the reticuloendothelial system, along with ferritin. The regulation of plasma iron concentration and iron storage is governed by hepcidin, which is secreted by hepatocytes. It regulates the transport activity of the cellular iron exporter ferroprotein (FPN1) in proximal duodenal enterocytes and macrophages of the reticuloendothelial system through a negative feedback loop. FPN1 is the sole identified cellular iron transporter that facilitates the export of iron from duodenal enterocytes to the bloodstream, in addition to mobilizing iron from hepatocytes and resident macrophages within the reticuloendothelial system. The former reprocesses iron from engulfed senescent red blood cells. Following duodenal absorption, iron is either retained as mucosal ferritin or transferred to the bloodstream via ferroportin 1 (FPN1). Elevated hepcidin levels result in heightened intracellular iron binding to ferritin and a reduction in plasma iron levels. The iron associated with mucosal ferritin is not available for systemic utilization and is lost through the shedding of mucosal endothelial cells over time. This is the sole pathway for the excretion of bodily iron (Lanser et al. 2021).

Fe transport in the bloodstream predominantly occurs bound to the β-globulin transferrin, generated by the liver. Immune activity in response to microorganisms triggers the production of several pro-inflammatory cytokines, leading to disrupted iron homeostasis characterized by enhanced iron uptake and diminished iron release by macrophages in the reticuloendothelial system. Inflammation additionally enhances the production of the iron-regulatory protein hepcidin. Low hepcidin levels are typically observed, allowing for adequate iron absorption in the duodenum and appropriate iron release from macrophages and storage (refer to Lanser et al. 2021 for further details).

Above facts were relevant for our idea to study serum Fe concentration and UIBC in a group of healthy foals and a group of *R.equi* infected foals. Using available repository material, we ran tests to quantify serum iron (Fe) and unbound iron binding capacity (UIBC). Available data on serum fibrinogen concentration (Fbg) and haematology data were also included in the analysis.

Since the physiological concentration of serum Fe is lower than the minimum required for pathogen survival, some microorganisms developed mechanisms to capture the scarcely available Fe from various sources (Vail et al. 2021). Hence, the host response commonly lowers serum Fe concentration to limit pathogen proliferation. Given the previous studies reporting decreased serum or plasma iron concentration as an indicator of acute inflammatory response in horses, a decrease in serum iron concentration can also be a feature of *R.equi* infection (Smith et al. 1987; Borges et al. 2007). This actually appers to be a feature, since decreased serum or plasma iron concentrations were identified as an indication for an acute inflammatory response in horses

This observational study was based on the hypothesis that *Rhodococcus equi* infection and/or the treatment protocol may affect serum iron (Fe) and unsaturated iron-binding capacity (UIBC) levels in foals. The hypothesis was evaluated by measuring Fe and UIBC levels in previously collected biological samples.

MATERIALS and METHODS

This study was approved by Kastamonu University Animal Experiments Local Ethics Committee and an approval certificate (Decision no: 2025/49) was obtained.

Samples from the Case Group

Repository samples were used from foals of a study already in 2024 by Deniz et al. (2024). Briefly, the samples came from 15 foals suffering from confirmed R. equi pyogranulomatous pneumonia that were treated orally for 10 days with rifampin and azithromycin after the diagnosis including physical exam, thoracic ultrasonography and bacteriology from transtracheal fluid according to ACVIM consensus (Giguère et al. 2011a).

Control Group

The foals were randomly selected from the population on the stud. Moreover, at the start of the study, fibrinogen levels were within the reference range (<400 mg/dL) of all 10 foals. Moreover, at the start of the study fibrinogen levels were within the reference range (<400 mg/dL) of all 10 foals.

Sampling, Sampling Material and Sample Handling

Serum iron (Fe) and unsaturated iron-binding capacity (UIBC) were assessed using a DiaSys respons® 910 Vet analyser (DiaSys Diagnostic Systems, USA, Wixom, MI), while fibrinogen (Fbg) contents were determined with an automated Start St Art coagulation analyser (Diagnostica Stago Inc., Asnieres, France).

Treatment

Foals in the case group were treated with oral azithromycin (Azomax, Kocak Farma) 10 mg/kg orally once daily for 15 days and rifampicin (RIFCAP, Kocak Farma) 5 mg/kg orally twice daily for 15 days. For more details see Deniz et al. (2024).

Statistical Analysis

Data were displayed as boxplots and descriptive statistcs was used to characterize the groups. The statistical software used was SPSS version 25. Inferal statistics was by one-way ANOVA and by post-hoc Tukey HSD. Correlations were calculated by Pearson. Additionally, ROC (Receiver Operating Characteristic) analysis was performed to examine the discriminatory power of biochemical parameters between the groups. The classification performance of the parameters was Sampling techniques and handling were conducted in accordance with standard practices in horse stud medicine, as thoroughly detailed by Deniz et al. (2024). evaluated by calculating the AUC (Area Under the Curve) of the ROC. Significance level were set at $p<0.05$ for all statistical tests. The results were expressed as mean ± standard deviation (SD) plus their 95% confidence intervals. Graphs were prepared using Graph Pad Prism 9.0 software (Graph Pad Software Inc., San Diego, CA, USA).

RESULTS

Deniz et al. (2024) extensively described the population characteristics of cases and controls and deemed them sufficiently comparable regarding the study's foal population.

The effect of treatment on the concentrations of Fe (µg/dl), TIBC (µg/dl), and Fbg (mg/dl) in foals infected with *R. equi* is given in Table 1. As Fig 1, already suggest UIBC and Fbg (between the groups were significantly different ($p=0.004$ and $p<0.001$, respectively). Serum Fe concentration between the groups over the treatment time was not significantly different ($p=0.138$).

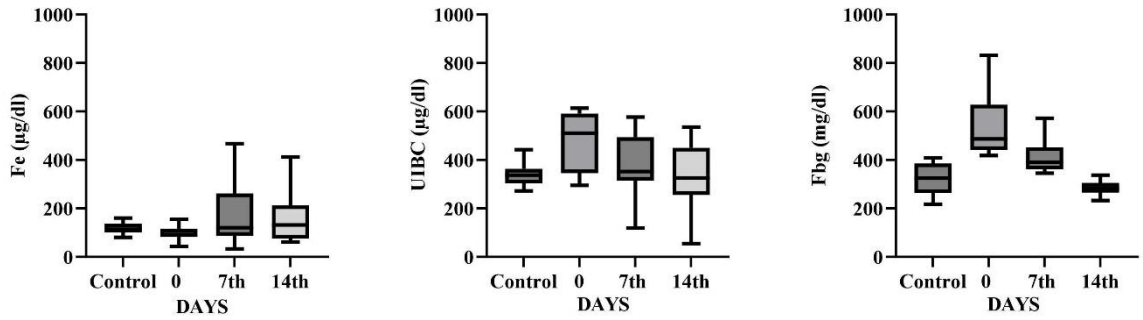

Figure 1: Boxplots of serum Fe, UIBC, and Fbg values once for the control group (dark blue) as reference and of the case by group during the 15 days of treatment.

Table 1. Data were expressed as mean ± standard deviation (95% CI). Fe: Iron, UIBC: Unsaturated Iron Binding Capacity, Fbg:Fibrinogen

Variable	Control	0 (n=15)	7th (n=15)	14th (n=15)	p
Fe (ug/dl)	117±26 (96-158)	101±29 (76-117)	165±118 (80-269)	159±160 (70-248)	-
UIBC (ug/dl)	338±45 (304-376)^{b}	482±116 (344-600)^{a}	376±125 (300-513)^{ab}	333±129 (250-493)^{bc}	*
Fbg (mg/dl)	321±66 (230-400)^{c}	538±124 (441-675)^{a}	412±66 (358-488)^{b}	283±26 (260-308)^{c}	**

a, b, c: Statistical difference was found in columns with different letters ($p<0.05$), :$p>0.05$, *: $p<0.05$, **: $p<0,001$

DISCUSSION

UIBC and Fbg concentrations were significantly higher at the start of the treatment in the case group. The control group was not monitored any longer than at the start of treatment of the case group. All other comparisons are within the case group and are affected by time and treatment. Concentrations of UIBC and Fbg increased after one week of treatment and decreased in the second week, likely due to therapy.
The serum Fe concentration difference between the two groups at the start of treatment, although not statistically significantly, still showed the inflammatory negative marker effect of serum Fe (Fig 1).
When iron stores are low, transferrin levels increase and vice versa when transferrin concentration is low there is too much iron. Transferrin in the case group calculated from UIBC and Fe was about 26 g/dl, whereas in the control group this was about 13 g/dl. So, we concluded as others reported (Smith and Cipriano 1987; Brosnahan et al. 2012) that the innate immunity during infection competes with *R.equi* for Fe by increasing serum transferrin concentration. The large spread in concentrations is likely due to individual responses and different times between the onset of infection and the sampling based on occurrence of clinical symptoms. However, some iron studies in man show diurnal variation of as much as 30%; therefore. If this applies to horses is not known, but we collected our samples during the morning, as recommended by Dale et al. (2002). The individual responses may also concern the genetic background of various genetically determined transferrins. Transferrin is highly polymorphic in most species, with 15 variants identified for horses using biochemical methods, and may be responsible for variation in susceptibility to bacterial pathogens (Mousel et al. 2003).
Regarding haematopoiesis, Faramarzi and Rich (2019) showed that in foals mean cellular volume (MCV) decrease up to month 6 which is related to the elimination of the foetal erythrocytes and increasing number of microcytes (Harvey et al. 1987). It is believed that microcytosis is mainly due to a relative iron deficiency in foals, which is largely because of low iron concentration in the dams' milk and limited iron storage in foals. Faramarzi and Rich (2019) showed the lowest MCV and MCH values at day 90 followed by a steady increase up to day 365. Since these authors did not measure iron levels in their study it is not possible to conclude that foals' temporarily anaemia is indeed Fe dependent. Suitable age-related reference values for foal could not be found in literature. With respect to our observation on microcytosis, a study by Kohn et al. (1990) described that during the first 6 weeks after birth, foal erythrocytes were smaller than adult horse erythrocytes. Mean serum iron concentration was lower than that in adult horses, In foals at birth and during the first 4 months and total iron-binding capacity values were above the adult reference range.
Similar to Mycobacterium tuberculosis, *R equi* is slow growing and clinical signs do not develop for months (Hondalus and Mosser 1994) apparently leaving opportunities for spontaneous recovery, since many foals recover from subclinical *R. equi* infections (Arnold-Lehna et al. 2018). Between 18 and 50 % of infected foals develop pyogranulomatous pneumonia and need antimicrobial treatment (Vail et al. 2021). Till now, treatment is mostly is successful, since 2–5 % of the clinically apparent cases perish (Vail et al. 2021).

Since *R.equi* pneumonia is an insidious disease and early detection may be difficult suitable biomarkers may aid discission to treat. In a previous study Ekinci et al. (2024) tried to identify set of putative lung tissue biomarkers to help the decision to treat or not to treat foals infected with *R.equi*. This was done in the context of preventing overtreatment with antimicrobials. However, concentrations of collectin-11 (CL-11), surfactant protein A (SP-A) and surfactant protein A (SP-D) as single criterium of pulmonary damage appeared not useful. Nevertheless, linking collection - 11 concentration to a general inflammatory marker as plasma fibrinogen suggested improved detection. Since serum iron concentrations are frequently included in blood biochemical diagnostic packages, adding the concentrations of serum iron to the diagnostic algorithm as marker of putative bacterial infection (Ratledge et al. 2000) could refine diagnostic precision. However, our initial data suggest that this is not worth the try. The high specificity and sensitivity of serum fibrogen concentrations (> 400 mg/mL) to detect inflammation has been show before in a the foals included in previous study (Ekinci et al. 2024) and performed as expected. The decrease in fibrinogen levels during the treatment period suggests that the inflammatory response was brought under control. The weak correlation between UIBC and fibrinogen concentrations indicates independent processes during infection. While fibrinogen primarily indicates the inflammatory response, UIBC represents the body's defense mechanism in regulating iron metabolism during infection.
Increased UIBC and elevated fibrinogen levels during infection indicate that iron metabolism and inflammation are simultaneously activated in infected foals. However, the fact that UIBC is more active in the early stages of infection and fibrinogen rises as part of the acute-phase response suggests that these two parameters play different roles at various stages of infection (Inoue et al. 2005). The impact of *R. equi* infection on the host's immune system further complicates these biochemical processes; in particular, chronic infections may lead to inconsistent biochemical responses (Prescott, 1991). This explains why the correlation between serum iron, UIBC, and fibrinogen levels is not strong and highlights the multifaceted effect of infection on biochemical

pathways. The current findings are in line with previous observations on the dynamic nature of iron and protein metabolism in horses, both in pathological and environmental contexts (Aragona et al., 2024; Deniz et al., 2025).

CONCLUSION

In conclusion, changes in serum iron, UIBC, and fibrinogen levels in foals infected with *R. equi* reflect the impact of the infection on biochemical processes, and these parameters serve as important biomarkers for monitoring the infection during treatment. However, a more detailed examination of these biochemical processes at different stages of disease along with larger sample sizes, would help clarify these findings and provide a more comprehensive understanding.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: OD Data curation, Writing – original draft, Methodology. BA Writing– review & editing.

Ethical approval: This study was approved by Kastamonu University Animal Experiments Local Ethics Committee and an approval certificate (Decision no: 2025/49) was obtained.

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RESEARCH ARTICLE

Investigation of the Effects of Polydatin on Gentamicin-Induced Renal Toxicity in Rats

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ABSTRACT

Gentamicin (GNT), an aminoglycoside antibiotic, induces nephrotoxicity through mechanisms like tubular apoptosis and inflammation. Polydatin (Poly), a natural polyphenolic compound with antioxidant and anti-inflammatory properties, has shown potential in alleviating renal damage. This study aimed to investigate the protective effects of Poly in rats with GNT-induced kidney injury using biochemical, molecular, and histopathological methods. 35 *Wistar albino* rats were divided into 5 groups (7 rats/group), including control, Poly (100 mg/kg), GNT (100 mg/kg), and two combined treatment groups (GNT+Poly at 50 mg/kg and 100 mg/kg). After 7 days of treatment, kidney tissues and blood were collected for analysis of renal function markers, oxidant-antioxidant parameters, gene expression (NF-ϰB, TNF-α, Caspase-3, Bax, Bcl-2, KIM1, AQP2), and histopathological evaluation. GNT increased serum urea and creatinine levels ($p<0.001$), increased MDA levels ($p<0.001$) and decreased antioxidants ($p<0.001$); also increased the expression of NF-ϰB and TNF-α ($p<0.001$), increased Caspase-3 and Bax ($p<0.001$) and decreased Bcl-2 levels ($p<0.001$). When administered together with GNT, Poly decreased MDA levels ($p<0.001$) and increased GSH levels ($p<0.001$), decreased inflammation markers (NF-ϰB and TNF-α) ($p<0.01$), decreased Caspase-3 and Bax ($p<0.01$) and increased Bcl-2 levels ($p<0.01$), and also improved histological damage and decreased histological score ($p<0.05$). In GNT-induced renal toxicity, Poly 100 treatment provided renal protection by reversing oxidative stress, inflammation, and apoptosis.

Keywords: Apoptosis; Gentamicin; Nephrotoxicity; Oxidative Stress; Polydatin

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Ratlarda Gentamisin Kaynaklı Böbrek Toksisitesi Üzerine Polidatin' in Etkilerinin Araştırılması

ÖZ

Bir aminoglikozid antibiyotik olan gentamisin (GNT), tübüler apoptoz ve inflamasyon gibi mekanizmalar yoluyla nefrotoksisiteye neden olur. Antioksidan ve anti-inflamatuar özelliklere sahip doğal bir polifenolik bileşik olan polidatin (Poly), böbrek hasarını hafifletme potansiyeli göstermiştir. Bu çalışma, biyokimyasal, moleküler ve histopatolojik yöntemler kullanarak GNT kaynaklı böbrek hasarı olan sıçanlarda Poly'nin koruyucu etkilerini araştırmayı amaçlamıştır. 35 *Wistar albino* sıçan, kontrol, Poly (100 mg/kg), GNT (100 mg/kg) ve iki kombine tedavi grubu (GNT+Poly 50 mg/kg ve 100 mg/kg) olmak üzere 5 gruba (grup başına 7 sıçan) ayrıldı. 7 günlük tedaviden sonra böbrek fonksiyon belirteçleri, oksidan-antioksidan parametreler, gen ekspresyonu (NF-ϰB, TNF-α, Kaspaz-3, Bax, Bcl-2, KIM1, AQP2) ve histopatolojik değerlendirme analizi için böbrek dokuları ve kan toplandı. GNT serum üre ve kreatinin düzeylerini ($p<0,001$), MDA düzeylerini ($p<0,001$) arttırdı ve antioksidanları ($p<0,001$) azalttı; ayrıca NF-ϰB ve TNF-α ekspresyonunu artırdı ($p<0,001$), Kaspaz-3 ve Bax'ı artırdı ($p<0,001$) ve Bcl-2 düzeylerini azalttı ($p<0,001$). GNT ile birlikte uygulandığında Poly, MDA düzeylerini düşürdü ($p<0,001$) ve GSH düzeylerini artırdı ($p<0,001$), inflamasyon belirteçlerini (NF-ϰB ve TNF-α) azalttı ($p<0,01$), Kaspaz-3 ve Bax'ı azalttı ($p<0,01$) ve Bcl-2 düzeylerini artırdı ($p<0,01$) ve ayrıca histolojik hasarı iyileştirdi ve histolojik skoru azalttı ($p<0,05$). GNT kaynaklı böbrek toksisitesinde, Poly 100 tedavisi oksidatif stres, inflamasyon ve apoptozu tersine çevirerek böbrek koruması sağlamıştır.

Anahtar kelimeler: Apoptozis; Gentamisin; Nefrotoksisite; Oksidatif Stres; Polidatin

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INTRODUCTION

Ketosis A sudden and usually reversible decrease in renal function is defined as acute renal failure (ARF). Although its pathogenesis is complex, ischemia, toxins, and nephrotoxic drugs cause ARF. Nephrotoxic drugs such as aminoglycoside antibiotics cause approximately 20% of all ARF cases in intensive care units (Saeedavi et al. 2023). Gentamicin (GNT) is an aminoglycoside derivative antibiotic frequently used in urinary tract, eye, and soft tissue infections, especially against gram-negative bacteria. It is known that nephrotoxicity and ototoxicity develop in patients treated with GNT for 7 days or longer. However, the rapid bactericidal effect still allows broad-spectrum GNT to be used in treatment (Pakfetrat et al. 2022).

In GNT kidney injury, tubular apoptosis and necrosis are induced, leukocyte and inflammatory cell infiltration increases, and glomerular congestion develops. However, increased excessive reactive oxygen species (ROS) production and induction of inflammatory and cell death pathways are considered to be the basic mechanisms of GNT-induced renal dysfunction (Abukhalil et al. 2025). Considering all these mechanisms involved in pathogenesis, effective therapeutic approaches are needed to prevent or reduce GNT-induced renal injury. One of these approaches is the use of plant-based antioxidants that are natural and have few side effects (Şimşek et al. 2024; Keleş et al. 2014).

Polydatin (Poly), which is Resveratrol-3-β-mono-D-glucoside and is generally used as a food flavoring agent, is a natural polyphenolic compound with significant nutritional value obtained from Polygonum cuspidatum roots, grapes, peanuts, cocoa products, and hop flowers (Dahran et al. 2025; Highab et al. 2024). Poly, which is widely consumed in Asian populations, has been reported to be frequently used in hepatorenal toxicities and to improve damage due to its antioxidant and anti-inflammatory effects (Abdul-Hamid et al. 2023; Dahran et al. 2025).

The presented study aimed to investigate the possible mechanisms of action of the Poly in rats with GNT-induced kidney damage using biochemical, molecular, and histopathological methods.

MATERIALS and METHODS

Chemicals

This GNT (40 mg/1 ampoule) used in the study was supplied by Deva Drug Company (Istanbul, Turkey), and Poly (98%, Cas No: 65914-17-2) was supplied by Aktin Chemical Company (Chengdu, China).

Experimental Animals

Thirty-five male *Wistar albino* rats (10-12 weeks and 220–250 grams), were used in the experiment. A seven-day adaptation period preceded the experimental phase to ensure acclimatization to the laboratory environment. All animal-related procedures were conducted at the KONUDAM Center (Konya/Türkiye) in compliance with institutional ethical standards.

Ethics Committee Approval

The study received ethical approval from the Necmettin Erbakan University KONÜDAM Experimental Medicine Research and Application Center Directorate, with decision number 2024-63, issued on 11.07.2024.

Experimental Design

Wistar albino rats were randomly divided into 5 groups with 7 rats in each group.

1. **Control group (C):** Animals received oral saline once daily for 7 consecutive days.
2. **Polydatin group (Poly):** Polydatin was administered orally at a dose of 100 mg/kg/day for 7 days, as described by Ali et al. (2022).
3. **Gentamicin group (GNT):** Rats were given intraperitoneal gentamicin at 100 mg/kg/day for 7 days, following the protocol of Hakyemez et al. (2022).
4. **Gentamicin + Polydatin 50 group (GNT+Poly50):** Gentamicin (100 mg/kg/day, i.p.) was administered for 7 days, alongside oral Polydatin at a dose of 50 mg/kg/day for the same period.

Gentamicin + Polydatin 100 group

5. **(GNT+Poly100):** Animals received gentamicin intraperitoneally at 100 mg/kg/day for 7 days, in combination with oral Polydatin at 100 mg/kg/day.

Twenty-four hours after the final dose, the rats were euthanized under light sevoflurane anesthesia, and blood samples from the jugular vein, along with kidney tissues, were collected for further analysis. Blood samples were centrifuged at 1,507 x g for 10 minutes at 4°C and serum was separated.

Serum Renal Function Markers

Quantification of serum urea and creatinine levels was performed using commercially available diagnostic kits (Diasis Diagnostic Systems, Istanbul, Turkey), strictly adhering to the manufacturer's protocol. Creatinine was analysed according to the Jaffe reaction at 492 nm wavelength and 37°C temperature according to the manufacturer's instructions. Urea was analysed at 340 nm wavelength and 37°C according to the manufacturer's instructions.

Analysis of Oxidant-Antioxidant Parameters

Kidney tissue was weighed and homogenized at a ratio of 1/20 with 1.15% potassium chloride in a tissue homogenizer (IKA, T18 digital ultra-turrax, Germany). The supernatant obtained at 10000 rpm was used for the measurement of glutathione peroxidase (GPx) (Matkovics, 1988) activity and glutathione (GSH, Sedlak and Lindsay, 1968) levels, while the supernatant

obtained at 3500 rpm was used for the determination of malondialdehyde (MDA) (Placer et al. 1966), superoxide dismutase (SOD) (Sun et al. 1988), catalase (CAT) (Aebi, 1984) and protein (Lowry et al. 1951).

RT-PCR

At the end of the experiment, mRNA transcription levels of genes listed in Table 1 were analyzed by the RT-PCR method in kidney tissues obtained. RNA isolation was performed using commercially available QIAzol Lysis Reagent (Qiagen, 79306). Isolated total RNA was converted to cDNA with cDNA Synthesis Kit (ABM, G236, Richmond, Canada). Reactions were combined with 4 µL of 5X RT buffer, 1 µL of dNTP mix, 1 µL of primers, 1 µL of OneScript® Plus RTase, 2 µg of RNA, and nuclease-free water to a final volume of 20 µL on ice. These were followed by incubation at 50–55 °C for 15 min and enzyme inactivation at 85 °C for 5 min. Then, the PCR mixture was prepared by adding 2X qPCR MasterMix (ABM, G891, Richmond, Canada) with primer sequences, and the reaction started. The procedures were carried out in appropriate temperature cycles in the Rotor-Gene Q (Qiagen) device according to the protocol specified by the manufacturer. Gene expressions obtained from the analysis were normalized with the β-Actin reference gene and evaluated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Hematoxylin and Eosin

Rat kidney samples were kept in 10% buffered formalin solution for fixative purposes for 24 hours. After a routine paraffin follow-up procedure, 5 µm thick sections were taken from kidney tissues and stained with hematoxylin and eosin (H&E). The obtained images were evaluated blindly using a light microscope (Olympus Cx43; Japan). In addition, random areas were selected for each animal to score histopathological lesions, and lesions such as glomerular atrophy, inflammatory cell infiltration, vascular congestion, and degeneration of tubular cells were taken as a basis. Scores were as follows: 0: no damage, 1: mild damage, 2: moderate damage, 3: severe damage.

Statistical Analysis

Group comparisons were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple pairwise comparisons (SPSS, version 20.0; Chicago, IL, USA). A p-value of less than 0.05 was considered statistically significant. Data are expressed as mean ± standard deviation (SD).

Table 1. Primer sequences

Gene	Sequences (5'-3')
NF-κB	F: AGTCCCGCCCCTTCTAAAAC
	R: CAATGGCCTCTGTGTAGCCC
TNF-α	F: CTCGAGTGACAAGCCCGTAG
	R: ATCTGCTGGTACCACCAGTT
Caspase-3	F: ACTGGAATGTCAGCTCGCAA
	R: GCAGTAGTCGCCTCTGAAGA
Bax	F: TTTCATCCAGGATCGAGCAG
	R: AATCATCCTCTGCAGCTCCA
Bcl-2	F: GACTTTGCAGAGATGTCCAG
	R: TCAGGTACTCAGTCATCCAC
KIM1	F:TGGCACTGTGACATCCTCAGA
	R: GCAACGGACATGCCAACATA
AQP2	F: AGCTGCCTTCTATGTGGCT
	R: GCGTTGTTGTGGAGAGCATT
β-Actin	F: CAGCCTTCCTTCTTGGGTATG
	R: AGCTCAGTAACAGTCCGCCT

RESULTS

Kidney Function Tests

The effects of GNT and Poly applications on serum urea (Figure 1A) and creatinine (Figure 1B) levels of renal function tests were investigated. According to the obtained data, it was determined that there was no difference between the control and Poly groups in both parameters ($p>0.05$), GNT application provided a significant increase in these parameters compared to the control and Poly groups ($p<0.001$), and Poly50 and Poly100 doses administered together with GNT were effective in reducing urea and creatinine levels ($p<0.001$).

Oxidant-Antioxidant Status

The impact of GNT and Poly on oxidative stress markers—MDA (Figure 2) and GSH (Figure 3A)—as well as antioxidant enzymes GPx (Figure 3B), SOD (Figure 3C), and CAT (Figure 3D), was thoroughly examined. No significant differences were observed in MDA and GSH levels between the Control and Poly groups ($p>0.05$). However, GNT administration led to a marked elevation in MDA levels and a significant reduction in GSH levels compared to the Control and Poly groups ($p<0.001$). Co-administration of Poly with GNT, at both 50 and 100 mg/kg doses, significantly decreased MDA levels and restored GSH concentrations relative to the GNT group ($p<0.001$). Furthermore, GNT treatment substantially suppressed the activities of GPx, SOD, and CAT ($p<0.001$), while concurrent administration of Poly50 or Poly100 effectively enhanced the activity of these antioxidant enzymes, thereby reinforcing the antioxidant defense mechanisms ($p<0.001$)

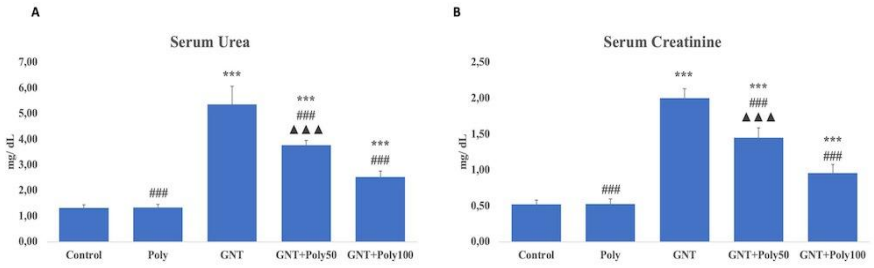

Figure 1: Effects of GNT and Poly on serum urea (A) and serum creatinine (B) levels. Values are given as mean ± SD. Control vs others: *p<0.05, **p<0.01, ***p<0.001, GNT vs others: #p<0.05, ##p<0.01, ###p<0.001, GNT+Poly50 vs GNT+Poly100: ▲p<0.05, ▲▲p<0.01, ▲▲▲p<0.001.

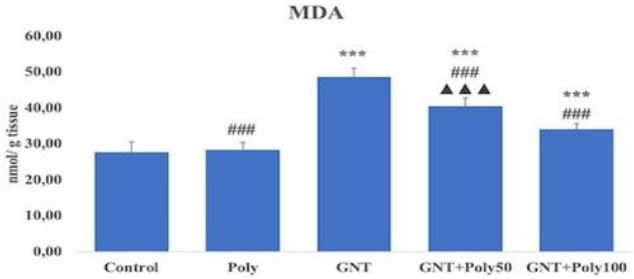

Figure 2: Effects of GNT and Poly on MDA levels in rat kidney tissues. Values are given as mean ± SD. Control vs others: *p<0.05, **p<0.01, ***p<0.001, GNT vs others: #p<0.05, ##p<0.01, ###p<0.001, GNT+Poly50 vs GNT+Poly100: ▲p<0.05, ▲▲p<0.01, ▲▲▲p<0.001.

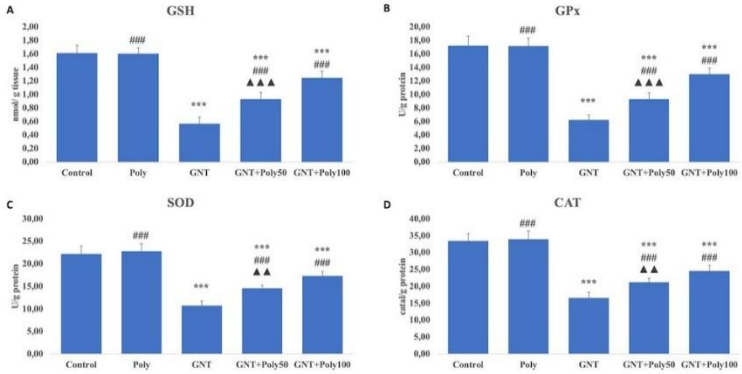

Figure 3. Effects of GNT and Poly on GSH (A) level and GPx (B), SOD (C), and CAT (D) activities and in rat kidney tissues. Values are given as mean ± SD. Control vs others: *p<0.05, **p<0.01, ***p<0.001, GNT vs others: #p<0.05, ##p<0.01, ###p<0.001, GNT+Poly50 vs GNT+Poly100: ▲p<0.05, ▲▲p<0.01, ▲▲▲p<0.001.

Inflammation

The mRNA expression levels of inflammatory markers NF-κB (Figure 4A) and TNF-α (Figure 4B) were analyzed. GNT treatment significantly upregulated the expression of both markers in renal tissue compared to the Control and Poly groups, indicating an inflammatory response (p<0.001). Co-treatment with Poly resulted in a dose-dependent reduction in NF-κB and TNF-α mRNA expression levels, demonstrating its anti-inflammatory potential (p<0.01).

Apoptosis

The impact of GNT and Poly on the expression of apoptotic markers Caspase-3 (Figure 5A) and Bax (Figure 5B), as well as the antiapoptotic marker Bcl-2 mRNA, was evaluated. The results revealed that GNT significantly elevated the mRNA expression of Caspase-3 and Bax (p<0.001) while reducing Bcl-2 expression (p<0.001), indicating the induction of apoptosis. The Poly50 dose administered with GNT had no significant effect on Caspase-3 and Bcl-2 levels (p>0.05). However, the Poly100 dose reduced Caspase-3 and Bax levels and enhanced Bcl-2 expression (p<0.01), suggesting an antiapoptotic role.

Effect of GNT and Poly on KIM-1 and AQP2

The effects of GNT and Poly on the mRNA expression levels of Kidney Injury Molecules-1 (KIM1) and Aquaporin 2 (AQP2), which are indicators of kidney damage, were examined. According to the results, it was determined that GNT administration increased the expression level of KIM1 (Figure 6A) compared to the control and Poly groups, while decreasing the expression level of AQP2 (Figure 6B) (p<0.001). It was determined that the Poly50 dose given together with GNT was not effective in both parameters (p>0.05), while the Poly100 dose decreased the expression level of KIM1 and increased the level of AQP2 (p<0.01).

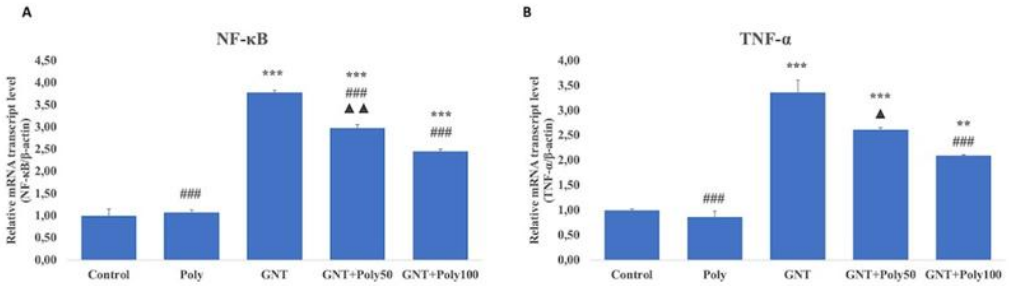

Figure 4: Effects of GNT and Poly on NF-κB (A) and TNF-α (B) mRNA transcription levels in rat kidney tissues. Values are given as mean ± SD. Control vs others: *p<0.05, **p<0.01, ***p<0.001, GNT vs others: #p<0.05, ##p<0.01, ###p<0.001, GNT+Poly50 vs GNT+Poly100: ▲p<0.05, ▲▲p<0.01, ▲▲▲p<0.001.

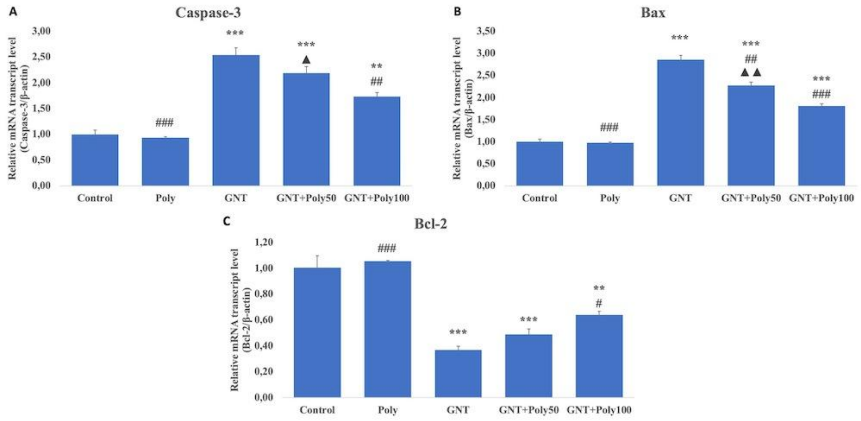

Figure 5: Effects of GNT and Poly on Caspase-3 (A), Bax (B), and Bcl-2 (C) mRNA transcription levels in rat kidney tissues. Values are given as mean ± SD. Control vs others: *p<0.05, **p<0.01, ***p<0.001, GNT vs others: #p<0.05, ##p<0.01, ###p<0.001, GNT+Poly50 vs GNT+Poly100: ▲p<0.05, ▲▲p<0.01, ▲▲▲p<0.001.

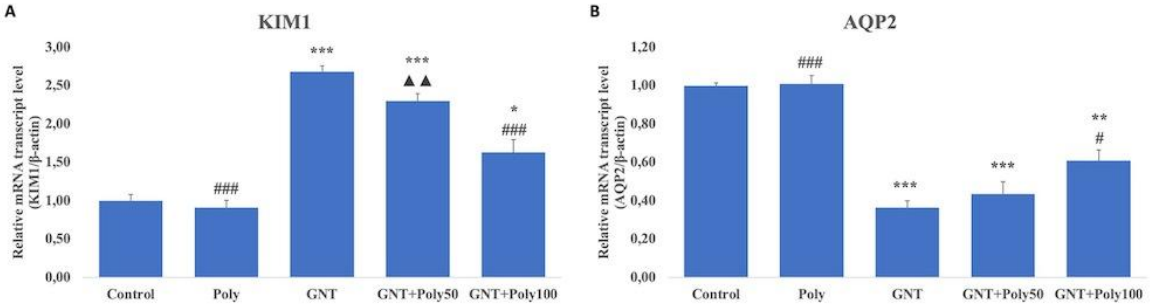

Figure 6: Effects of GNT and Poly on KIM1 (A) and AQP2 (B) mRNA transcription levels in rat kidney tissues. Values are given as mean ± SD. Control vs others: *p<0.05, **p<0.01, ***p<0.001, GNT vs others: #p<0.05, ##p<0.01, ###p<0.001, GNT+Poly50 vs GNT+Poly100: ▲p<0.05, ▲▲p<0.01, ▲▲▲p<0.001.

Effect of GNT and Poly on kidney morphology

H&E staining was performed to explain the histopathological effects of GNT and Poly applications on kidney tissue, and the results are presented in Figure 7. When representative photomicrographs were examined in the control and Poly-only groups, the Malpighian cortex and tubule structures localized in the renal cortex were normal, and there was no morphological difference between these two groups (Figure 7A, 7B). The images in the GNT-applied group included various pathological features such as tubular cell degeneration, inflammatory cell infiltration, vascular congestion, hemorrhage, and atrophic glomeruli (Figure 7C). On the other hand, the combined treatments of GNT and Poly increased the number of Malpighian corpuscles with intact morphology. In addition, there were occasional congested blood vessels and rare degenerative changes in tubular epithelial cells in these groups (Figure 7D, 7E). When the histopathological score results were evaluated, there was a significantly increased damage score in the GNT group, while the histopathological score in the GNT+Poly50 and GNT+Poly100 groups decreased significantly compared to the GNT group (p<0.05) (Figure 7F).

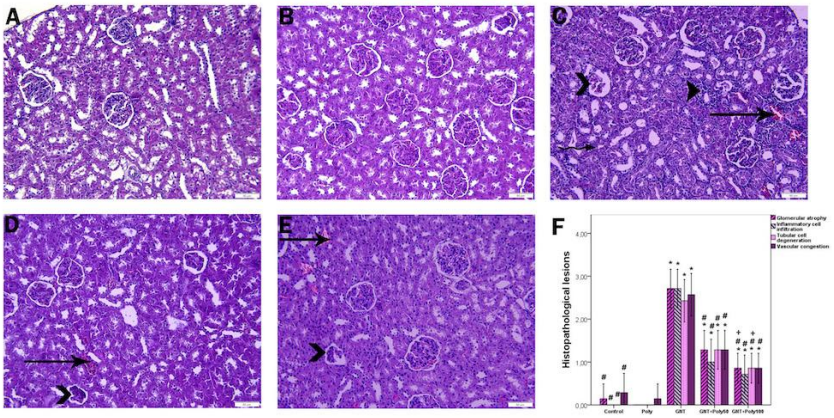

Figure 7: Microscopic images of rat kidney tissues treated with gentamicin and polydatin. Normal histological structure was observed in (A) Control and (B) Poly groups. (C) GNT toxicity caused atrophy of glomeruli (arrowhead), shedding of tubular epithelial cells (curved arrow), an increase in inflammatory cells (arrowhead), and congestion of interstitial blood vessels (arrow). (D) GNT+ Poly 50 and (E) GNT+ Poly 100 groups showed decreased atrophic glomeruli (arrowhead) and occasionally congested interstitial blood vessels (arrow). (F) Histopathological score analysis of H&E staining in five groups. (H&E, ×200; scale bar, 50 μm). Control vs others: *p<0.05, GNT vs others: #p<0.05, GNT+Poly 50 vs GNT+Poly 100: +p<0.05

DISCUSSION

Rapid deterioration of renal function due to exposure to nephrotoxic chemicals and drugs is called nephrotoxicity, and its pathogenesis involves various mechanisms, including ROS release, tubular and glomerular damage, and renal vasoconstriction (Tanyeli et al. 2020). GNT is an antibiotic that causes nephrotoxicity with morphological and biochemical changes in the proximal tubules (Abdel-Fattah et al. 2021). In the presented study, the effects of Poly, a natural antioxidant, were investigated in rats with GNT-induced nephrotoxicity.

Serum urea and creatinine are two important markers that show the structural integrity of the kidney (Akaras et al. 2023a). The first step in evaluating the toxic effects of various compounds is considered to be the increase in serum urea and creatinine levels (Akaras et al. 2023b; Şimşek et al. 2023). It has been revealed in different studies that GNT increases serum urea and creatinine levels (Hakyemez et al. 2022; Pakfetrat et al. 2022). In the presented study, it was determined that serum urea and creatinine levels increased in rats administered GNT, and Poly supportive treatment was effective in reducing these marker levels. Dahran et al. (2025) reported that Poly administration was effective in reducing serum urea and creatinine levels in rats with nephropathy.ROS is a key factor in the pathophysiology of kidney diseases (Kankılıç et al. 2024a; Bal et al. 2023). Both in vivo and in vitro studies demonstrate that GNT enhances ROS production by altering mitochondrial respiration, which subsequently accelerates the peroxidation of polyunsaturated fatty acids (PUFAs) (Aydın et al. 2009; Kandemir et al 2015). Antioxidant enzymes and compounds are essential for maintaining the balance between oxidants and antioxidants in the body (Tuncer et al. 2024). When antioxidant activity declines and antioxidant compounds are depleted, cells become more susceptible to oxidative stress, leading to an increase in oxidants (Aksu et al. 2019; İleritürk et al. 2022). In GNT-induced nephrotoxicity, oxidative stress manifests as elevated lipid peroxidation and reduced antioxidant enzyme activities (Kandemir et al. 2015; Bai et al. 2023). In the present study, GNT treatment resulted in increased MDA levels, reduced GSH levels, and decreased activities of SOD, CAT, and GPx, leading to oxidative stress in the kidneys. Previous studies have shown that Poly supplementation in kidney damage models lowers MDA levels and offers protection against oxidative stress by enhancing antioxidant enzyme activities (Zhou et al. 2022; Demirkapı et al. 2023). Similarly, this study observed that Poly treatment reduced GNT-induced lipid peroxidation and boosted antioxidant enzyme activities, thus mitigating oxidative stress.

The formation of reactive oxygen species causes the activation of pro-inflammatory pathways (Küçükler et al. 2024; Ileriturk et al. 2024). Among these, NF-ϰB signaling has been reported to be the main signal transduction pathway that plays a role in the regulation and activation of the genes of pro-inflammatory cytokines such as TNF-α (Çağlayan et al. 2022; Yeşildağ et al. 2022; Akaras et al. 2023c). Bai et al. (2023) reported that GNT increases the release of pro-inflammatory cytokines and triggers inflammation by activating the NF-ϰB signaling pathway. Hassanein et al. (2021) reported that GNT increases kidney tissue inflammation, which occurs through the activation of NF-ϰB. In the presented study, it was determined that NF-ϰB and TNF-α expression levels increased and inflammation was induced in the kidney tissue of rats

administered GNT, and that Poly, administered together with GNT, was effective in suppressing inflammation by reducing these expression levels. It has been demonstrated in different toxicity models that Poly reduces inflammation and has an anti-inflammatory effect, especially by suppressing NF-κB increases (Chen et al. 2021; Demirkapı et al. 2023).

Apoptosis, or programmed cell death, is a process that removes damaged, surplus, or potentially harmful cells from the body, often leading to cellular stress and/or injury in healthy cells (Yıldız et al. 2022; İleritürk et al. 2023; Akaras et al. 2024). Apoptosis begins with multiple events that lead to the activation of a caspase or protease family (Gür and Kandemir, 2022; Gencer et al., 2024). Caspases are responsible for the morphological and biochemical features of apoptotic cells (Kankılıç et al. 2024b). Caspase-3 has been reported to be one of the most important proteases that initiate both extrinsic and intrinsic apoptosis pathways and is also a marker of the irreversible point of apoptosis (Ekinci-Akdemir et al. 2018, Aksu et al. 2018; Yılmaz et al. 2024). It has been determined in different studies that GNT increases the levels of Caspase-3 and Bax and decreases the levels of Bcl-2 in kidney tissue in rats, and it has been reported that GNT induces apoptosis (Babaeenezhad et al. 2021; Laorodphu et al. 2022). In the presented study, it was determined that with GNT application, caspase-3 and Bax expression levels increased, while the expression of Bcl-2, an antiapoptotic marker, decreased, and apoptosis was induced, and Poly, applied together with GNT, was effective in suppressing apoptosis by regulating these values inversely.

CONCLUSION

As a result, it was determined that oxidative stress, inflammation and apoptosis mechanisms were induced in the kidney tissue of GNT-applied rats, that Poly supportive treatment was effective in protecting the kidney tissue by reversing these mechanisms, and that the use of Poly 100 dose would be more beneficial in GNT-induced kidney toxicities.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: ÖK, HŞ and FMK contributed to the experimental design, biochemical analysis. ÖK, drafted and wrote the manuscript. NA, performed histological analysis. All authors have read and approved the finalized manuscript.

Ethical approval: This study was carried out at Necmettin Erbakan University KONUDAM Experimental Medicine Application and Research Center (Konya / Turkey). This research was approved by The Ethics Committee of the Necmettin Erbakan University (dated 11.07.2024 and decision number 2024/63).

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RESEARCH ARTICLE

Data Management with Manda Yıldızı and Çolpan Software for Animal Improvement

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ABSTRACT

The basis of productivity and sustainability in animal husbandry relies on correct and systematic data recording. Traditional record-keeping methods impose serious limitations such as data loss, inconsistency, and challenges in analysis, which in turn reduce the effectiveness of national breeding programs. This study aims to present contributions of two computer software systems developed to support digital transformation and meet the needs of the "Community-Based Livestock Breeding Programme" conducted by the Ministry of Agriculture and Forestry. In this context, the web-based software Çolpan was developed for small ruminant breeding, while the Windows-based software Manda Yıldızı was created for buffalo and native cattle breeding. Çolpan was designed with a web architecture using the Delphi and IntraWeb framework, whereas Manda Yıldızı was coded as a native desktop application with Delphi VCL. The data management for both systems is integrated with database. Both software programs enable centralized and secure collection, processing, and analysis of pedigree and phenotypic data. Role-based access control ensures data security and authorization, and allows project participants to monitor and report field data in real time. In conclusion, these programs strengthen data-driven decision-making processes in breeding projects, accelerating progress and making significant contributions to sustainability.

Keywords: Community-Based Breeding, Çolpan, Data Management, Manda Yıldızı

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Manda Yıldızı ve Çolpan Yazılımları ile Hayvan Islahı Amaçlı Veri Yönetimi

ÖZ

Hayvancılıkta verimliliğin ve sürdürülebilirliğin temeli, doğru ve sistematik veri kaydına dayanmaktadır. Geleneksel kayıt tutma yöntemleri; veri kaybı, tutarsızlık ve analiz zorlukları gibi ciddi kısıtlamalar getirmekte, bu durum ulusal ıslah programlarının etkinliğini düşürmektedir. Bu çalışma, Tarım ve Orman Bakanlığı tarafından yürütülen Halk Elinde Hayvan Islahı Ülkesel Projeleri'nin ihtiyaçlarına yönelik geliştirilen ve dijital dönüşümü destekleyen iki bilgisayar yazılımının katkılarını ortaya koymayı amaçlamaktadır. Bu kapsamda, küçükbaş hayvan yetiştiriciliği için web tabanlı yazılım Çolpan, manda ve yerli sığır yetiştiriciliği için ise Windows tabanlı yazılım Manda Yıldızı geliştirilmiştir. Çolpan, Delphi ve IntraWeb framework'ü kullanılarak web mimarisi ile tasarlanmış, Manda Yıldızı ise Delphi VCL ile yerel masaüstü uygulaması olarak kodlanmıştır. Her iki sistemin veri yönetimi, veri tabanı ile entegre edilmiştir. Yazılımlar; soy kütüğü ve fenotipik ölçüm verilerinin merkezi ve güvenli bir yapıda toplanmasını, işlenmesini ve analiz edilmesini sağlamaktadır. Rol tabanlı erişim kontrolü sayesinde veri güvenliği ve yetkilendirme temin edilmekte, proje paydaşlarının sahadaki verileri anlık olarak izlemesine ve raporlamasına olanak tanınmaktadır. Sonuç olarak, bu programlar ıslah projelerinde veriye dayalı karar alma süreçlerini güçlendirerek ilerlemeyi hızlandırmakta ve sürdürülebilirliğe önemli katkılar sunmaktadır.

Anahtar Kelimeler: Halk Elinde Islah, Çolpan, Veri Yönetimi, Manda Yıldızı

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GİRİŞ

Hayvancılık sektörü ülke ekonomisinin gelişmesinde ve gıda güvenliğinin sağlanmasında stratejik bir rol üstlenmektedir. Modern uygulamalarda verimliliği artırmanın ve sürdürülebilirliği sağlamanın en temel unsurlarından biri, doğru ve düzenli kayıt tutma sistemlerinin işletilmesidir. Hayvanların kimlik bilgilerinden verim kayıtlarına, soy kütüklerinden sağlık geçmişine kadar pek çok verinin sistematik biçimde takip edilmesi, yalnızca işletme düzeyinde değil, aynı zamanda ulusal düzeyde yürütülen yetiştirme ve ıslah politikaları açısından da kritik öneme sahiptir (Simmons ve Ekarius 2009; Çelikyürek ve Aygün 2017). Verim kayıtlarının tutulması, hayvanların genetik potansiyellerinin belirlenmesi, üstün nitelikli damızlıkların seçilmesi ve sürülerin planlı şekilde geliştirilmesi için temel bir gerekliliktir. Bu veriler hayvan türleri ve yetiştirici işletmeler açısından farklılık göstermektedir (Bourdon 2013). Örneğin, koyun yetiştiriciliği yapılan bir işletmede; kuzuların doğum tarihi, doğum ağırlığı ve sütten kesim ağırlığı gibi temel değerler kaydedilirken, süt üretimi yapan işletmelerde buna ek olarak süt verimi ve süt bileşenlerine ilişkin veriler de kayıt altına alınmaktadır (Sönmez ve ark. 1977). Ayrıca, işletmenin yapısına bağlı olarak cidago yüksekliği, göğüs çevresi, sağrı yüksekliği, ultrason ve testis ölçümleri gibi detaylı veriler de kayıt altına alınabilmektedir. Hayvanların fenotipik ölçülerinin elle yazarak kaydedilmesi dolayısıyla geleneksel yöntemler, zaman kaybına yol açmakta ve risk oluşturmaktadır. Bu nedenle, verilerin hızlı ve doğru şekilde belgelendirilmesi, düzenli olarak yedeklenmesi ve geçmişten yola çıkılarak geleceğe yönelik planlama yapılabilmesi için dijital hayvancılık yönetim sistemlerine duyulan gereksinim artmaktadır. Birçok işletmenin damızlık hesaplamaları yapamaması ya da mevcut altyapısının bu analizleri gerçekleştirmeye elverişli olmaması zaten karmaşık olan süreci iyice içinden çıkılmaz bir hale getirmektedir. Bunun sonucunda hayvanların genetik potansiyeli yeterince değerlendirilememektedir. Nitekim bu olgu çiftlik yönetiminde verimliliğin düşmesine ve sürecin daha fazla emek ve zaman gerektiren bir yapıya dönüşmesine yol açmaktadır (Hamadani ve Ganai 2022; Kavurur 2023). Bilgisayar ve yazılım sektörlerindeki hızlı ilerleme hayvansal üretim sistemlerinde köklü bir dönüşümü beraberinde getirmiştir. Özellikle verilere bakarak karar alma süreçlerini ortaya koyan bir anlayış giderek yaygınlaşmaktadır. Sektörün endüstri haline gelmesinde yaşanan bu paradigma kayması önemli bir rol oynamaktadır. Bilinçli ve bilimsel yöntem ve verilere dayalı hareket eden yetiştiriciler kazanacak bunu tercih etmeyenler ise zaman içerisinde sektörden ayrılmak zorunda kalacaktır. Gelişmiş ülkelerde büyükbaş, küçükbaş ve kanatlı sektörlerinde üretim süreçlerinin planlanması, uygulanması ve izlenmesinde bilgisayar destekli yazılım ve veri tabanlarının kullanımı rutin hale gelmiştir. Dijital kayıt sistemleri, damızlık materyal seçimi, sürü sağlığı yönetimi, genetik ilerleme takibi ve ekonomik verimliliğin optimizasyonu gibi üretim zincirinin tüm aşamalarında etkin şekilde rol almaktadır (Bourdon 2013; Çelikyürek ve ark. 2019; Aydın ve Günlü 2010). İşletme verimliliğini artırmak için, ihtiyaçların doğru belirlenmesi ve bu ihtiyaçlara uygun yerli yazılımların geliştirilmesi önemlidir. Kayıt sistemlerinin kurgusu ve çatısı modern hayvan yetiştiricilik türü ve işletmenin büyüklüğüne göre farklılıklar gösterebilmektedir. Bu noktada, kullanılacak yazılımın hem işlevsel hem de gereksinimleri karşılar nitelikte olması beklenmektedir (Kavurur 2023). Manda yıldızı ve Çolpan yazılımları Türkiye genelinde Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü (TAGEM) tarafından yürütülen Halk Elinde Hayvan Islahı Ülkesel Projelerinde manda ve koyun üreticilerinin damızlık programları için en iyi genetik materyalleri belirlemelerine yardımcı olmak amacıyla tasarlanmıştır. Bu tür kamu destekli projelerde, sahadaki hayvanların performans ve soy kütüğü verilerinin toplanması, analiz edilmesi ve merkezi bir veri tabanında işlenmesi; doğru damızlık seçimi, genetik ilerlemenin izlenmesi ve hayvancılığın bölgesel gelişimi açısından büyük önem taşımaktadır. Özellikle yerli yazılımlar, proje teknik elemanlardan alınan geri dönüşler ve saha tecrübeleri ile çerçevesi çizilen kullanıcı dostu arayüzleri sayesinde farklı bölgelerde yürütülen projelere kolay entegrasyon imkânı sunarken yapay zeka ve nesnelerin interneti gibi yeni teknolojilere altyapı sağlayarak hayvancılıkta sürdürülebilirlik ve izlenebilirliği desteklemektedir. Böylece büyük çaplı kamu projelerin daha etkin, şeffaf ve bilimsel temelli yürütülmesine de imkan sağlamaktadır. Bu çalışma, işletmelerin yönetimini dijital altyapıyla güçlendiren ve Tarım ve Orman Bakanlığı'nın Ulusal ıslah projelerinde etkin biçimde kullanılan bir bilgisayar yazılımının yapısını, işlevselliğini ve sektöre sağladığı katma değeri kapsamlı biçimde ortaya koymaktadır. Çalışmanın temel amacı, kayıt sistemleri aracılığıyla hayvancılıkta dijital dönüşümü hızlandıran, ıslah süreçlerini optimize eden ve sahada uygulanabilirliği yüksek yenilikçi bir çözüm sunmaktır.

MATERYAL ve METOT

Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü (TAGEM) tarafından yürütülen Halk Elinde Hayvan Islahı Ülkesel Projeleri yürütülürken elde edilen verilerin veri depolama ortamlarında tutulması ve bunlardan damızlık hesaplarda yararlanılabilmesi gereksinimi doğmuştur. Projeler yürütülürken farklı yazılımlar kullanılmaya başlanmış ve son aşamada tüm alt projelerde kullanılmak üzere küçükbaşlar için daha sonra Ulusal Küçükbaş Hayvan Islahı Bilgi Sistemi (UKIBS) adını alacak Çolpan ve büyükbaşlar için de Manda Yıldızı arayüzleri geliştirilmiştir. Bu süreçte

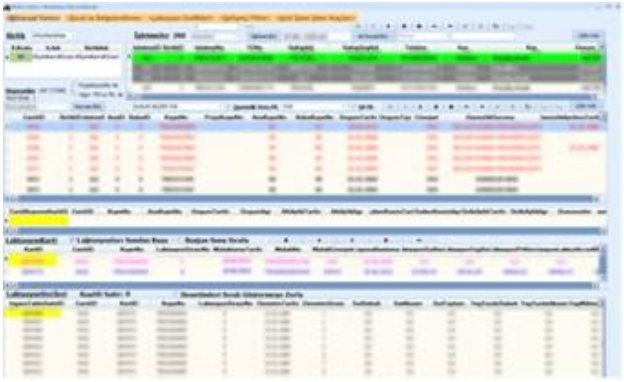
geliştirme, test ve derleme işlemleri, Intel Xeon işlemcili, 64 GB sistem belleği (RAM) ve 2 TB depolama kapasitesi ile donatılmış, Windows 11 işletim sistemi ile çalışan yüksek performanslı bir iş istasyonu (work station) bilgisayar kullanılmıştır. Yazılımların kodlama işlemi ve ekran tasarımları için Delphi ve C++ programlama dillerinden yararlanılmıştır. Bu amaçla Hızlı Uygulama Geliştirme (RAD Studio) platformu (Embarcadero, USA) kullanılmıştır. Projelerin farklı işlevsel gereksinimlerine ve hedeflenen kullanıcı kitlesine bağlı olarak Windows ve web tabanlı olmak üzere iki farklı mimari yaklaşım benimsenmiştir. Manda Yıldızı uygulaması, Windows işletim sistemi üzerinde maksimum performans, donanım kaynaklarına doğrudan erişim ve zengin bir kullanıcı arayüzü (UI) deneyimi sunmak amacıyla, Delphi'nin VCL (Visual Component Library) çatısı kullanılarak yerel (native) bir masaüstü uygulaması olarak tasarlanmıştır. Bu yaklaşım, derlenen kodun herhangi bir ara katman veya sanal makine olmaksızın doğrudan işletim sistemi "Application Programming Interface" (API)'leri ile etkileşerek yüksek verimlilikle çalışmasını sağlamaktadır. Çolpan yazılımında ise bağımsız erişim ve merkezi yönetim avantajları hedeflenmiş ve herhangi bir güncel web tarayıcısı üzerinden erişilebilen, sunucu taraflı bir web uygulaması olarak yapılandırılmıştır. Bu amaçla, Delphi geliştiricilerinin masaüstü uygulamalarına benzer durum bilgisi koruyan (stateful) bir programlama modeliyle karmaşık web çözümleri üretmesini kolaylaştıran IntraWeb framework'ünden faydalanılmıştır. Farklı ön yüz (front-end) mimarilerine sahip bu iki uygulamanın veri depolama ve yönetimi, kurumsal düzeyde ölçeklenebilirlik, veri bütünlüğü sağlayan ve güvenilir bir ilişkisel veri tabanı metodolojisi olan Microsoft SQL Server ile tümleşik hale getirilmiştir. Her iki uygulamanın güvenlik mimarisi, rol tabanlı erişim kontrolünü (program yöneticisi, proje lideri, proje teknik elemanı, bakanlık yetkilisi, il müdürlüğü yetkilisi gibi) merkeze alan çok katmanlı bir yapıda tasarlanmıştır. Programı açıldığında kullanıcının yetkisi yazılımın bu özelliği ile tespit edilmekte ve buna uygun işlemler gerçekleştirilerek kullanıcılar takip edilmektedir. Bu kapsamda, native masaüstü uygulaması olan Manda Yıldızı'nda yetkilendirme, kullanıcı rolüne göre arayüz bileşenlerinin dinamik kontrolü ile sağlanırken; web tabanlı Çolpan uygulamasında ise buna ek olarak, HTTPS ile iletişim şifrelenmiş ve SQL Enjeksiyonu gibi kritik zafiyetlere karşı parametrik sorgularla sunucu tarafında katı önlemler alınmıştır. Bu mimarinin temelini ve en dış güvenlik halkasını ise, her iki uygulamanın da bağlandığı Microsoft SQL Server üzerinde "en az yetki prensibi" ile yapılandırılan kısıtlı veri tabanı rolleri oluşturarak veri düzeyinde bütünlük ve gizlilik temin edilmiştir.

BULGULAR

Her iki yazılım da Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü (TAGEM) tarafından yürütülen Halk Elinde Islah projelerinin değişen gereksinimlerine cevap vermek üzere geliştirilmiştir. Bu programlar, projeye dahil olan işletmelerde bireysel hayvan verilerinin güvenli, erişilebilir ve merkezi bir yapıda organize edilerek saklanmasını sağlamak amacıyla tasarlanmıştır. Saha uygulamalarında ortaya çıkan yeni durumlar karşısında dönüşebilir olarak tasarlanan platformlar, hem proje teknik elemanının iş yükünü azaltmakta hem de veri yönetimi süreçlerinin daha esnek, etkin ve sistematik bir biçimde yürütülmesine imkan sağlamaktadır.

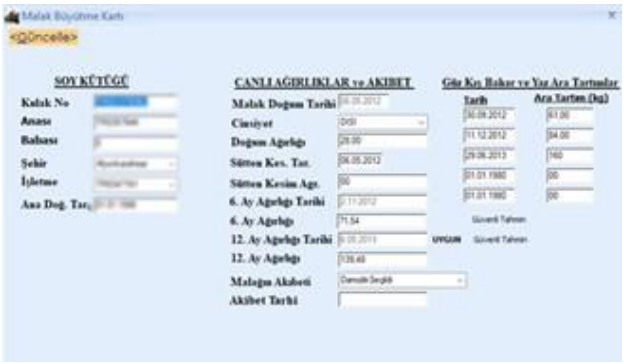
Manda Yıldızı'nın geliştirilme süreci 2008 yılında Afyon Kocatepe Üniversitesi Hayvancılık Araştırma ve Uygulama Merkezi mandalarını kayıt altına almak üzere yazılan KÜHKAY adlı yazılımla başlamış ve zaman içerisinde Manda Yıldızı adını alarak ilk versiyonu yayınlanmıştır (Tekerli ve Koçak 2013). Daha sonraları yazılım belirli aralıklarla çıkartılan farklı versiyonlar, yeni arayüzler, daha kullanıcı dostu veri girişi ve ftp serverları ile başlayan ve web üzerinden aynılaştırma (merge replication) mekanizmasına kadar ilerleyen bir dönüşüm yaşamıştır. Halk Elinde Anadolu Mandasının ve Yerli Sığırların ıslahı projelerinde kullanılmak üzere geliştirilmiş olan Manda Yıldızı yazılımı proje teknik elemanlarının sahada topladıkları fenotipik verilerin sayısal bir ortamda saklanması imkan sağlamaktadır. Programa ulaşım her yetkiliye tanınan kullanıcı adı ve şifreler vasıtasıyla sağlanmaktadır (Resim 1). Veriler kişisel bilgisayarlar aracılığıyla merkezi bir sunucuya gönderilmektedir. Bu amaçla Microsoft SQL Server tarafından sunulan aynılaştırma altyapısı kullanılmaktadır. Sistem hem sunucu hem de kişisel bilgisayarda depolanan veriler ile çalışmakta olup, burada çok sayıda veriye tek seferde çabuk bir şekilde erişilmesi mümkün olmuştur. Üzerinde "Birlik Listesi" yazan butona tıklanarak Resim 2'de görüldüğü gibi işletmelerdeki hayvanlara ilişkin kayıtlara ulaşılmaktadır.

Resim 1: Manda Yıldızı temel giriş ekranı
Figure 1: Manda Star basic login screen

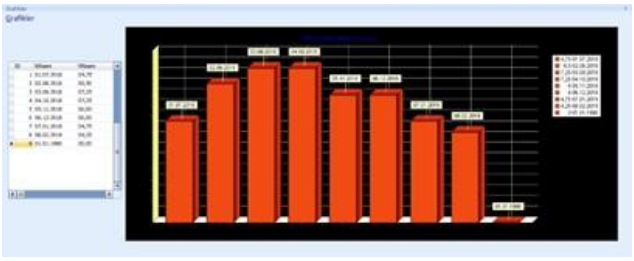


Resim 2: Manda Yıldızı Birlik Listesi
Figure 2: Mandate Star Troop List

Bunlar doğum, büyüme, laktasyon, damızlık olma ve ölüm ile ilgili verilerden müteşekkildir. Bu bilgiler kullanıcılar tarafından girilebilir ve izlenebilir (Resim 3). Büyüme verilerinin yanında anaç hayvanlara ilişkin süt verim kontrolleri de kaydedilebilmekte ve grafikleştirilebilmektedir (Resim 4).



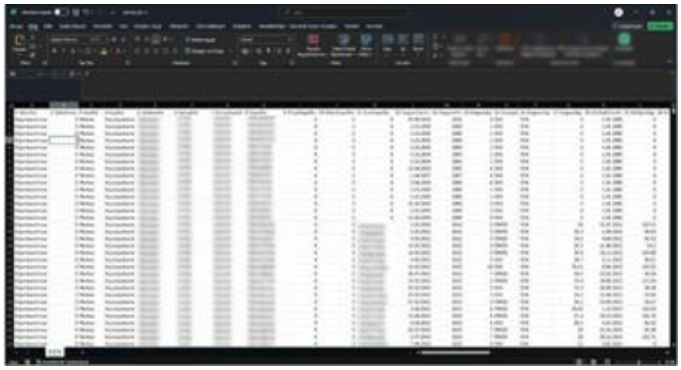
Resim 3: Manda Yıldızı Veri giriş ve takip ekranı
Figure 3: Manda Star Data entry and tracking screen



Resim 4: Laktasyon verilerinin bireysel olarak grafik haline getirilmesi
Figure 4: Individual graphing of lactation data

Veri girişlerinin yanında damızlık seçimleri ve araştırmalarda kullanılmak üzere Manda Yıldızı içerisinden veriler kolaylıkla dışa aktarılabilmektedir. Resim 5 ve Resim 6'da malaklara ilişkin büyüme verileri ile analarının ilgili laktasyondaki süt ve bileşen verimlerinin Excel programına uygun formatta dışarıya aktarılmasını sağlayan modül görülmektedir.

Resim 5: Sistemde bulunan verilerin filtrelenerek dışarı Excel formatında aktarılması
Figure 5: Filtering the data in the system and exporting it in Excel format



Resim 6: Excel formatında dışarı aktarılmış bir veri seti örneği
Figure 6: An example of a dataset exported in Excel format

Çolpan, TAGEM tarafından yürütülen Ülkesel Küçükbaş Hayvan Islahı Projesi kapsamında geliştirilmiştir. Toplam 58 ilde yürütülen bu çatının alt projelerinde çalışan teknik elemanlar, sahada topladıkları fenotipik verileri Excel aracılığı ile sisteme aktarmaktadırlar (Resim 7). Bu sayede veriler UKIBS'te toplanmaktadır. Tüm bu süreç Microsoft SQL Server altyapısı kullanan merkezi bir sunucudan yönetilmektedir.

Resim 7: Excel dosya aktarım ekranı
Figure 7: Excel file transfer screen

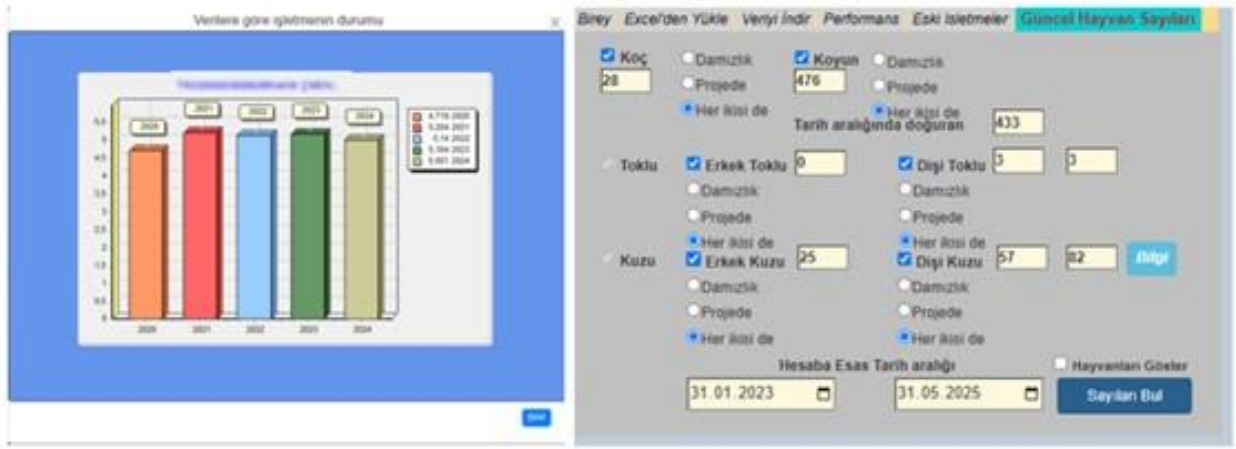
Web tabanlı yapısı sayesinde sisteme internet bağlantısı olan herhangi bir cihaz üzerinden erişim sağlanabilmektedir. Programın sunduğu çevrimiçi erişim sayesinde projenin diğer paydaşları da (proje lideri, TAGEM, Tarım İl Müdürlüğü yetkilileri) sisteme giriş yaparak sahadan alınan verilerin anlık denetimi, raporlanması veya analizlerinin yapılabilmesi sağlanmaktadır (Resim 8).

Resim 8: Çolpan yazılımının ana ekranı
Figure 8: Main screen of the Çolpan software

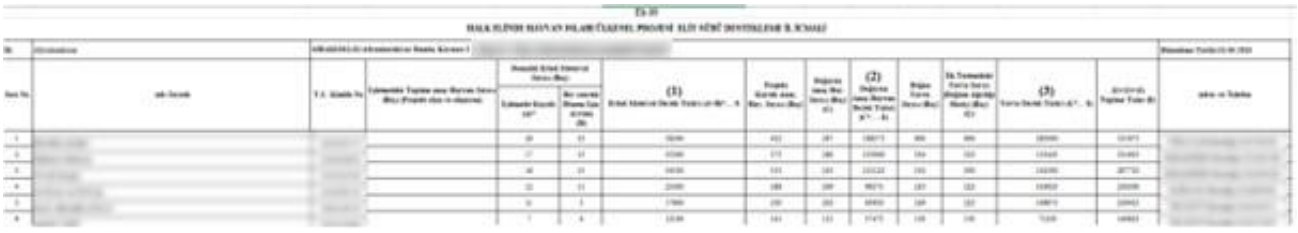
Farklı kullanıcılar eş zamanlı olarak sisteme erişebilmekte ve kullanıcıya özel yetkilendirme sağlanmaktadır (Resim 9). Bu sayede kullanıcı kendine tanımlı olan işlemleri gerçekleştirebilmektedir.

Resim 9: Çolpan giriş ekranı
Figure 9: Çolpan login screen

Program, veri girişine ek olarak, hayvan kayıtlarına ve projenin genel durumuna ilişkin tanımlayıcı raporlar oluşturulabilmekte bu sayede analiz edilebilir ve yorumlanabilir bir veri yapısı sunmaktadır (Resim 10). Yazılım, projenin yürütülme sürecinde önemli yer tutan hayvan transferi gibi işlemlerin de sistem üzerinden izlenmesine ve yönetilmesine olanak tanımaktadır. Aynı zamanda proje kapsamında verilen desteklemelerin hesaplarının yapılması, belgelendirilmesi ve yönetmelik formatına uygun hale getirilmesini sağlamaktadır (Resim 11). Bu yönüyle Çolpan hem veri tabanı aracı hem de proje uygulamalarını destekleyen bir platform niteliği taşımaktadır.



Resim 10: Çolpanda yer alan verilerin raporlanma örnekleri
Figure 10: Examples of reporting data in Çolpan



Resim 11: Çolpan tarafından oluşturulan icmal örneği
Figure 11: Example of summary created by Çolpan

TARTIŞMA

Diğer sektörlerde olduğu gibi Türk tarım ve hayvancılık sektöründe de dijitalleşmenin önemi her geçen gün artmakta ve veri yönetimi alanında modern sistemlerin kullanılması yönünde talep oluşmaya başlamaktadır. Geleneksel kayıt tutma yöntemleri (defter, matbu formlar, Excel dosyaları, fotoğraflar vb.) veri bütünlüğü, erişilebilirlik ve analiz açısından önemli kısıtlamalar getirmektedir. Bu eksikliklere çözüm olarak geliştirilen ÇOLPAN ve Manda Yıldızı gibi sistemler sahaya yönelik pratik yapılarıyla geleneksel yöntemlere göre daha avantajlı konumdadır. Hayvancılıkta bireysel verilerin düzenli, güvenli ve analiz edilebilir şekilde kaydedilmesi, ıslah çalışmaları ve sürdürülebilirlik açısından kritik önem taşımaktadır. Web tabanlı olan Çolpan sayesinde geleneksel yöntemlerde sıkça karşılaşılan veri kaybı, tutarsızlık ve erişim sorunları ortadan kalkmakta ve veriye erişim kolaylaşmaktadır. Çok kullanıcılı erişim, toplu veri girişi ve çıkışı, şehir, proje, işletme ve birey bazlı yaklaşımı ile izlenebilirlik, analiz edilebilir veri yapısı gibi özellikler, sahada etkin bir veri yönetimini ve sürdürülebilirliği mümkün kılmaktadır. Manda Yıldızı, manda yetiştiriciliği alanında soy kütüğü, verim takibi ve destekleme başvuruları için geliştirilmiş kapsamlı bir programdır. Geleneksel yöntemlerin aksine, bu sistem kullanıcılar arası veri paylaşımını kolaylaştırmakta, teknik personelin veri girişini standartlaştırmakta ve desteklemelerde yardımcı olmaktadır. Buzağılama aralığı, süt verimi ve kompozisyonu ve döl verimi takibi gibi önemli verilerin sistem üzerinden anlık olarak izlenebilmesini sağlamakta ve böylece karar alma süreçlerini kısaltmaktadır. Dijitalleşme sürecinde bu tür entegre sistemlerin yaygınlaştırılması, hayvancılığın verimlilik ve izlenebilirlik açısından ilerlemesine önemli katkı sağlayacaktır. Her iki yazılımda son yıllarda önem kazanmaya başlayan nesnelerin interneti (Internet of Things) teknolojisinin Türkiye'de kullanılabilmesi için ortam oluşturmaktadır. Çolpan web tabanlı olmasıyla farklı platformlardan erişimi kolaylaştırırken, Manda Yıldızı Windows platformu üzerinden tek seferde çok sayıda veriyi hızla elde etme imkanı sağlamaktadır. Böylece Türkiye'de ilk defa iki farklı yazılım teknolojisi kullanıcıların istifadesine sunulmuş olmakta ve alanda bu yönleriyle bir ilk olma özelliğindedir.

Dünyada benzer amaçlarla kullanılan hayvan kayıt sistemleriyle karşılaştırıldığında özgün bir yapıya sahip olan bu yazılımlar performans verilerinin alınması ve işlenmesi özellikleriyle Amerika'da Ulusal Kimliklendirme Sisteminden (National Animal Identification System - NAIS) bu sistemin temel olarak hayvan hastalıklarıyla mücadeleye yönelik bir sistem olması ve salgınlarda izleme yapma yönüyle ayrılmaktadır (USDA 2006). Hindistan'da geliştirilen INAPH (Information Network for Animal Productivity and Health) sistemi, saha personelinin dijital cihazlar aracılığıyla verileri anlık olarak kaydetmesini ve merkezi sunucuyla eş zamanlı çalışmasını sağlar. Bu sistem, üreme, sağlık, beslenme ve danışmanlık gibi birçok alanı kapsar ve mobil cihazlarla veri toplama yönüyle dikkat çekmektedir (INAPH, 2025). Bu çalışmadaki yazılımlar henüz bu düzeyde entegre saha mobilizasyonu sağlamasa da işletme düzeyinde verilerin merkezi sistemde işlenmesini ve kullanılabilir hale getirilmesiyle benzerlik göstermektedir. Bu açıdan, Çolpan ve Manda Yıldızı'nın gelecekte mobil uygulama desteğiyle sahadaki veri giriş hızını artırma potansiyeli yüksektir. Avustralya'daki Sheep Genetics Australia (SGA) sistemi, BLUP yöntemiyle genetik üstünlükleri belirlemekte ve Avustralya Koyun Damızlık Değerleri (ASBV) sistemine dayalı tahminler yapmaktadır (SGA, Breeder's Quality Assurance Manual, 2025; Brown ve ark. 2006). Çolpan ve Manda Yıldızı yazılımları ise damızlık değer tahmini için gereken altyapıları sunmakta olup, gelişmeye açıktır. İrlanda'da da Sheep Ireland programı adı altında verilerin program yetkilileri tarafından üreticilerden toplanması da dahil bir kayıt sistemi oluşturulmuştur. Genetik değerlendirme modülleri sağlık, analık, doğum ve üretim olmak üzere dört ana başlık altında yapılmakta; veri kalitesi ve güncellemeler de düzenli olarak denetlenmektedir (McDermott ve ark. 2018). Ayrıca geliştirilen mobil uygulamalar ve Veri Kalitesi İndeksi (DQI) gibi yeniliklerle, sahadaki kayıt kalitesi ve katılım düzeyi artırılmaktadır. Çolpan'ın İrlanda modeliyle benzer biçimde, kamusal veri altyapısı ve yetiştirici katılımı bakımından geliştirilebileceği yönleri bulunmaktadır. Smart Sheep Breeder sistemi ise veri işleme sürecinde yapay zekâ (AI) ve IoT teknolojilerini entegre etmesi bakımından dikkat çekicidir. Derin öğrenme modelleri, otomatik tartım sistemleri, elektronik tanımlama (RFID) ve entegre sensörler sayesinde çiftliklerde tam otomatik bir veri toplama ve analiz ortamı oluşturulmuştur (Hamadani ve Ganai 2022). Ayrıca, bireysel damızlık değerlerinin hesaplanması gibi yüksek düzeyli analizler bu sistemi bir karar destek platformuna dönüştürmüştür. Çolpan ve Manda Yıldızı mevcut haliyle sistem olarak benzer şekilde yerli üreticiye uygun, sadeleştirilmiş ama genişletilebilir bir altyapı sunmaktadır.

SONUÇ

Çolpan ve Manda Yıldızı sistemleri, Ülkesel Hayvan Islahı Projesi kapsamında sürdürülen ıslah faaliyetlerinin daha etkin, sistematik ve izlenebilir bir biçimde yürütülmesini sağlayan bir veri yönetim sistemleridir. Veriye dayalı karar alma süreçlerini destekleyen bu yapılar hem damızlık seçimindeki verimliliği artırmakta hem de uzun vadede sürdürülebilir hayvancılık uygulamalarına katkı sunarak izlenebilirlik sağlamaktadır. Sistemlerin kendi hitap ettikleri projelere odaklı tasarımı ve altyapısı, mevcut ihtiyaçlara çözüm üretmenin yanı sıra, gelecekteki gelişmelere uyum sağlayabilecek ve

kendini güncelleyerek dijitalleşen üretim sistemleri içerisinde yer alamaya devam edebilecektir. Bu sistemlerin geliştirilmesi ve modern teknolojilerle desteklenmesi ıslah çalışmalarının başarısını artırmak açısından önerilmektedir.

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

Yazar Katkıları: MT, projenin fikrine, tasarımına ve yürütülmesine katkıda bulunmuştur. MD, SÇ, YK ve KA, verilerin toplanmasına katkıda bulunmuştur. MT, MD, SÇ, KÇ ve OE, verileri kontrol etmiştir. MT, MD ve OE, makalenin taslağını hazırlamış ve yazmıştır. MT, OE, MD, SÇ, KÇ, YK ve KA, makaleyi eleştirel bir şekilde incelemiştir. Tüm yazarlar, nihai makaleyi okuyup onaylamıştır.

Etik izin: Bu çalışma "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik" Madde 8 (k) gereği HADYEK iznine tabi değildir. Bu yazıda sunulan veri, bilgi ve belgeler akademik ve etik kurallar çerçevesinde elde edilmiştir.

Teşekkür: Yazılımların hazırlanması sırasında verdikleri katkılardan dolayı başta Dr. Serdar YAĞCI, Dr. İrfan GÜNGÖR ve Dr. Nejla GÜLOĞLU olmak üzere TAGEM personeli, Afyonkarahisar Manda Yetiştiricileri Birliği, Kütahya Koyun – Keçi Yetiştiricileri Birliği ve başta Osman ÇELİK olmak üzere tüm proje teknik elemanlarına teşekkürü borç biliriz.

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RESEARCH ARTICLE

Determination of Risk Factors in Beekeeping Enterprises in Burdur Province

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ABSTRACT

Türkiye holds a significant place in global honey production rankings; however, its yield per hive remains below the world average. According to 2022 data, there is a 3.78-fold difference in hive yield between Türkiye, which ranks third globally in the number of hives, and China, which holds the second position. This situation highlights the need to improve hive productivity to ensure the sustainability of the Turkish beekeeping sector. Numerous direct and indirect risk factors contribute to the sector's inability to achieve adequate productivity. Türkiye's diverse and varied geographical structures result in these risk factors varying from region to region. In this context, the study aims to identify the risk factors encountered by beekeeping enterprises in Burdur province, located in the Western Mediterranean Region, throughout the production to marketing process. As part of the research, beekeeping enterprises included in the 2025 sample were visited, and the "Beekeeping Risk Factor Scale" was administered. As a result of the exploratory factor analysis conducted on the applied scale, a four-factor structure was identified, explaining a total variance of 71.413%. This structure comprises 25 items. The variance explained by each factor is as follows: 21.826% by socioeconomic factors, 19.394% by technical factors, 12.767% by factors related to itinerant beekeeping, and 10.769% by factors related to marketing. As a result, it is crucial for the sustainability of the sector that both beekeeping enterprises and policymakers acknowledge the risk factors identified in Burdur province. Developing solution-oriented policies and implementing measures to mitigate these risks are of great importance.

Keywords: Beekeeping sector, Burdur province, Factor analysis, Risk factors

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Burdur İli Arıcılık İşletmelerinde Risk Faktörlerinin Belirlenmesi

ÖZ

Türkiye, bal üretim miktarı bakımından dünya sıralamasında önemli bir konuma sahiptir. Ancak, kovan başına verim düzeyi, dünya ortalamasının altında kalmaktadır. 2022 yılı verilerine göre, kovan sayısı bakımından dünya sıralamasında üçüncü sırada yer alan Türkiye ile ikinci sırada bulunan Çin arasında, kovan başına verim açısından yaklaşık 3,78 katlık bir fark bulunmaktadır. Bu durum, Türkiye arıcılık sektörünün sürdürülebilirliği açısından, kovan başına verimliliğin artırılmasının gerekliliğini ortaya koymaktadır. Sektörün yeterli verimliliğe ulaşamamasında birçok doğrudan ve dolaylı risk faktörü etkili olmaktadır. Türkiye'nin sahip olduğu geniş ve farklı coğrafi yapılar, bu risk faktörlerinin bölgelere göre değişiklik göstermesine neden olmaktadır. Bu bağlamda, çalışmanın amacı Batı Akdeniz Bölgesi'nde yer alan Burdur ilindeki arıcılık işletmelerinin, üretimden pazarlama sürecine kadar karşı karşıya kaldıkları risk faktörlerini belirlemektir. Araştırma kapsamında, 2025 yılı içerisinde belirlenen örneklem dâhilinde arıcılık işletmeleri ziyaret edilmiş ve "Arıcılık Risk Faktörü Ölçeği" uygulanmıştır. Uygulanan ölçek üzerinde gerçekleştirilen açıklayıcı faktör analizi sonucunda, dört faktörlü bir yapı elde edilmiştir. Toplamda %71,413 oranında varyans açıklanmış olup, bu yapı 25 maddeden oluşmaktadır. Faktörlerin açıklamış oldukları varyans oranları sırasıyla; sosyoekonomik faktörler için %21,826, teknik faktörler için %19,394, gezgin arıcılıkla ilgili faktörler için %12,767 ve pazarlama ile ilgili faktörler için %10,769'dur. Sonuç olarak, Burdur ili özelinde tespit edilen risk faktörlerinin hem arıcılık işletmeleri hem de politika yapıcılar tarafından dikkate alınarak çözüm odaklı politikalar geliştirilmesi ve bu risklerin azaltılmasına yönelik önlemler alınması, sektörün sürdürülebilirliği açısından büyük önem arz etmektedir.

Anahtar Kelimeler: Arıcılık sektörü, Burdur ili, Faktör analizi, Risk faktörleri

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INTRODUCTION

The beekeeping sector plays several vital roles globally due to its production structure. Its most important functions include directly and indirectly meeting humanity's nutritional needs, supporting pollination essential for the continuity of nature and vegetation, and enabling individuals with limited resources in rural areas to sustain their livelihoods through production. Although beekeeping yields various products, honey production remains the primary focus of beekeeping. According to FAO data from 2023, total global honey production reached 1,893,805.48 tons (FAO 2025). China holds the top position in production, followed by Türkiye in second place (TAB 2025).

In this context, beekeeping holds a significant position within Türkiye's livestock sector due to its potential and status as an alternative production branch. The relatively low investment costs and shorter depreciation periods of beekeeping enterprises compared to other agricultural activities, along with the rapid conversion of investments into income, make this sector strategically important for rural development in Türkiye (Apimondia 2025; Bingöl Beekeeping Report 2011; Uzun et al. 2022).

According to TURKSTAT data for 2024, there are 97,984 beekeeping enterprises in Türkiye, with 4,580 located in the TR61 region and 481 in Burdur province. In the same year, Türkiye had 8,717,162 new-type hives and 244,813 old-type hives. In the TR61 region, the number of new-type hives was 315,277, and the number of old-type hives was 470; in Burdur province, these figures were 36,213 and 160, respectively. Honey production in 2024 totalled 95,492.311 tons nationwide, 2,465.114 tons in the TR61 region, and 185.45 tons in Burdur province (TÜİK 2025). Analysis of the 2004–2024 period shows that honey production in Türkiye increased by 29.17%, whereas it decreased by 19.53% in the TR61 region and by 67.05% in Burdur province (TÜİK 2025).

While honey production in Türkiye increased by approximately 30% between 2004 and 2024, it decreased by 67% in Burdur province. This significant decline suggests the presence of substantial risks that are affecting production in the region. Many factors influence these production levels, and it is important to recognize that these factors may pose risks to beekeeping enterprises. According to Varalan (2023), risk factors impacting beekeeping activities include global climate change (Rai and Ravuiwasa 2019; Giannini et al. 2020; Vercelli et al. 2021), diseases and harmful organisms (Higes et al. 2010; Çukur 2014), the biological condition of the queen in colonies (Van Engelsdorp et al. 2013; Çakmak and Seven Çakmak 2016), invasive species (Rai and Ravuiwasa 2019), nomadic beekeeping practices (Pilati and Prestamburgo 2016; Simone-Finstrom et al. 2016), economic, financial, and marketing challenges (Seven and Akkılıç 2005; Öztürk et al. 2014; Çevrimli and Sakarya 2018), as well as various technical problems (Van Engelsdorp et al. 2008; Van der Zee et al. 2014; Söğüt et al. 2019).

This study aims to identify the risk factors affecting beekeeping products in enterprises located in Burdur province throughout the production-to-marketing process. The research aims to analyze the impact of these risks on beekeeping activities and their influence on the sector's sustainability.

MATERIAL and METHODS

The research focuses on beekeeping enterprises operating in the central district of Burdur province and the districts of Ağlasun, Altınyayla, Bucak, Çavdır, Çeltikçi, Gölhisar, Karamanlı, Kemer, Tefenni, and Yeşilova in 2025. The beekeeping enterprises included in the study comprise those registered with the Burdur Provincial Directorate of Agriculture and Forestry, as well as the Burdur Bee Breeding Union, and those that volunteered to participate.

Within the scope of the research, the main mass constituting the universe is 330 beekeeping enterprises. Based on a 90% confidence level in the research, the minimum sample size, considered statistically sufficient, was determined to be 56. This sample size was determined through calculations designed to achieve reliable results. The formulas for the calculation methods used are presented below. [Table value corresponding to the confidence level (z=1.64); Observation rate in the population (p=0.5) (in cases where this rate is unknown, the highest value was taken as 0.5); Acceptable deviation tolerance (d=0.01); N: Population size, n: Sample size].

$$n_0 = \frac{z^2 \times p \times (1-p)}{d^2} = \frac{1.96^2 \times 0.5 \times 0.5}{(0.01)^2} = 67.24$$

$$n = \frac{n_0}{1 + \frac{n_0}{N}} = \frac{67}{1 + \frac{67}{330}} \approx 56$$

In addition to determining the minimum sample size, potential issues that could arise during data collection were considered. Accordingly, nine additional enterprises were included, bringing the total number of participants to 65. However, two of these enterprises were excluded from the study due to incomplete data, resulting in the analysis being conducted on 63 enterprises.

Within the scope of the research, 'Beekeeping Risk Factor Scale' questions were asked of the business owners to determine the risk perceptions of the enterprises. The 'Beekeeping Risk Factor Scale' used

in the research was developed by Varalan and Çevrimli (2024). In the beekeeping risk factor scale, there are 51 questions under four headings (socioeconomic factors, technical factors, environmental and climatic factors, and factors related to itinerant beekeeping) to identify risk factors. These 51 items in the scale form consist of a 5-point Likert-type scale (Very risky, Risky, Neither Risky nor No Risk, No Risk, No Risk at all). These scale questions, prepared by Varalan and Çevrimli, were reduced to 27 items as a result of their factor analysis (Varalan and Çevrimli 2024). In our study, due to the nature of the sample groups, the responses to two items (31, 34) were not evaluated, and the scale questions were reduced to 25 items in total.

In this study, the dataset collected from beekeeping enterprises in Burdur province was analysed using IBM SPSS Statistics Standard Concurrent User Version 27 (IBM Corp., Armonk, New York, USA) for exploratory factor analysis. In this analysis, factors are defined as dimensions derived from linear combinations of observed variables, representing hypothetical constructs formed by these observed variables. To determine whether the data is suitable for factor analysis, the correlation matrix is examined. If many correlation coefficients are below 0.30, factor analysis may not be the most suitable approach. Bartlett's test of sphericity is applied to statistically assess whether correlations among variables exist by testing if the correlation matrix is an identity matrix. Additionally, the Kaiser-Meyer-Olkin (KMO) measure, which is based on correlation and partial correlation coefficients, is used as an essential indicator of data adequacy for factor analysis. In this study, the principal components method was employed to extract the factors.

In determining the optimal number of factors, the selection was guided by the criterion that retained factors should have eigenvalues greater than one. To enhance interpretability, a rotation procedure was employed, and the Varimax method was utilized to identify the specific variables associated with each factor. Subsequently, a confirmatory factor analysis (CFA) was conducted to evaluate the adequacy of the factor structure derived from the exploratory factor analysis (EFA) in relation to theoretical or hypothesized models. It is widely acknowledged that EFA is generally conducted as a preliminary step in scale construction and in evaluating construct validity (Karahan, 2014; Durutürk, 2015; Çınar Özdemir, 2015).

While exploratory factor analysis determines the optimal number of factors based on the data, confirmatory factor analysis is used to verify a known or hypothesized factor structure. In this study, the open-source statistical software JAMOVI (Version 2.3.28) was used for confirmatory factor analysis (Lachin 2000; Obuchowski 2002).

RESULTS

Within the scope of the study, the initial findings related to the application of the Beekeeping Risk Factor Scale in Burdur province, along with the distribution of the factors and corresponding items, are presented in Table 1.

As shown in Table 1, the Burdur Beekeeping Risk Factor Scale comprises 25 items distributed across four factors: Socioeconomic Factors, Technical Factors, Factors Related to Migratory Beekeeping, and Factors Related to Marketing. The findings related to the validity and reliability of the Beekeeping Risk Factor Scale are presented in Table 2.

To evaluate the suitability of the dataset for factor analysis, the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity were applied. The KMO value was calculated as 0.656, indicating a borderline level of suitability according to Kaiser's (1974) classification. Additionally, Bartlett's test of sphericity was significant (χ^2=731.700; $p<0.001$), confirming that the data meet the assumption of multivariate normality and supporting the appropriateness of factor analysis.

The exploratory factor analysis revealed a four-factor structure for the scale, explaining a total variance of 71.413%. This indicates acceptable construct validity, as explained variance between 60% and 80% is generally considered sufficient in the social sciences. Examining the variance explained by each factor separately: socioeconomic factors accounted for 21.826%, technical factors for 19.394%, itinerant beekeeping factors for 12.767%, and marketing-related factors for 10.769% of the variance.

According to Table 2, the factor loadings for items in the first dimension range from 0.456 to 0.681, in the second dimension from 0.403 to 0.762, in the third dimension from 0.525 to 0.810, and in the fourth dimension from 0.484 to 0.519. Based on the $\alpha \geq 0.70$ threshold suggested by Nunnally and Bernstein (1994), the technical factors and marketing dimensions demonstrate sufficient internal consistency, while the socioeconomic factors and itinerant beekeeping dimensions are borderline. However, since the overall Cronbach's Alpha (α) exceeds 0.70, the scale's reliability is considered adequate.

Therefore, it can be concluded that the four dimensions effectively measure their respective sub-features, indicating that the questionnaire is a reliable measurement tool.

Table 1. Factors and Items Related to the Beekeeping Risk Factor Scale

Factors	Name of the Factor	Item Number	Question Items
Factor 1	*Socioeconomic Factors*	4	Low income from the beekeeping sector
		7	Inadequate tool-equipment assets of the enterprise
		9	Failure to keep records in the enterprise
		10	Changes in the country's economy
		11	Rise in exchange rates
		12	Inadequate credit facilities
		13	Changes in the interest rates of loans that can be obtained
		15	Increase in indebtedness of enterprises
		16	Inadequate organization among producers
Factor 2	*Technical Factors*	18	The productivity/adaptation level of the bee breed you breed in the region
		19	Prevalence of bee diseases and pests
		20	Insufficient knowledge in the fight against bee diseases and pests
		22	Use of old queen bees in hives
		23	The problem of obtaining quality queens for hives
		26	Inadequate care and feeding conditions of bees
		27	Neglect of autumn feeding and spraying
		28	Insufficient technical knowledge on beekeeping
Factor 3	*Factors Related to Migratory Beekeeping*	37	Too close proximity of apiaries to each other in accommodation during migratory beekeeping
		38	Exclusion from village land during migratory beekeeping
		39	Demand for high land prices in the hospitality region
		40	Colony losses during transport of beehives
		41	Inadequate labour supply related to beekeeping
Factor 4	*Factors Related to Marketing*	44	Insufficient product marketing opportunities for enterprises or beekeepers
		45	Products cannot be sold at the desired time
		46	Inadequate quality/price relationship in products

Table 2. Validity and Reliability Results of the Beekeeping Risk Factor Scale

Item Number	Socioeconomic Factors	Technical Factors	Factors Related to Migratory Beekeeping	Factors Related to Marketing
4	0.482			
7	0.512			
9	0.519			
10	0.639			
11	0.632			
12	0.592			
13	0.642			
15	0.681			
16	0.456			
18		0.403		
19		0.762		
20		0.674		
22		0.567		
23		0.460		
26		0.505		
27		0.608		
28		0.495		
37			0.671	
38			0.810	
39			0.591	
40			0.576	
41			0.525	
44				0.484
45				0.440
46				0.519
Explained Variance %	21.826	19.394	12.767	10.769
Cronbach's Alpha (α)	0.612	0.716	0.612	0.759
Total Disclosed Variance Rate=71.413				
Kaiser Meyer Olkin (KMO)=0.656				
Bartlett test value=731.700; ***p*<0.001**				
Cronbach's Alpha (α)=0.753				

The scale model (χ^2=290, df=242) consists of four dimensions (see Table 3). The fit indices indicate that the model demonstrates an acceptable level of fit. According to the threshold values suggested by Hu and Bentler (1999) -where CFI and TLI≥0.90 indicate good fit- both indices meet the acceptable criteria. Specifically, the CFI value of 0.92 suggests a good fit between the model and the data. Additionally, the RMSEA value of 0.056, with the upper limit of its confidence interval not exceeding 0.079, further supports a good model fit. Since RMSEA values≤0.05 are considered a perfect fit and values≤0.08 an acceptable fit in the literature, these results confirm that the model provides an acceptable fit to the data (see Table 3). Confirmatory factor analysis was conducted on the beekeeping risk factor scale, which includes twenty-five (25) items and four (4) sub-dimensions. The model is visually presented in Figure 1.

Table 3. Statistical Values Regarding the Fit of the Structural Equation Model

						RMSEA 90% CI	
χ^2	df	p	CFI	TLI	RMSEA	Lower	Upper
290	242	0.018	0.920	0.901	0.056	0.025	0.079

RMSEA= Root Mean Square Error of Approximation; CFI= Comparative Fit Index; TLI= Tucker- Lewis Index

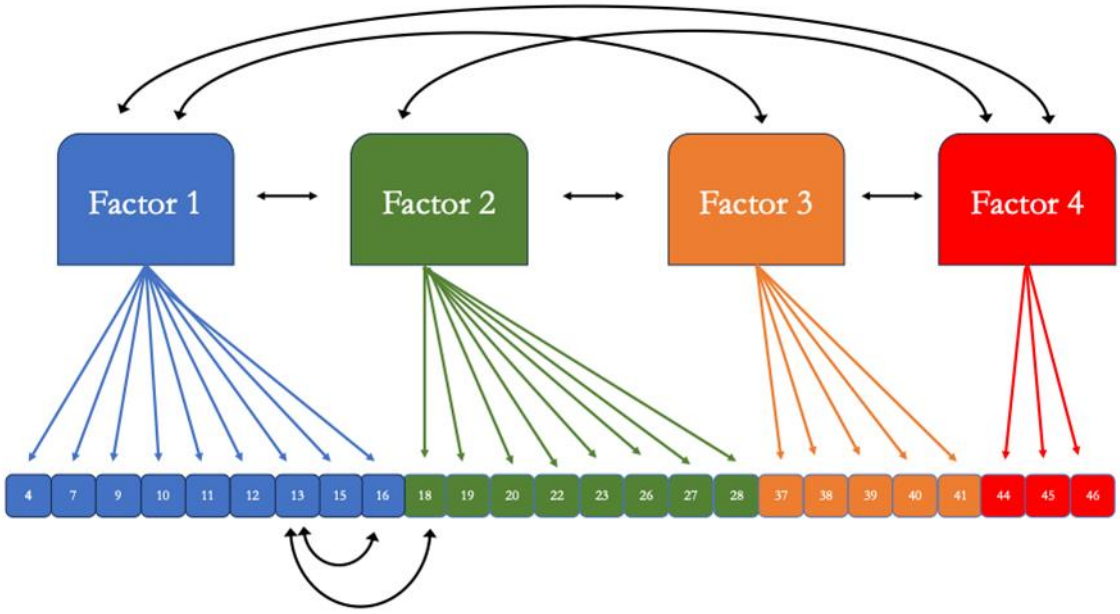

Figure 1: Confirmatory Factor Analysis Model Illustrating the Relationships Among the Subscales of the Scale

DISCUSSION

In beekeeping enterprises in Burdur province, the "Beekeeping Risk Factor Scale" was initially developed by Varalan and Çevrimli (2024) as a 51-item questionnaire. However, a total of 24 items (1, 2, 3, 5, 6, 8, 14, 17, 21, 24, 25, 29, 30, 32, 33, 35, 36, 42, 43, 47, 48, 49, 50, and 51) were subsequently removed from the scale as they were not compatible with the factor structure. When we administered the "Beekeeping Risk Factor Scale" to beekeeping enterprises in Burdur province, in addition to the 24 items that Varalan and Çevrimli (2024) had already excluded, the responses to two more items were not evaluated due to the characteristics of the sample group. This situation is consistent with the study conducted in Kars province, where the necessity of removing items from the scale was associated with the sample size. It has been noted that the total number of questions is relatively high compared to the minimum sample size recommended for factor analysis (Varalan and Çevrimli 2024). In other words, it has been suggested that studies with smaller sample sizes are better conducted using scales with fewer items (Büyüköztürk 2002).

When the percentages of the total variance explained by factors in studies addressing risk factors in the Turkish beekeeping sector are examined, it is observed that the studies conducted in different provinces produce varying results. For example, in a study conducted among migratory beekeeping enterprises in Türkiye, this value was found to be 74.48% (Aksoy et al. 2022). In Kars province, the explained variance was 73.96% (Varalan and Çevrimli 2024), whereas in Ordu province it was 64.57% (Öztürk 2013). Two studies conducted in Iğdır province reported 69% (Karadaş and Birinci 2018) and 74.80% (Kaya and Kılıç Topuz 2023), respectively. Our study, conducted in Burdur province, explained 71.413% of the total variance.

An examination of the scales used in these studies shows that the study conducted in migratory beekeeping enterprises involved 30 items and 10 risk factors (Aksoy et al. 2022); the study in Kars province used 27 items and 8 risk factors (Varalan and Çevrimli 2024); the study in Ordu province had 24 items and 8 risk factors (Öztürk 2013); and the two studies in Iğdır province included 25 items and 8 risk

factors (Karadaş and Birinci 2018) and 27 items and 7 risk factors (Kaya and Kılıç Topuz 2023), respectively. Although our study in Burdur province used the same scale items as the study in Kars, it ultimately consisted of 25 items and four risk factors.

When the Cronbach's alpha and KMO (Kaiser-Meyer-Olkin) values reported in studies conducted in Türkiye are examined sequentially, it is observed that the study conducted by Varalan and Çevrimli (2024) in Kars reported values of 0.857 and 0.648, respectively, while Öztürk (2013) reported 0.657 and 0.617. Similarly, Aksoy et al. (2022) indicated these values as 0.534 and 0.573. According to the study by Kaya and Kılıç Topuz (2023), these values were reported as 0.608 and 0.544. In our study, the corresponding values were found to be 0.753 and 0.656.

It is observed that numerous risk factors are present in beekeeping activities in Türkiye, and the relative importance of these risk factors varies across regions and provinces. In a study conducted in Muğla province, the most critical risk factors in beekeeping activities were reported as "high input costs," followed by "losses due to diseases" and "nutritional/feeding deficiencies" (Akbağ et al. 2025). In a study conducted in İzmir province, the top three risks faced by beekeepers were identified as "adulteration and imitation of honey," "input costs," and "climatic conditions-drought" (Onuç et al. 2019). In a study conducted in Erzincan province, the three most significant issues identified were "bear attacks," "adverse climatic conditions," and "marketing problems," respectively (Alkaya and Candemir 2025).

In a study conducted in Muğla province, the most significant source of risk faced by beekeepers participating in the research was reported to be the high cost of inputs (Akbağ et al. 2025). In this context, when examining the impact values of factors that could affect economic indicators in the beekeeping sector, it is observed that the results vary and that related items can appear under different factor headings. In our study conducted in Burdur province, Item 15 (increase in the indebtedness of enterprises), which falls under "socioeconomic factors," had the highest value within this factor group at 0.681. This was followed by Item 13 (changes in interest rates of available loans) at 0.642 and Item 10 (changes in the national economy) at 0.639. These items are similar to those in the study conducted in Kars, where Items 15 and 13 were included under "financing-related risk factors," with values of 0.793 and 0.814, respectively. In Kars, both items had higher values compared to Burdur. Item 10, on the other hand, was classified under "economic, organizational, and global risk factors" in Kars and received a value of 0.911, which was higher than its corresponding value in Burdur (Varalan and Çevrimli 2024). In a study conducted in Iğdır, the items receiving the lowest scores under "social sustainability factors" were "investment using income from beekeeping and the role of women in honey production," with a value of 0.195, and the "satisfaction factor," with a value of 0.682. It is observed that most items under the headings of investment and satisfaction are, directly or indirectly, associated with the economic structure and indicators (Kaya and Kılıç Topuz 2023). In a study on migratory beekeeping enterprises in Türkiye, under the "marketing factor," the items with the highest values were "increase in debt amount" at 0.830 and "instability in interest rates" at 0.794. Under the heading of "economic structure and natural conditions factor," "increase in input costs" at 0.819 and "inability to obtain loans" at 0.607 were the items with the highest values in this group (Aksoy et al. 2022). In the study conducted in Iğdır, "changes in government policies regarding beekeeping" (0.682) and "changes in the country's economic conditions" (0.670) were identified as economic and political risk factors (Karadaş and Birinci 2018). In a study conducted in Ordu, the items under the "economic and natural conditions factor" that explained risk were "changes in product prices" with a value of 0.790 and "changes in the country's economic situation" with a value of 0.594 (Öztürk 2013).

In a study conducted in Muğla province on the risks faced by beekeeping enterprises, "losses caused by diseases" were identified as the second most significant risk factor, after "high input costs" (Akbağ et al. 2025). In our study on beekeeping enterprises in Burdur province, under the risk category associated with "technical factors," Item 19 (prevalence of bee diseases and pests) ranked first with a value of 0.762. It was followed by Item 20 (insufficient knowledge in combating bee diseases and pests) with 0.674, and Item 27 (neglecting autumn feeding and treatment) with 0.608. When comparing these items to the results from Kars province, Item 19 was classified under the "disease monitoring and control" factor with a value of 0.800, Item 20 under "queen bee and knowledge-related risk factors" with 0.783, and Item 27 under "insufficient care and feeding conditions for bees" with 0.822 (Varalan and Çevrimli 2024). In a study conducted among migratory beekeepers in Türkiye, the "disease factor" included "disease and wintering losses," which had a value of 0.755. Under the "climatic conditions factor," the "nutritional deficiency in hives" item scored 0.800. Under the "operator characteristics factor," the items "inability to combat diseases" and "lack of technical knowledge" scored 0.860 and 0.475, respectively (Aksoy et al. 2022). In another study conducted in Iğdır province, the two items most closely related to our focus -"diseases and losses during wintering" and "nutritional deficiency"- received values of 0.517 and 0.583, respectively (Karadaş and Birinci 2018). In the study conducted in Ordu province, the "disease and wintering losses" within the disease factor had a value of 0.798, and "inability to combat diseases and pests" had a value of 0.738, while the item "food

deficiency," under the operational conditions factor, was identified with a value of 0.703 (Öztürk 2013). In a study conducted in Muğla, the insufficiency in combating diseases and pests was identified as the sixth most significant risk factor among twelve (Akbağ et al. 2025).

Regarding "migratory beekeeping" in Burdur, the highest-scoring item was Item 38 (being denied access to villages or land during migratory beekeeping) with a value of 0.810, followed by Item 37 (apiaries being located too close to each other during migration or lodging) with 0.671, and Item 39 (high land rental fees in the lodging area) with 0.591. In Kars, the corresponding values were 0.841, 0.568, and 0.825, respectively. The first two items fell under the category of "risks arising from migratory beekeeping," while the last one was classified under "risks arising from enterprises and the region" (Varalan and Çevrimli 2024). In a study conducted among migratory beekeepers in Türkiye, the "attitudes toward beekeepers" factor identified "charging fees to beekeepers" (0.928) and "transportation fees" (0.891) as major risks (Aksoy et al. 2022). In Muğla, the item "lack of guidance in selecting suitable beekeeping locations," associated with migratory beekeeping, was identified as the least significant risk factor among all risk items (Akbağ et al. 2025).

Within the category of risk factors related to marketing, the item with the highest value is item 46, which refers to the inadequacy of the quality/price relationship in products, with a value of 0.519. In the study conducted in Kars province, the same item was recorded as 0.621, representing the lowest value among the three items constituting the marketing-related risk factor. In Burdur province, the order from the highest to the lowest value was item 46 (inadequacy of the quality/price relationship in products), item 44 (insufficient marketing opportunities for enterprises and beekeepers), and item 45 (inability to sell products at the desired time), whereas in Kars the order was 45, 44, and 46 respectively (Varalan and Çevrimli 2024). Another study reported that the item concerning marketing problems under the marketing factor had a value of 0.816, while the item reflecting the instability of product prices under the policies factor was recorded as 0.510 (Aksoy et al. 2022). In the study conducted in Iğdır province, insufficient marketing opportunities received a value of 0.481 (Karadaş and Birinci 2018). In contrast, in Ordu province, the item reflecting low marketing opportunities under the operating conditions factor had a value of 0.500 (Öztürk 2013). In the sector, the marketing of products is negatively affected by counterfeiting and adulteration. A study conducted in Muğla province identified counterfeiting and adulteration as a medium-level risk factor for unfair competition (Akbağ et al. 2025).

CONCLUSION

Beyond honey production, which meets a fundamental nutritional need for humanity, the beekeeping sector offers numerous important benefits, including supporting plant pollination, fostering rural development, and generating employment with relatively low investment costs. However, despite these multifaceted advantages, beekeeping is subject to various risks throughout the entire production process, from flower to table. It is therefore crucial to assess these risk factors within the context of elements that directly or indirectly impact the sector and to develop appropriate solutions—or at least mitigate their effects.

In this context, Türkiye's geographical features, climatic conditions, and socioeconomic factors must be carefully considered in risk assessments. Regional classifications should be made based on production structures, geographic characteristics, and development levels. For the beekeeping sector to contribute effectively to rural development in Türkiye, it is essential that support policies are designed with attention to regional risk factors, ensuring sustainable production and the implementation of effective strategies.

Conflict of interest: The author has no conflicts of interest to report

Authors' Contributions: ACA contributed to the project idea, design, and execution of the study. ACA contributed to the acquisition of data. ACA analyzed the data. ACA drafted and wrote the manuscript. ACA reviewed the manuscript critically. ACA has read and approved the finalized manuscript.

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RESEARCH ARTICLE

Determination of Fattening Performance, Some Slaughter and Carcass Traits of Blonde d'Aquitaine, Charolais, Limousin and Simmental cattle

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ABSTRACT

This study aims to evaluate the fattening performance and some slaughter and carcass characteristics of four different cattle breeds (Blonde d'Aquitaine, Charolais, Limousin, and Simmental) fattened in a private enterprise in Türkiye. Data from a total of 120 imported male cattle were used in the research. These animals were placed on a fattening with an average initial live weight of 294.10 kg. In this study, parameters such as average daily live weight gain, slaughter weight, carcass weight, and dressing percentage were examined. Statistical analyses were performed using analysis of variance (ANOVA) and multiple comparison tests. The average daily live weight gains for Blonde d'Aquitaine, Charolais, Limousin, and Simmental bulls were found to be 1.416±0.035, 1.641±0.060, 1.457±0.024, and 1.538±0.082 kg, respectively, with a significant difference among breeds ($p<0.05$). The dressing percentages for these breeds were determined as 58.90±0.41%, 58.20±0.80%, 59.10±0.30%, and 56.50±1.00%, respectively. The results revealed statistically significant differences among breeds in terms of initial live weight, daily live weight gain, and slaughter weight ($p<0.05$). In particular, the Charolais breed stood out for its higher average daily live weight gain. It was also concluded that the Simmental breed shows similarities to specialized beef breeds in certain traits and has potential as an alternative. The findings obtained from this study can provide valuable insights to producers regarding productivity and performance when selecting cattle breeds for fattening.

Keywords: Beef cattle, Daily live weight gain, Dressing percentage

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Blonde d'Aquitaine, Şarole, Limuzin ve Simental Sığırların Besi Performansı, Bazı Kesim ve Karkas Özelliklerinin Belirlenmesi

ÖZ

Bu çalışmada, Türkiye'deki özel bir işletmede besiye alınan dört farklı sığır ırkının (Blonde d'Aquitaine, Şarole, Limuzin ve Simental) besi performansı, bazı kesim ve karkas özelliklerinin ortaya konulması amaçlanmıştır. Araştırmada toplam 120 baş ithal erkek sığırın verileri kullanılmıştır. Bu sığırlar ortalama 294,10 kg canlı ağırlıkla besiye alınmıştır. Bu çalışmada günlük canlı ağırlık artışı, kesim ağırlığı, karkas ağırlığı ve randımanı gibi parametreler incelenmiştir. İstatistiksel analizler varyans analizi (ANOVA) ve çoklu karşılaştırma testleri ile gerçekleştirilmiştir. Blonde d'Aquitaine, Şarole, Limuzin ve Simental boğalarda günlük canlı ağırlık artışı değerleri sırasıyla; 1,416±0,035, 1,641±0,060, 1,457±0,024 ve 1,538±0,082 kg bulunmuş olup ırklar arasındaki fark önemli ($P<0,05$) olmuştur. Aynı ırklarda karkas randımanı sırasıyla; %58,90±0,41, %58,20±0,80, %59,10±0,30 ve %56,50±1,00 olarak tespit edilmiştir. Sonuç olarak, ırklar arasında besi başı ağırlığı, günlük canlı ağırlık artışı ve kesim ağırlığı bakımından istatistiksel olarak anlamlı farklar olduğu ortaya konulmuştur ($p<0,05$). Özellikle Şarole ırkı yüksek günlük canlı ağırlık artışı bakımından öne çıkmıştır. Simental ırkının da etçi ırklarla belirli özellikler bakımından benzerlikler gösterebildiği ve bir alternatif sunma potansiyeli olduğu sonucuna varılmıştır. Elde edilen bulgular, etçi sığır ırkı seçiminde üreticilere verimlilik ve performans bakımından fikir verebilecek niteliktedir.

Anahtar Kelimeler: Etçi sığır, Günlük canlı ağırlık artışı, Karkas randımanı

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INTRODUCTION

Animal husbandry holds significant importance both world and in Türkiye, as it serves as a vital source of nutrition, creates employment opportunities, and contributes to the national economy through exports (Ergün and Bayram, 2021). In Türkiye, the distribution of red meat production in 2023 was 70.1% beef, 23.9% sheep meat, 5.4% goat meat, and 0.6% buffalo meat (TUIK, 2025). Cattle stand out as one of the main sources of adequate and balanced nutrition for humans worldwide, as well as in Türkiye (Duru and Sak, 2017). Their ability of cattle to efficiently utilize roughages rich in cellulose has made them products an indispensable source of nutrition for people. With the increasing population and changing demands, variations in the productive traits of cattle have also emerged (Pınarbaşı and Yazgan, 2020). Considering Türkiye's growing population and the associated demand for high-quality, healthy food, the importance of cattle breeding becomes evident (Göncü and Bozkurt, 2019). Cattle fattening is of great importance due to its economic advantages and its role in the production of meat, which is essential for ensuring adequate and balanced nutrition for the population (İmik et al., 2000). In order to meet the resulting demand, there is a need for beef breeds with high fattening performance and the ability to produce high-quality carcasses. The importation of live animals for fattening has laid the groundwork for the development of a livestock sector that relies on imported beef cattle in Türkiye (Arıkan and Gökhan, 2018). Over the past two decades, significant progress has been made in beef production in Türkiye: farm capacities have grown, and greater emphasis has been placed on technological innovations in housing, feeding, health management, and marketing. Today, cattle fattening is increasingly concentrated in large, specialized farms that meet modern entrepreneurial standards (Göncü and Bozkurt, 2019). The consumption of beef is influenced by economic conditions, health concerns, socio-cultural backgrounds, traditions, and habits (Cankurt et al., 2010). Identifying variations in fattening performance and carcass characteristics among breeds is of great importance for improving the efficiency of meat production systems and ensuring carcass quality that meets market demands. In this context, production parameters such as average daily live weight gain, feed conversion ratio, slaughter weight, dressing percentage, and carcass conformation emerge as key indicators for determining economic profitability. This study aims to determine the fattening performance, as well as some slaughter and carcass characteristics, of Blonde d'Aquitaine, Charolais, Limousin, and Simmental cattle raised under private farm conditions.

MATERIALS and METHODS

The material of this study consisted of records on the fattening performance and some slaughter and carcass characteristics of a total of 120 male cattle imported to a private enterprise in Afyonkarahisar province, including 70 Limousin, 33 Blonde d'Aquitaine, 11 Charolais, and 6 Simmental animals. After pre-fattening health procedures (antiparasitic treatments, vaccination, etc.) and an adaptation period, the animals were weighed and placed in a fattening. Animals were fed with a diet including corn silage, concentrate, crushed barley, alfalfa, oats, corn flake, straw, and ryegrass in the routine feeding practiced by the farm. During the fattening period, they were housed in free stall-semi-open barns and weighed monthly. Descriptive statistics for some extra carcass characteristics were also determined in 5 Limousin bulls.

For initial live weight and daily live weight gain;

$Y_{ij} = \mu + genotype_i + e_{ij}$

For hot carcass weight, dressing percentage, and slaughter weight;

$Y_{ij} = \mu + genotype_i + b_1(initial\ weights) + b_2(fattening\ period) + e_{ij}$

In the model, Y represents the observation value; genotype refers to Blonde d'Aquitaine, Charolais, Limousin, and Simmental; and initial weights (b_1) and fattening period (b_2) were considered as covariates for carcass weight, dressing percentage, and slaughter weight. Descriptive statistics, analysis of variance (ANOVA), and multiple comparison tests were performed using the SPSS 18.0 for Windows software (SPSS Inc., Chicago, IL, USA).

RESULTS

The average daily live weight gain, slaughter weight, hot carcass weight, and dressing percentages of Blonde d'Aquitaine, Charolais, Limousin, and Simmental bulls are presented in Table 1. The initial live weights at the start of fattening for Blonde d'Aquitaine, Charolais, Limousin, and Simmental bulls were determined as 294.65±10.60, 361.86±18.37, 271.52±7.28, and 248.35±24.87 kg, respectively. The differences among breeds were found to be statistically significant ($p<0.001$). According to the results of the multiple comparison test, Charolais bulls were observed to be distinct from the other breeds. Additionally, Simmental bulls had the lowest initial live weight among the breeds. Regarding average daily live weight gain, values for Blonde d'Aquitaine, Charolais, Limousin, and Simmental were 1.416±0.035, 1.641±0.060, 1.457±0.024, and 1.538±0.082 kg, respectively, and the differences among breeds were found to be

significant ($p<0.05$). Charolais bulls were found to be statistically different from Limousin and Blonde d'Aquitaine, while Simmental bulls did not differ from the other breeds. Hot carcass weights for Blonde d'Aquitaine, Charolais, Limousin, and Simmental were 383.86±5.56, 393.36±10.29, 398.07±3.84, and 406.21±13.22 kg, respectively, and dressing percentages were 58.90±0.41%, 58.20±0.80%, 59.10±0.30%, and 56.50±1.00%, respectively. For both traits, genotype effect was not statistically significant, while initial live weight and fattening period as covariates were significant. Slaughter weights were 650.87±8.37, 676.55±15.50, 673.54±5.79, and 719.17±19.91 kg, respectively. For this trait, breed, initial live weight, and fattening period were all found to be significant ($p<0.05$). Simmental bulls, showing the highest value, were separated from Blonde d'Aquitaine with the lowest value in the multiple comparison test, while Charolais and Limousin were similar to both breeds.

Table 1. Fattening performance, some slaughter and carcass traits of Blonde d'Aquitaine, Charolais, Limousin and Simmental bulls

Factor	n	Initial live weight, kg	Daily live weight gain, kg	Hot carcass weight, kg	Dressing percentage, %	Slaughter weight, kg
µ	120	294.10±8.37	1.513±0.027	395.37±4.36	58.18±0.30	680.03±6.57
Breed		***	*			*
Blonde d'Aquitaine	33	294.65±10.60^{b}	1.416±0.035^{b}	383.86±5.56	58.90±0.41	650.87±8.37^{b}
Charolais	11	361.86±18.37^{a}	1.641±0.060^{a}	393.36±10.29	58.20±0.80	676.55±15.50^{ab}
Limousin	70	271.52±7.28^{b}	1.457±0.024^{b}	398.07±3.84	59.10±0.30	673.54±5.79^{ab}
Simmental	6	248.35±24.87^{b}	1.538±0.082^{ab}	406.21±13.22	56.50±1.00	719.17±19.91^{a}
Initial live weight^{c}		-	-	***	*	***
Fattening period^{c}		-	-	***	***	***

*: $p<0.05$, ***: $p<0.001$

^{ab}: Different superscripts in same column are significantly different ($P<0.05$)

^{c}: The initial live weights and fattening period were fitted to the model as a covariate in hot carcass weight, dressing percentage and slaughter weight

Descriptive statistics for some slaughter and carcass characteristics of Limousin bulls are presented in Table 2. Slaughter weight ranged from 648 to 748 kg, with an average of 686 kg. Hot carcass weight varied between 395.00 and 480.40 kg, averaging 428.36 kg. Similarly, cold carcass weight ranged from 389.40 to 473.60 kg, with an average of 421.85 kg. Bone weight in the carcass ranged from 57.50 to 60.20 kg, with a mean of 58.74 kg. The weight of the unconsumed amount in the carcass ranged from 13.50 to 16.40 kg, with an average of 14.74 kg.

Table 2. Descriptive statistics on Some slaughter and carcass traits of Limousin bulls

Trait	Mean	Std. Error	Min	Max
Slaughter weight, kg	686.00	19.424	648.00	748.00
Hot carcass weight, kg	428.36	16.163	395.00	480.40
Cold carcass weight, kg	421.85	15.989	389.40	473.60
Bone weight in carcass, kg	58.74	0.514	57.50	60.20
Unconsumed amount weight in carcass, kg	14.74	0.585	13.50	16.40
Muscle weight in carcass, kg	348.38	14.939	317.80	397.00
Ribeye weight, kg	17.00	1.045	14.40	20.40
Striploin weight, kg	11.74	0.678	10.60	14.20
Tenderloin weight, kg	5.78	0.360	5.10	6.80
Chilling loss percentage, %	1.52	0.075	1.40	1.80

DISCUSSION

In this study, the average initial live weight at the start of fattening was determined as 294.10 kg, and the effect of genotype on this trait was found to be statistically significant ($p<0.001$). The value of 294.65 kg recorded for Blonde d'Aquitaine falls between the 278 kg and 323 kg values reported by Chambaz et al. (2001). For Charolais and Simmental, the present study found initial weights of 361.86 kg and 248.35 kg, respectively, which were higher for Charolais (276.7 kg) but lower for Simmental (261.6 kg), reported by Duru and Sak (2017). Additionally, the Simmental's initial weight exceeded the 118.00–230.5 kg range reported in other studies (Altuntaş and Arpacık 2004; Üstüner et al., 2020). The initial weight (271.52 kg) for Limousin found in this study aligns with the 249.05–297.14 kg range reported by Arıkan and Gökhan (2018) and Duru and Sak (2017). Such differences may result from variations in the origin of animal material, management conditions, and feeding programs.

In this study, the average daily live weight gain was found to be 1.51 kg, with statistically significant differences observed among breeds ($p<0.05$). The value found for Blonde d'Aquitaine was 1.42 kg, which exceeded the 0.87–1.20 kg range reported by various researchers (Chambaz et al., 2001; Sochor et al., 2005; Vinet et al., 2021) in pure and crossbred Blonde d'Aquitaine. For Limousin, the 1.46 kg

recorded in this study is at the upper limit of the 0.95–1.46 kg range reported in the literature (Duru and Sak, 2017; Arıkan and Gökhan, 2018; Chambaz et al., 2001; Alberti et al., 2008; Şenyüz et al., 2020). For Charolais, the mean average daily weight gain was 1.64 kg, which was within the 1.10–2.30 kg range reported by other studies (Chambaz et al., 2001; Sochor et al., 2005; Barton et al., 2006; Alberti et al., 2008; Duru and Sak, 2017; Şenyüz et al., 2020; Strydom and Hope-Jones, 2022). For Simmental, the average daily weight gain was 1.54 kg, which was higher than the range of 1.04–1.49 kg previously reported (Chambaz et al., 2001; Altuntaş and Arpacık, 2004; Sochor et al., 2005; Barton et al., 2006; Alberti et al., 2008; Duru and Sak, 2017; Şenyüz et al., 2020). These differences between the present study and the literature may be due to genetic factors, management conditions, and variations in feeding programs.

In this study, the average slaughter weight was determined as 680.03 kg, and genotype affected significantly ($p<0.05$). For Blonde d'Aquitaine, this value was 650.87 kg, which is within the 227–820 kg range reported by other researchers for pure and crossbred Blonde d'Aquitaine (Chambaz et al., 2001; Sochor et al., 2005; Vinet et al., 2021). For Limousin, the value was 673.54 kg, close to the upper limit of the 565.4–697.0 kg range reported in the literature (Alberti et al., 2008; Salamonczyk et al., 2022; Lunesu et al., 2024), and consistent with previous findings. Average slaughter weight for Charolais was 676.55 kg, which is higher than the range of 620.7–653.3 kg reported in previous studies (Sochor et al., 2005: Barton et al., 2006; Alberti et al., 2008). For Simmental, the mean slaughter weight was 719.17 kg, and this value is higher than the 593.66–694.1 kg range reported in earlier studies (Sochor et al., 2005; Barton et al., 2006; Alberti et al., 2008; Üstüner et al., 2020). These differences may be from variations in management conditions, feeding strategies, and genetic factors. Particularly, the fattening period, initial weight, and other environmental factors can affect slaughter weight.

Dressing percentage was calculated 58.18 % for all fattening young bulls. The value of 58.9% determined for Blonde d'Aquitaine was in the range between 56.7% and 64.3% reported by researchers (Chambaz et al. 2001; Sochor et al., 2005; Vinet et al., 2021). Average dressing percentage for Charolais was 58.2%, which was within the range of the 57.4-61.0% reported in previous studies (Chambaz et al. 2001; Sochor et al., 2005; Barton et al., 2006; Alberti et al., 2008; Duru and Sak, 2017; Şenyüz et al., 2020; Strydom and Hope-Jones, 2022). For Limousin, the dressing percentage (59.1%) was with the range of 57.2% and 63.7% reported by previous studies (Chambaz et al. 2001; Alberti et al., 2008; Duru and Sak, 2017; Şenyüz et al., 2020; Salamonczyk et al., 2022; Lunesu et al., 2024). The dressing percentage of simmental was 56.5%, which was between the 53.40% and 62.02% values reported by different researchers (Chambaz et al. 2001; Altuntaş and Arpacık, 2004; Sochor et al., 2005; Barton et al., 2006; Alberti et al., 2008; Duru and Sak, 2017; Şenyüz et al., 2020; Üstüner et al., 2020). These differences may be attributed to variations in management practices, feeding strategies, and genetic factors. This study despite Simmental bulls having lower initial weights compared to other breeds, the analysis of fattening performance, slaughter and carcass weights shows that they achieved superior carcass and slaughter weights.

When Limousin bulls were evaluated in terms of some carcass characteristics, muscle weight in the carcass ranged from 317.80 to 397.00 kg, with an average of 348.38 kg. Ribeye weight varied among individuals from 14.40 to 20.40 kg, with an average of 17 kg. Similar variation was observed in striploin weight, which ranged from 10.60 to 14.20 kg and averaged 11.74 kg. Tenderloin weight ranged from 5.10 to 6.80 kg, with an average of 5.78 kg. Meanwhile, chilling loss percentage varied between 1.40% and 1.80%, with an average of 1.52%. These findings highlight the phenotypic diversity among Limousin bulls and reveal differences in the evaluated carcass characteristics.

CONCLUSION

In this study, the fattening performance and certain slaughter and carcass characteristics of Blonde d'Aquitaine, Charolais, Limousin, and Simmental bulls raised under the same environmental and feeding-management conditions were evaluated. The findings showed significant differences among breeds. These results highlight the importance of breed selection in countries like Türkiye that aim to improve meat production efficiency and quality. Charolais was found to offer a suitable alternative for fattening enterprises due to its high meat yield and economic potential. The Simmental breed, known for its dual-purpose characteristics, also showed similarities to specialized beef breeds in certain traits and presents an alternative potential. Future studies conducted in different regions could help provide a more comprehensive assessment of the performance of these breeds.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: SK, MD, SÇ and KY contributed to the study design and conduct of the study. Data organization and statistical analysis were performed by SK, MD, SÇ and KY. SK, MD and SÇ drafted and wrote the manuscript. SK, MD and SÇ reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules."

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RESEARCH ARTICLE

3D Reconstruction of Some Limb Bones in New Zealand Rabbits with 3D Scanning and Computed Tomography: Morphological Investigation

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ABSTRACT

In recent years, there has been a notable increase in the number of studies utilizing three-dimensional (3D) scanners and computed tomography in the field of anatomy. However, the literature reveals that studies evaluating the compatibility between these two methods remain limited. In this study, a total of 12 adult rabbits were used. Following maceration, selected limb bones were subjected to 3D reconstruction using both computed tomography (CT) and 3D scanner. The resulting models were analyzed morphologically. On the scapula, the ventral end of the acromion was observed to terminate at the proc. hamatus and extend caudally as the metacromion. In the humerus, the tuberculum majus slightly surpassed the caput humeri, and a foramen supratrochleare was present at the distal end. The antebrachial skeleton consisted of the radius and ulna, with the ulna being more developed. The radius displayed a distinct fovea capitis radii and processus styloideus radii, while the ulna exhibited a prominent tuber olecrani and a proc. styloideus ulnae. The femur, the trochanter major extended beyond the femoral head, with the crista intertrochanterica located between the trochanter major and trochanter minor. At the distal femur, facies poplitea were clearly visible. The crural skeleton consisted of the tibia and fibula, which were fused into a single structure. In conclusion, the anatomical landmarks identified in the 3D models generated by both methods were consistent. While CT excels in scanning entire tissues and providing rapid data, 3D scanners offer advantages portability, color imaging, and cost. Combined use of methods is considered beneficial for veterinary anatomy education.

Keywords: 3D scanner, 3D reconstruction, Computed tomography, Method comparison

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Yeni Zelanda Tavşanlarında Bazı Ekstremite Kemiklerinin 3 Boyutlu Tarayıcı ve Bilgisayarlı Tomografi ile 3 Boyutlu Rekonstrüksiyonu: Morfolojik İnceleme

ÖZ

Son yıllarda, anatomi alanında 3 Boyutlu (3B) tarayıcılar ve bilgisayarlı tomografi kullanan çalışmaların sayısında belirgin bir artış olmuştur. Ancak literatürde, bu iki yöntem arasındaki uyumluluğu değerlendiren çalışmaların sınırlı olduğu gözlenmiştir. Yapılan çalışmada toplam 12 yetişkin tavşan kullanılmıştır. Maserasyonun ardından, seçilen ekstremite kemikleri hem bilgisayarlı tomografi (BT) hem de 3B tarayıcı kullanılarak 3B rekonstrüksiyona tabi tutulmuştur. Elde edilen modeller, morfolojik olarak analiz edilmiştir. Scapula'da, acromion'un ventral ucunun proc. hamatus'ta sonlandığı ve metacromion olarak caudal olarak uzandığı gözlemlendi. Humerus'ta, tuberculum majus'un caput humeri'yi hafifçe aştığı ve distal uçta foramen supratrochleare gözlendi. Önkol iskeleti radius ve ulna'dan oluştuğu ve ulna'nın daha gelişmiş olduğu gözlendi. Radius, belirgin bir fovea capitis radii ve proc. styloideus radii sahipken, ulnae'da belirgin bir tuber olecrani ve processus styloides ulnae varlığı tespit edildi. Femur'da, trochanter major'un caput femoris düzeyini aştığı ve crista intertrochanterica trochanter major ile trochanter minor arasında yer aldığı gözlendi. Distal femurda, facies poplitea belirgindi. Ossa cruris, tek bir yapı halinde birleşmiş tibia ve fibula şeklinde olduğu tespit edildi. Sonuç olarak, her iki yöntemle oluşturulan 3B modellerde belirlenen anatomik noktalar benzerlik göstermekteydi. CT, tüm dokuları tarama ve hızlı veri sağlama konusunda üstünlük sağlarken, 3B tarayıcılar taşınabilirlik, renkli görüntüleme ve maliyet açısından avantajlar sunmaktadır. Yöntemlerin birlikte kullanılması, veteriner anatomi eğitimi için faydalı olabileceği düşünülmektedir.

Anahtar Kelimeler: 3B rekonstrüksiyon, 3B tarayıcı, Bilgisayarlı tomografi, Metot karşılaştırma

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INTRODUCTION

Medical imaging techniques facilitate the diagnosis, treatment and management of disease by health professionals. In the present era, techniques such as X-ray, Computed Tomography (CT), Magnetic Resonance Imaging (MRI) and ultrasound are employed with considerable frequency. In recent years, three-dimensional scanning technology with high accuracy and no negative side effects has become increasingly prevalent.

Although the foundations of three-dimensional scanning technology can be traced back to 1960, the initial generation of devices incorporated light, a single camera, and a single projection. Given the lack of technological advancement at the time, the scanning process was inherently time-consuming. Scanners that permit the detailed scanning of objects at high resolution and speed were first made available for sale in 1994 (Edl et al. 2018). Three-dimensional (3D) scanners are employed extensively in the medical domain, including human medicine (Schmalz et al. 2012), veterinary anatomy (Hackmann et al. 2019), archaeology (Remondino 2011), and dentistry (Rosicky et al. 2016). Despite the perception that the areas where techniques such as photogrammetry, laser scanners, structured light scanners and stereoscopy are used today are distinct, many scanners employ a similar underlying principle (Raja and Fernandes, 2007). The development of computed tomography (CT) technology can be traced back to the discovery of X-rays. The discovery of X-rays is generally accepted as having occurred in 1895, although its emergence can be traced back to 1850 (Mould, 1995). The first commercially available CT scanner was produced in 1972, and since then, numerous studies have been conducted on this technology. This study aims to utilise a 3D scanner and CT sections to create a 3D model of some extremity bones in New Zealand rabbits. Computed tomography is frequently used in the field of veterinary anatomy. The aim of the study is to compare the anatomical points of 3D models obtained with a 3D scanner with those obtained by computed tomography and to contribute to veterinary anatomy education.

MATERIALS and METHODS

Experimental Animal Model

In this study, six male and six female adult New Zealand rabbits were used and subsequently euthanised following the administration of general anaesthesia (Lipman et al. 1990; Oguntoye and Oke 2014; Gökmen et al. 2019). The animals were subjected to classical maceration, as described by Taşbaş (1965). Study permissions were obtained from Selçuk University Animal Experiments Local Ethics Committee (Decision no: 2020-57 (Appendix-1)).

CT Scan and 3D Modelling

In the study, a multislice spiral tomography device (Siemens Dual Source, Somatom Definition Flash, Germany) was employed for bone scans. Imaging was conducted using the following parameters: 120 kV, 300 mA, 0.6 mm slice thickness, 512 x 512 matrix. Three-dimensional modelling was conducted using the 3D Slicer 5.0.3 software.

3D Modelling with 3D Scanner Device

In the study, Shining 3D EinScan Pro 2X (2020) + Shining 3D EinScan Colour Pack was used for 3D scanning. EXScan Pro v.3.6.0.5 software was used for modelling. Device calibration was performed with an accuracy of 0.026 mm and 0.039 pixels. The process was completed by obtaining 18 images in a single scan in fixed scan mode. After the first scan was completed, the position of the bone was changed and images were obtained from different angles. Imaging was performed from four different angles. The 3D models were then obtained and exported in ".stl and .obj" formats.

RESULTS

In this study, three-dimensional models obtained from CT scans and three-dimensional scanner models were compared morphologically. The findings obtained from both methods were found to be parallel to each other.

Scapula

It was observed that the scapula was smooth, flat, and narrowed until the collum scapulae, with the dorsal part of the latter resembling a triangle. It was observed that the spina scapulae divided the bone into the fossa supraspinata and the fossa infraspinata. The fossa infraspinata was observed to narrow from the angulus caudalis to the collum scapulae. The spina scapulae was observed to ascend from dorsal to ventral, extending over the fossa infraspinata. It was observed that the spina scapulae terminated in a ventral position, in proximity to the acromion. It was established that the acromion terminates as the proc. hamatus at the ventral end of the bone and extends caudally as the metacromion. The fossa subscapularis is observed to have a wide distribution in the facies medialis. It was observed that the cavitas glenoidalis in the ventral part of the scapula resembled a gourd, narrowed by the incisura glenoidalis and bounded by the tuberculum supraglenoidale. It was established that the processus corocoideus in the cranial region of the bone exhibited a hook-shaped configuration, terminating in a bending motion towards the facies costalis (Figure 1).

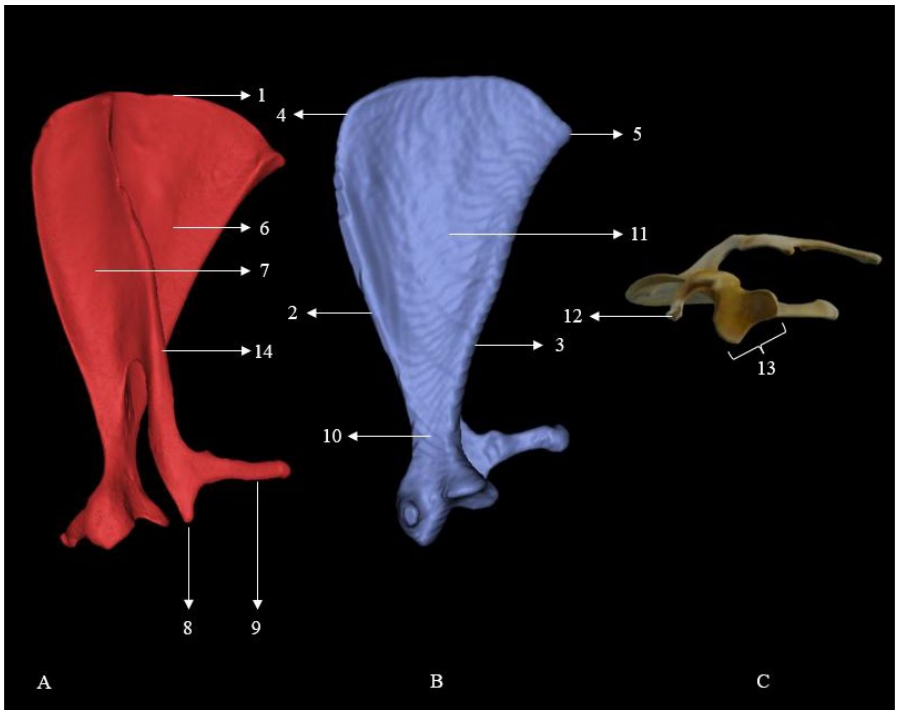

Figure 1. Reconstruction image and anatomical landmarks of the scapula. A: 3D Scanner model .stl file format; B: CT 3D model; C: 3D Scanner color scanned 3D model. 1. Margo dorsalis, 2. Margo cranialis, 3. Margo caudalis, 4. Angulus cranialis, 5. Angulus caudalis, 6. Fossa infraspinata, 7. Fossa supraspinata, 8. Acromion, 9. Metacromion, 10. Collum scapulae, 11. Fossa subscapularis, 12. Processus coracoideus, 13. Cavitas glenoidealis, 14. Spina scapulae

Humerus

The caput humeri was observed to be markedly wide. The tuberculum majus was observed in the cranio-lateral aspect of the caput humeri, while the tuberculum minus was noted in the cranio-medial aspect. The tuberculum majus exhibited a slight prominence relative to the caput humeri. The sulcus intertubercularis was observed to be situated ventrally between the tuberculum majus and the tuberculum minus. The collum humeri, situated in a ventral position relative to the caput humeri of the tuberculum majus bone, was identified. The tuberositas deltoidea, which descends ventrally from the border of the tuberculum majus located on the cranial aspect of the humerus, was observed to be relatively blunt. As the tuberositas deltoidea descended distally, the corpus became rounded and the final third of the corpus was flattened by lateral expansion, subsequently joining the trochlea humeri. The presence of articular faces, designated as the condylus medialis and condylus lateralis in the distal region of the extremity, and the trochlea humeri articulating with the ossa antebrachii in the area between these articular faces, was established. The presence of the foramen supratrochleare, which provides articulation between the fossa olecrani and the fossa radialis located distal to the extremities, was determined (Figure 2).

Ossa antebrachii

It was observed that the skeleton antebrachii was formed by the radius and ulna. The radius developed to a lesser extent than the ulna, exhibiting a broad proximal section that narrowed towards the corpus and terminated in a thickened distal section.

Radius

The radius, which exhibited a slight arching motion in a forward direction and extended to the cranio-lateral aspect of the ulna, was observed to establish contact with the ulna at both the proximal and distal regions. The caput radi exhibited two distinct fovea capitis radii, while the trochlea radii and proc. styloideus radii (medialis) were clearly discernible. The presence of the facies articularis carpeae was evident on the distal aspect (Figure 3).

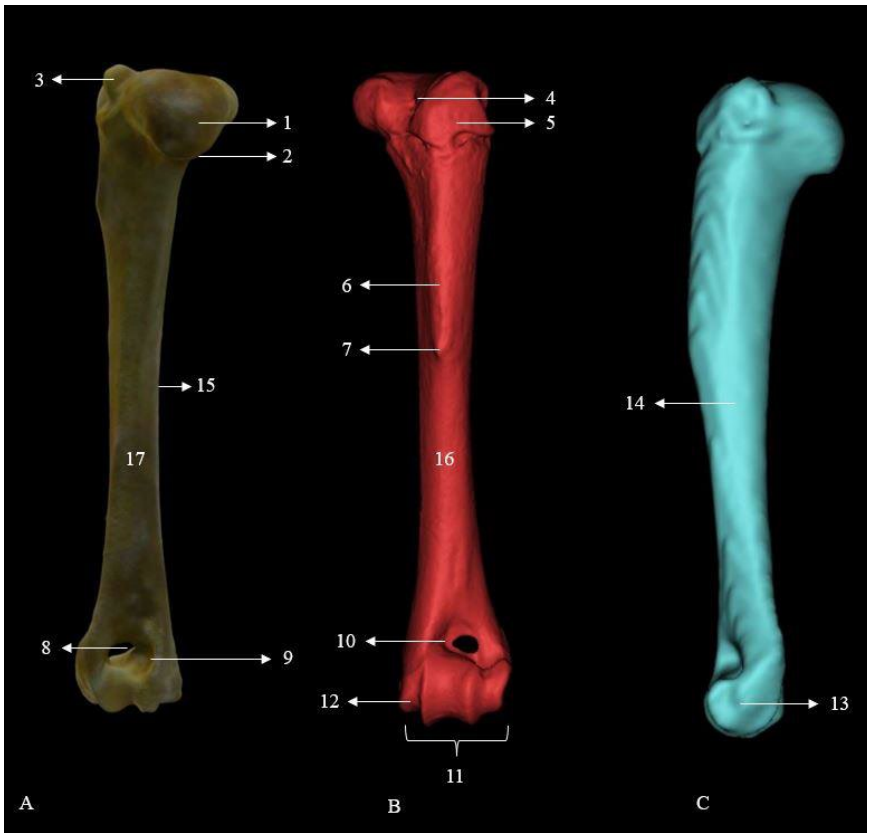

Figure 2. Reconstruction image and anatomical landmarks of the Humerus. A: 3D Scanner color scanned 3D model; 3D Scanner model .stl file format; C: CT 3D model. 1. Caput humeri, 2. Collum humeri, 3. Tuberculum majus, 4. Sulcus intertubercularis, 5. Tuberculum minus, 6. Crista humeri, 7. Tuberositas deltoidea, 8. Foramen supratrochleare, 9. Fossa olecrani, 10. Fossa radialis, 11. Trochlea humeri, 12. Epicondylus medialis, 13. Epicondylus lateralis, 14. Facies lateralis, 15. Facies medialis, 16. Facies cranialis, 17. Facies caudalis.

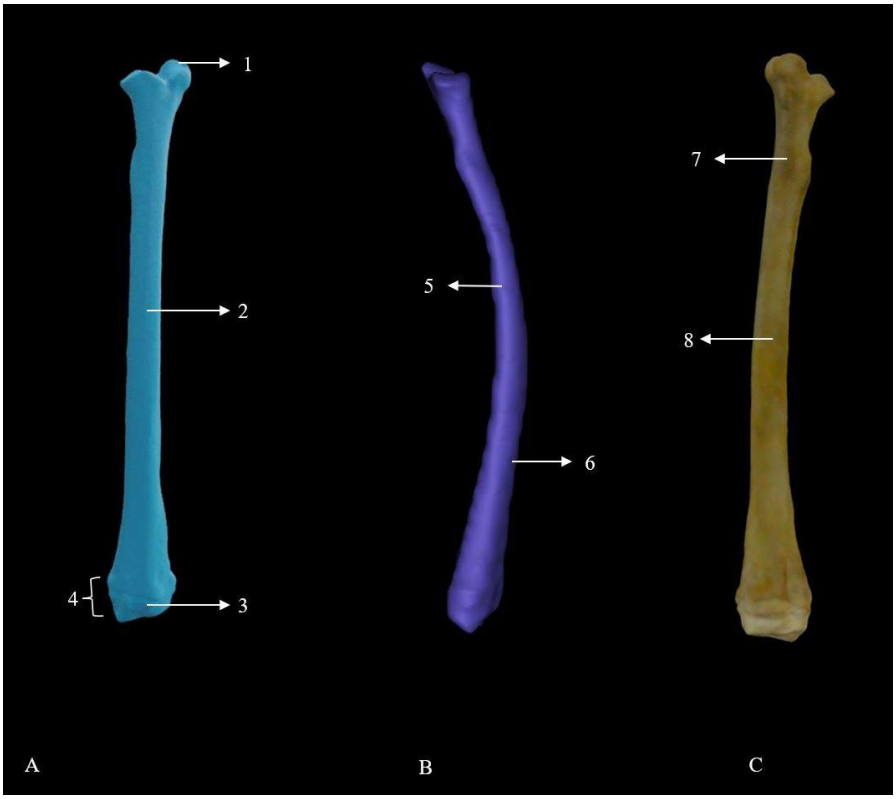

Figure 3. Reconstruction image and anatomical points of the radius. A: 3D Scanner model .stl file format; B: CT 3D model; C: 3D Scanner colour scanned 3D model. 1. Fovea capitis radii, 2. Corpus radii, 3. Proc. styloideus radii (medialis), 4. Trochlea radii, 5. Facies medialis 6. Facies cranialis, 7. Facies caudalis 8. Facies caudalis.

Ulna

In its proximal position, the bone runs caudal to the radius, while in its distal position, it is situated caudo-laterally to the radius. The proximal part of the bone is characterised by a prominent olecranon, which is terminated by three projecting tuber olecrani. The incisura trochlearis was observed to be concave, terminating proximally with the processus ancenous and distally with the processus coronoideus medialis and processus coronoideus lateralis. The incisura radialis, which separates the processus coronoideus medialis and processus coronoideus lateralis, was observed. Additionally, the corpus ulnae exhibited the presence of facies cranialis, facies medialis, and facies caudalis. Distally, the processus styloideus ulnae (lateralis) was also observed (Figure 4).

Femur

The caput femoris was observed in the proximal part of the femur, and the trochanter major was observed to be in a state of flexion, with its superior aspect extending beyond the level of the caput femoris. The fovea capitis femoris was clearly discernible on the caput ossis femoris. The femur exhibited three distinct protrusions: the trochanter major, the trochanter minor, and the trochanter tertius. A deep fossa trochanterica was observed between the caput femoris and the trochanter major, as well as a crista intertrochanterica between the trochanter major and the trochanter minor. The distal epicondyle medialis and the epicondyle lateralis were observed. The fossa intercondylaris and horizontal linea intercondylaris were observed between the two condylus. The trochlea ossis femoris was observed on the cranial aspect of the femur, allowing the patella to fit. The fossa extensoria was prominently located on the condylus lateralis. The tuberositas supracondylaris and facies poplitea were prominent (Figure 5).

Skeleton cruris

It consists of tibia and fibula. The proximal cross-sectional surface resembles a triangle. When viewed from the proximal side, it was observed that the surface was divided into two as condylus medialis and condylus lateralis and eminantia intercondylaris were located between the surfaces in the form of protrusions. Area intercondylaris centralis was present between the two eminantia intercondylaris. The presence of a tuberositas tibia was observed cranial to the bone. The tuberositas tibia was found to continue distally as crista tibia. Sulcus extentorius was found to be present. It was observed that the fibula was located caudo-lateral to the midpoint of the tibia. A wide spatium interosseum cruris was observed between the fibula and tibia. Linea m. poplitei was prominent. It was observed that the cochlea tibia located in the distal extremities was rectangular, parallel to the medial axis, and had prominent malleolus lateralis and malleolus medialis projections (Figure 6).

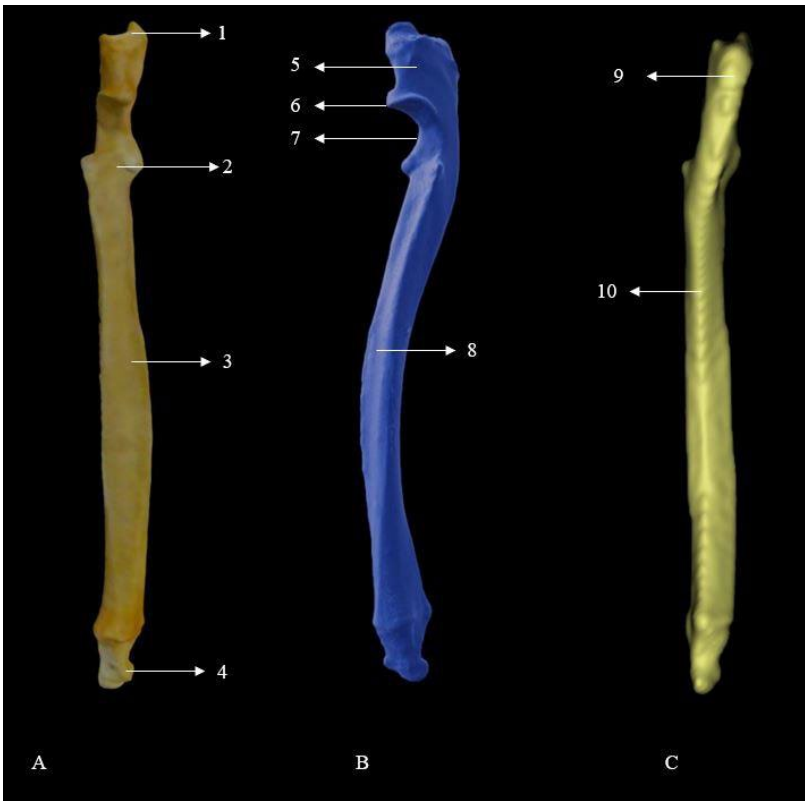

Figure 4. Reconstruction image and anatomical points of the ulna. 3D Scanner colour scanned 3D model; B: 3D Scanner model .stl file format; C: CT 3D model. 1. Tuber olecrani, 2. Proc. coronideus medialis ve lateralis, 3. Corpus ulnae, 4. Proc. styloideus ulnae (lateralis), 5. Olecranon, 6. Proc. anconeus, 7. Incisura trochlearis, 8. Margo lateralis, 9. Margo caudalis. 10. Facies caudalis.

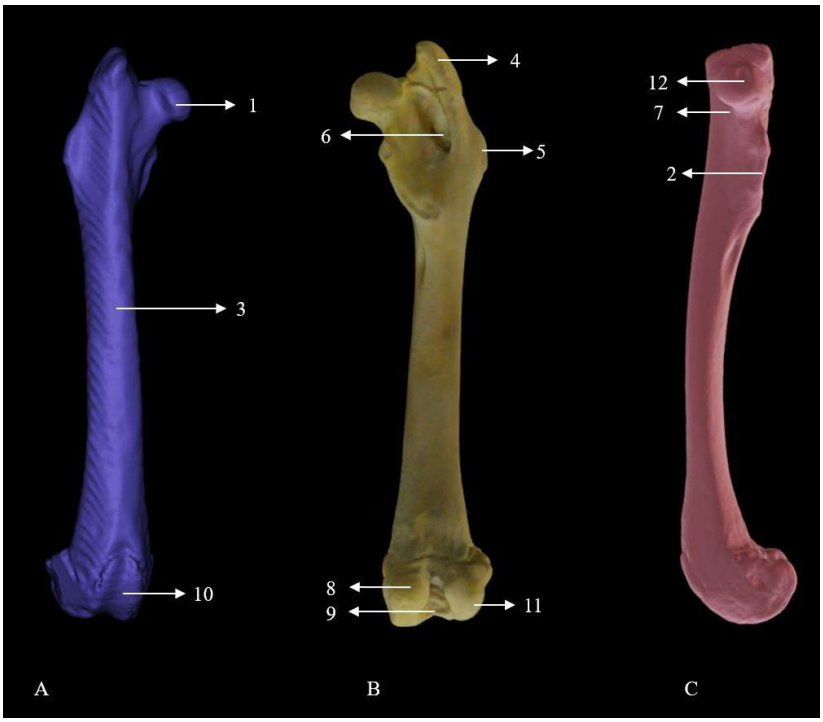

Figure 5. Reconstruction image and anatomical points of the femur.A: 3D Scanner colour scanned 3D model; B:3D Scanner model in .stl file format; C: CT 3D model. 1. Caput femoris, 2. Trochanter minus, 3. Corpus ossis femoris, 4. Trochanter major, 5. Trochanter minor, 6. Fossa intertrochanterica, 7. Collum femoris, 8. Condylus medialis, 9. Fossa intercondylaris, 10. Trochlea femoris, 11. Condylus lateralis, 12. Fovea capitis femoris.

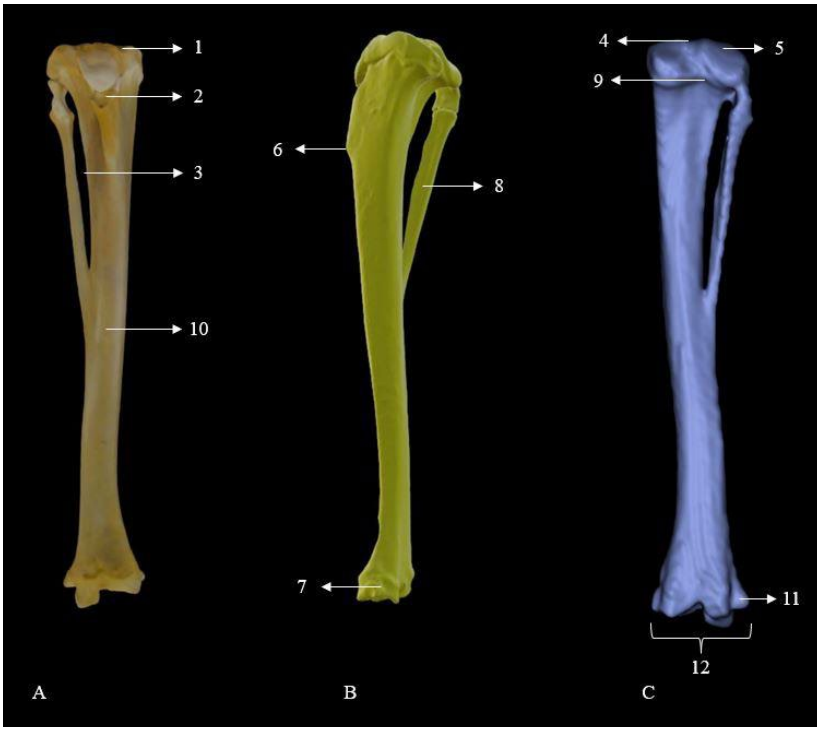

Figure 6. Reconstruction image and anatomical points of the skeleton cruris. A: 3D Scanner colour scanned 3D model; B: 3D Scanner model in .stl file format; C: CT 3D model. 1. Condylus medialis, 2. Tuberositas tibia, 3. Spatium interosseum cruris, 4. Eminentia intercondylaris, 5. Condylus lateralis, 6. Crista tibiae, 7. Malleolus medialis, 8. Fibula, 9. Inc. Poplitea, 10. Corpus tibiae, 11. Malleolus lateralis, 12. Cochlea tibiae.

DISCUSSION

3D models are used in many different fields, including the medical field, and their use in veterinary anatomy and anatomy education has become widespread in recent years. In the studies conducted, the models obtained using 3D scanners are physically printed by 3D printers and used as teaching materials and in areas such as plates and fixators in surgical procedures. Software can be used to take measurements and make adjustments on the models.

Tomography slices assist physicians in the diagnosis, detection and treatment of diseases. In recent years, 3D models have been created using tomography slices, providing us with more information about the scanned tissue, enabling us to take measurements and obtain preliminary information for surgical operations.

The scapula is the first bone connected to the body by muscles. In a study conducted on wild rabbits and domestic rabbits, it was found that the scapula is a flat bone consisting of lateral and medial surfaces and forms a joint with the cavitas glenoidalis, which is consistent with the findings of the study (Hussein Al-Ubaidy et al. 2020).

It has been reported that the scapula in rats (Salami et al. 2011) and rabbits (El-Ghazali and El-Behery 2018) is triangular in shape, while in marsupials, there are two types: triangular and quadrangular (Argot 2001). The models obtained show that the scapula is triangular in shape, which is consistent with the literature (Barone 1986). In a study conducted on wombats (Saber 2013), it was determined that the fossa infraspinata is wider than the fossa supraspinata in models obtained from both methods (El-Ghazali and El-Behery 2018). The spina scapulae, which starts between the two fossae and continues to rise to the level of the cavitas glenoidalis, ending at the acromion and continuing caudally as the metacromion, is consistent with the literature (Chin Jr 1957; El-Ghazali and El-Behery 2018; Doubell et al. 2020). The protrusion located on the lateral part of the scapula, called the metacromion, which continues caudally in a narrow and long shape, was clearly observed in the study. It has been reported that the muscles attached to this protrusion assist in the extension of the arm (Seckel and Janis 2008; El-Ghazali and El-Behery 2018). The cavitas glenoidalis was observed to be wide, developed in accordance with the surface of the caput humeri , and consistent with the literature (El-Ghazali and El-Behery 2018). The authors reported that the incisura glenoidalis was not detected in the study conducted on cats, but was present in the studies conducted on the African giant pouched rat (Cricetomys gambianus) and mole-rat (Özkan 2002a; Olude et al. 2010; Yılmaz et al. 2020a). In the present study, the incisura glenoidalis was clearly observed. In a study conducted on the wombat (Saber 2013), the cavitas glenodealis was reported to be oval in shape, while in studies conducted on the African giant rat (Cricetomys gambianus) (Olude et al. 2010) and squirrel (Kazeem et al. 2020), the processus coroideus was reported to be hook-shaped. Similar findings were observed in the models obtained in the present study.

Studies conducted on rabbits, guinea pigs, mongooses (Shunmugam and Sundaram 2022), Van cats (Yılmaz et al. 2020b), agoutis (Sundaram et al. 2015), and wild cats (Palanisamy et al. 2020) reported the presence of a distinct caput humeri, tuberculum majus, and tuberculum minus were reported by the authors. Shunmugam and Sundaram (2022) reported the presence of the caput humeri, tuberculum majus, tuberculum minus, and sulcus intertubercularis on the humerus in rabbits, guinea pigs, and Egyptian mice in their comparative study. Chiarello et al. (2021) reported the presence of the greater tubercle and lesser tubercle in opossums, along with a wide sulcus intertubercularis separating them. In wombats, the greater tubercle is divided into the cranial and caudal parts, and the presence of a very prominent deltoid tuberosity has been reported (Saber 2013). In the 3D models obtained, the tuberculum majus slightly exceeded the caput humeri, was separated from the tuberculum minus by the sulcus intertubercularis, and a less prominent tuberositas deltoidea was observed. In studies conducted on different species such as guinea pig (Witkowska et al. 2014, Shunmugam and Sundaram 2022), wombat (Saber 2013), African giant rat (Olude et al. 2010), Coypu (Şeicaru 2019), porcupine (Hystrix Cristata) (Yılmaz et al. 1998), mongoose (Shunmugam and Sundaram 2022) in the distal part of the humerus, it has been reported that the trochlea humeri consists of the lateral epicondyle and the medial epicondyle, and that the foramen supratrochleare, which connects the fossa radialis and the fossa olecrani, is located above these anatomical structures. In the present study, it was determined that the trochlea humeri consists of the condylus lateralis and condylus medialis, and that the supratrochlear foramen is located above these condyles and connects the fossa olecrani and the fossa radialis.

It was determined that the forearm consists of two bones, the radius and ulna, and that the ulna is more developed than the radius. It has been reported that the radius is concave towards the cranium and that the fovea capitis radii is located in its proximal section for the humerus joint surface to fit (El-Ghazali and El-Behery 2018, Akgün et al. 2021). The styloid process of the radius located at the distal end of the bone is consistent with the literature (Chin Jr 1957). The olecranon, which is clearly observed in studies conducted on wombats, cats, rats, and giant rats, is located in the proximal section of the ulna, which is more developed than the radius. The tuber olecrani, which ends proximal to the olecranon, and the proc. ancenous, and the inc. trochlearis, where the ulna articulates with the humerus, are reported by the authors (Saber 2013). In the three-dimensional models obtained in the study, the tuber olecrani was found to

have three protrusions, and the olecranon, incisura trochlearis, and processus ancenous were clearly observed in both methods (Yılmaz et al. 1998, El-Ghazali and El-Behery 2018, Yılmaz et al. 2020b). It was determined that the ulna terminates distally with the of the processus styloideus ulnae (Van Staden 2014).

Bakici et al. (2021) reported in their study on rabbits using micro-CT sections that the trochanter majör exceeded the level of the femoral head, the trochanter minor and the trochanter tertius were at the same level, and there was a deep fossa trochanterica (Bakıcı et al 2021). Using magnetic resonance imaging, it was reported that the rabbit femur has a caput femoris, trochanter major, trochanter minor, trochanter minor, and a deep fossa trochanterica (Wang et al 2009). In mole-rat, the authors reported the presence of the trochanter major, trochanter minor, trochanter tertius, and a deep fossa trochanterica, along with the crista intertrochenterica, and additionally, the presence of the fovea capitis in mole-rat (Özkan 2002b; Özkan 2002c). The absence of the trochanter tertius in chinchillas and the fovea capitis in African giant rats has been reported (Çevik-Demirkan et al. 2007; Olude et al. 2023). In studies conducted on different rodent species (de Araújo et al. 2013) and the African giant rat (Olude et al. 2023), as well as the chinchilla (Çevik-Demirkan et al 2007), it was reported that the distal part of the femur is divided into two parts, the lateral and medial condyles, with the intercondylar fossa between them, and that the lateral epicondyle and medial epicondyle were observed for muscle attachment, which is consistent with the findings of the present study. A distinct extensor fossa was identified on the trochanteric fossa and lateral condyle in the cranial part of the distal region.

The ossa cruris, consisting of the tibia and fibula, assist in stabilising the foot and, depending on the fusion status of the fibula, are thought to contribute to ankle movement. (Polly and Hall, 2007). In studies conducted on cats and chinchillas, the authors reported that the proximal section of the tibia is triangular and that the fibula develops separately (Çevik-Demirkan et al. 2007; El-Ghazali and El-Behery 2018). In burrowing species, the tibia and fibula fuse, and this is thought to contribute to bone strength, preventing bending during digging (Montoya-Sanhueza et al. 2022). Studies on rats (Salami et al. 2011), African mole-rats (Sahd et al 2019), and hedgehogs (Girgiri et al. 2016) have reported that the fibula fuses with the tibia. It was found that the tibia takes on a rectangular shape distally and that the cochlea tibia is in a sagittal position and consists of two parts, which is consistent with the literature (El-Ghazali and El-Behery 2018).

3D scanners have advantages in areas where tomography and MR scanners cannot be used because they are portable, inexpensive, and obtain data in color from the scanned surface (Wilhite and Wölfel 2019, da Silveira et al 2021). It has disadvantages compared to medical imaging devices due to modeling time and scanning only the surface (Wilhite and Wölfel 2019).

CONCLUSION

In this study, 3D reconstruction of some selected bones was performed using CT and 3D scanner. Both 3D scanner and CT results were observed, showing that different methods can be used in the field of veterinary anatomy. In addition to the advantages of the 3D scanner device, such as low sensitivity and high resolution, the fact that it can produce models in color, that it produces models quickly and efficiently, and that the 3D surface models and morphometric measurements obtained are closely related to tomography, have proven that 3D scanners can be used in the field of veterinary anatomy. We believe that the models obtained from 3D scanners will make important contributions to the teaching of veterinary anatomy by creating virtual models in color and printing them with 3D printers. Traditional textbooks may not be sufficient to understand the relationship between anatomical structures because they contain 2-dimensional images. Cadavers are time-consuming, limited in number, and not always available due to ethical concerns. 3D models fill these gaps. In this study, 3D scanning may be preferred because it is less expensive than tomography, is portable, can scan in free mode, has a lower margin of error, is easy to use, has no access limitations compared to medical imaging modalities such as CT and MRI, allows modeling of soft tissues, produces high-resolution 3D models, has no harmful side effects, and can scan in color. As a result of the study, we believe that the two methods should be integrated in use.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: AK and MOD contributed to the project idea, design and execution of the study. AK, MOD contributed to the acquisition of data. AK analysed the data. AK and MOD drafted and wrote the manuscript. AK, MOD and MK reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: Study permissions were obtained from Selçuk University Animal Experiments Local Ethics Committee (Decision no: 2020-57 (Appendix 1)).

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Explanation: *This article is summarized from the PhD thesis.

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RESEARCH ARTICLE

The Functional, Nutritional and Biological Properties of Ice Cream Produced Using Kefir Culture Inoculation

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ABSTRACT

In this study, changes in the physicochemical, microbiological and technological values of ice cream produced from ice cream mixes ripened with kefir culture inoculation at three different rates (0.5%, 1% and 1.5%) were investigated. Adding kefir culture to the mix decreased its firmness, cohesiveness, and viscosity index values and increased its consistency value. Furthermore, the pH, aw, % dry matter, first drop, complete melting, volume increase and all TPA (texture profile analysis) values were lower in ice creams produced using these mixes in comparison to the control sample. Furthermore, these changes increased in parallel with the amount of culture added. The ice cream produced using mixes ripened with 1.5% kefir culture inoculation yielded the lowest pH (5.74), aw (0.710), % dry matter (11.35), first drop (15.25 min.), complete melting (71.28 min.) and overrun (43.67%) values, as well as the lowest hardness (1759.32 N), springiness (0.90), cohesiveness (0.23), gumminess (742.42 N), chewiness (374.73 N) and resilience (0.698) results.
Keywords: Ice Cream, Fermentation, Kefir, Starter Culture

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Kefir Kültürü İnokulasyonu ile Üretilen Dondurmanın Fonksiyonel, Besinsel ve Biyolojik Özellikleri

ÖZ

Bu araştırmada üç farklı oranda (% 0.5, % 1 ve % 1.5) kefir kültürü inokülasyonu ile olgunlaştırılan dondurma mikslerinden üretilen dondurmaların fizikokimyasal, mikrobiyolojik ve teknolojik değerlerinde meydana gelen değişimler incelenmiştir. Dondurma miksine kefir kültürü ilavesi miksin sertlik, yapışıklık ve viskozite indeksi değerlerinin azalmasına kıvam değerinin ise artmasına neden olmuştur. Ayrıca bu mikslerden üretilen dondurmalarda kontrol örneğine kıyasla pH, aw, % kuru madde, ilk damlama, tamamen erime, overrun ve tüm TPA(tekstür profil analizi) değerleri de azalış göstermiştir. Ek olarak tespit edilen bu değişimler, ilave edilen kültür miktarına paralel şekilde artış göstermiştir. Örnekler arasında en düşük pH (5.74), aw (0.710), % kuru madde (11.35), ilk damlama (15.25 dk.), tamamen erime (71.28 dk.), overrun (43.67 %) değerleri en düşük örnek % 1.5 kefir kültürü inokülasyonu ile olgunlaştırılan mikslerden üretilen dondurma örnekleri olmuştur. Benzer şekilde en düşük sertlik (1759.32 N), esneklik (0.90), yapışıklık (0.23), yapışkanlık (742.42 N), çiğnenebilirlik (374.73 N) ve dayanıklılık (0.698) değerleri de aynı örneklerde tespit edilmiştir.
Anahtar Kelimeler: Dondurma, Fermentasyon, Kefir, Starter Kültür

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INTRODUCTION

Ice cream, thanks to its unique flavor, cooling effect, and nutritional value, is a popular dairy product consumed by people from all age groups around the world (Göktaş et al., 2022). Ice cream mixes mainly consist of milk, emulsifiers, vegetable oils, stabilizers, flavorings, and fruits. Artificial sweeteners, food dyes, pulp, dietary fibers, and probiotic bacteria are also added in some mixes (Marshall et al., 2003). The increasing number of consumers demanding natural, nutritious, and functional foods has encouraged producers to develop new ice cream products (Cruz et al., 2009).

Fermented dairy products have high nutritional value; they constitute a significant portion of fermented products and are preferred worldwide for their traditional and/or universal characteristics (Petrova et al., 2021). Fermented dairy products are categorized by considering production methods, animal species, and microbiological, physicochemical, and/or production technologies. Examples of these products include cheeses, fermented beverages, yoghurt, kefir, buttermilk, sour cream, and acidophilus milk (Shiby and Mishra, 2013).

Ice cream can be modified into a fermented product by introducing lactic acid bacteria and yeasts. This process enhances and changes its sensory, microbiological, and textural characteristics and increases its functional and nutritional value (Akarca et al., 2024).

Kefir is a fermented milk product produced through fermentation by lactic acid bacteria and yeasts (Gao and Li, 2016). Today, kefir can be produced in two ways: using kefir grains or a starter culture combination. Its aromatic, microbiological, and chemical characteristics are formed through the interaction of various bacteria and yeasts present in kefir grains or the combination of cultures (Farnworth, 2008).

This study aims to investigate alterations in the physicochemical, microbiological, and technological characteristics of samples produced using ice cream mixes ripened with kefir culture inoculation at three different rates.

MATERIALS and METHODS

Materials

The raw cow's milk was procured from a local producer in the Afyonkarahisar province. Salep, cream, and granulated sugar (beet sugar) were obtained from a local market in the same province.

Cultures

The Vivo Activ LLC 07400 (Ukraine) kefir cultures containing *Lactococcus lactis subsp. diacetylactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Leuconostoc mesenteroides subsp. cremoris,* and *Streptococcus thermophilus bacteria* and *Debaromyces hansenii* and *Kluyveromyces marxianus subsp. Marxianus* yeasts were used in this study. The culture contains 10 log CFU/g of living microorganisms.

Ice Cream Production

The ice cream production method used in this study (see Fig. 1) was modified from the method described by Akarca et al. (2024). Following production of the ice cream mixes, the kefir culture mixture in three different ratios (0.5%, 1% and 1.5%) was added to the mix, which was then ripened. The mixes were then processed into ice cream at -5 °C using a freezing machine (CRM-GEL 25C, Italy). Then, 250 g from each mix was placed in sterile glass containers and hardened at -24 °C. The resulting samples were kept at -18°C until analysis was completed.

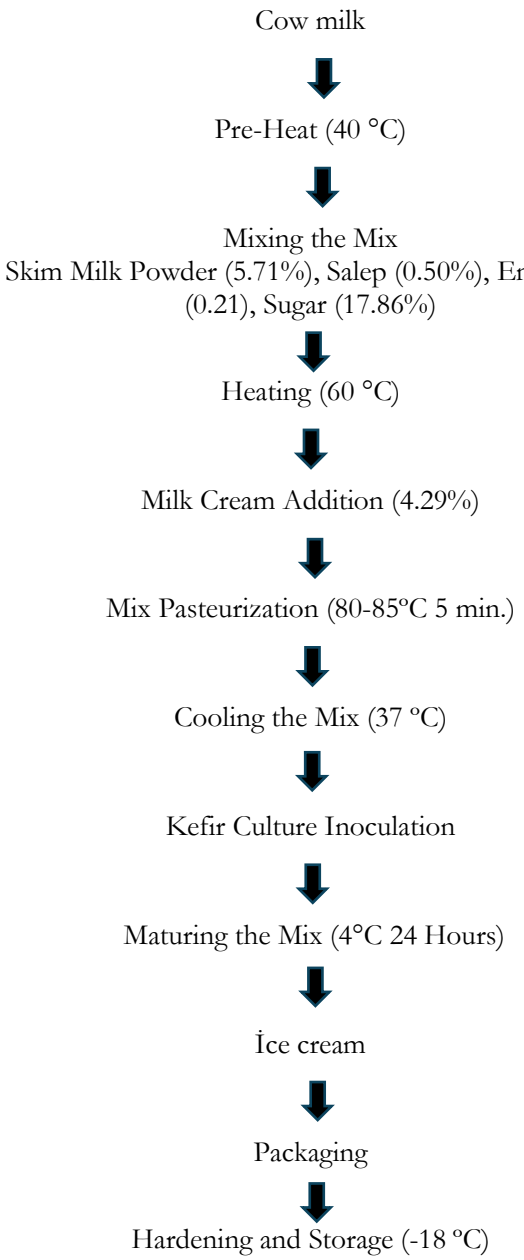

Figure 1: Ice Cream Production

Texture analysis of ice cream mix
Texture values (consistency, cohesiveness, viscosity index, and firmness) were measured utilizing a TA.XT Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) with back extrusion rig hardware (Sert et al., 2017).

The first drip time
Ten grams of ice cream sample were put into a stainless-steel wire strainer over glass containers that had been weighed on a precision balance (Shimadzu AUX 220, Japan), and were left to melt at 20 °C. A stopwatch was then started, and the moment the ice cream began to melt and the first drops fell was recorded (Akarca et al., 2024).

Time to complete melting
Ice cream samples that had been hardened in a deep freezer (Uğur UDD560 BK, Turkey) at -18 °C were taken out and allowed to melt at 20 °C on a stainless-steel wire grid with 0.2 cm wires on a 500 ml glass beaker. The stopwatch was then started. The samples were then left to completely melt. The time (in minutes) taken for complete melting was recorded to determine the melting time (Güven and Karaca, 2002).

Overrun
The samples were first placed into a 500 ml tared glass measuring cylinder. The same samples were then placed into a 500 ml beaker and melted in a water bath. The resulting mixture was then transferred to a measuring cylinder of the same volume and weighed again (Jiménez-Florez et al., 1993).

pH value
The samples were first mixed with one-tenth of sterilized pure water, and then homogenized utilizing a homogenizer (Daihan Wisestir HS-30T, South Korea). The pH values were then measured using a pH meter (Hanna HI 2215 pH/ORP) (Akarca et al., 2024).

Water activity (aw)
These values were measure using a water activity analyzer (Novasina LabTouch-aw, Lachen, Switzerland) (AOAC, 2016a).

Dry Matter Analysis (%)
The % dry matter values of the ice cream samples were determined using an oven (Nüve, Turkey), according to the AOAC (2016b) method, and calculated using the following formula:
% dry matter = (m1-m2)/(m1-m0) x 100
m1: Sample container + weighed sample weight
m2: Weighed container + weighed sample weight after analysis
m0: Initially weighed sample weight

Color values (*L*, a*, b)**
The color values of the samples were measured by utilizing a colorimeter (Minolta Co., Osaka, Japan) by making use of the Hunter color measurement system (Akarca et al., 2024).

Texture analysis of ice cream
The samples were cut into 22±0.5 mm diameter and 20±0.5 mm length cylinders at -18 °C. TPA values were determined at room temperature using a texture analyzer (TA-XT2i; Stable Micro Systems, Surrey, UK) with a 30 kg load cell. Measurements were performed utilizing a spherical probe (1" Spherical Probe, Part Code: P/1S, batch no. 13155, Stable Micro Systems Ltd., Godalming, UK). The pre-test, test and post-test speeds were set at 1, 5 and 5 mm/s, respectively. The samples were compressed to 40 % of their original height, with an interval of 5 s between each compression. To determine the TPA profile of each sample, the measurements were performed three times and the values obtained were averaged (Isleroglu et al., 2015).

Lactic acid bacteria (LAB) count
De Man Rogosa Sharpe (MRS) agar (Merck, 110660, Germany) was used when counting lactic acid bacteria introduced to the mixes. Serial dilutions of samples were prepared before analysis by utilizing 0.1% buffered peptone water (Merck, 107228, Germany), and analyses were performed by following the spread plate method. The inoculated Petri dishes were incubated under anaerobic conditions in jars (Merck, 116387, Germany), supplemented with Anaerocult® A (Merck, 113829, Germany), at 37 °C for 72 hours in an oven (Shori et al., 2022).

Total number of yeasts
The number of yeasts added to the ice cream mixes was determined using potato dextrose agar (Merck, Germany). Prior to microbiological analysis, serial dilutions were prepared utilizing 0.1% buffered peptone water (Merck, 107228, Germany), and analyses were performed by following the smear plate method. The inoculated Petri dishes were incubated under aerobic conditions at 25 °C for 5-7 days in an oven (Campos and Cristianini, 2007).

Statistical Analyses
ANOVA and Duncan's test ($p<0.05$) were performed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA) to determine the significance of differences in results obtained in this study. In addition, interactions between sample variation and results were determined by correlation and variation analysis.

RESULTS

The textural characteristics of the samples are presented in Table 1. The samples with the lowest and highest firmness and consistency values were found in the mixes fermented with kefir culture at 1.0 % (20.02

g and 294.43 g sec, respectively) and 1.5 % (14.69 g and 293.66 g sec, respectively).

Table 1. Textural Analysis Results of Ice Cream Mixes

Sample	Firmness (g)	Consitency (g sec)	Cohesiveness (g)	Index of Viscosity (g sec)
Control	15,03±0.42^{c}	283,28±1.80^{a}	-7,56±0.48^{a}	-2,65±0.16^{a}
0,5% C	17,18±0.68^{b}	276,99±2.30^{a}	-7,16±0.21^{a}	-8,21±0.85^{b}
1.0% C	20,02±0.21^{a}	294,43±12.28^{a}	-10,07±0.27^{b}	-12,81±0.56^{c}
1.5% C	14,69±0.47^{c}	293,66±2.12^{a}	-10,75±0.55^{b}	-14,38±1.93^{c}
P Value	0.001	0.130	0.0082	0.001
R	0.096	0.632	-0.885**	-0.961**

a - c (↓): Values shown with the same lower case letters in the same column for each analysis are significantly different (P<0.05). p <0.0001: Statistically very significant, p < 0.01: Statistically very significant, p < 0.05: Statistically significant, p >0.05: Not statistically significant, ns: Not statistically significant.

The cohesiveness and viscosity index values decreased in line with the kefir culture addition rate. The lowest values were found in the samples with 1.5% kefir culture, with sec values of -10.75 g and -14.38 g, respectively.

The physical analysis results are shown in Table 2. The lowest and highest first drip times were found in samples containing 1.5 % kefir culture (15.25 min.) and in the control sample (18.88 min.). Similarly, the lowest and highest complete melting times were found in the same samples (71.28 min. and 85.54 min.).

The sample with the lowest pH (5.74) was the one to which 1.5 % kefir culture was added, whereas the sample with the highest pH (6.46) was the control sample. The lowest and highest overrun values were found in the 1.5% kefir culture (43.67 %) and control (50.56%) samples.

Table 2. Physical Analysis Results of Ice Cream Samples

Sample	First Droplet (min.)	complete melting time (Min.)	Overrun
Control	18,88±0.37^{a}	85,54±1.24^{a}	50,56±2.30^{a}
0,5% C	17,97±0.67^{ab}	74,28±1.23^{b}	49,03±1.40^{ab}
1.0% C	16,88±0.45^{b}	73,62±0.28^{bc}	45,69±1.57^{ab}
1.5% C	15,25±0.35^{c}	71,28±0.87^{c}	43,67±2.19^{b}
P Value	0.006	<0.0001	0.069
R	-0.961**	-0.873**	0.244

a - c (↓): Values shown with the same lower case letters in the same column for each analysis are significantly different (P<0.05). p <0.0001: Statistically very significant, p < 0.01: Statistically very significant, p < 0.05: Statistically significant, p >0.05: Not statistically significant, ns: Not statistically significant,

The water activity and % dry matter values varied between 0.756 and 0.710, and between 32.22% and 31.35%, respectively, depending on the culture addition rate (p<0.05). The highest water activity and % dry matter values were found in the control sample, and the lowest ones were found in the sample to which 1.5% kefir culture was added (p<0.05).

Table 3. Physicochemical and Microbiological Analysis Results of Ice Cream Samples

Sample	pH	a_w	% Dry matter	LAB (log cfu/g)	yeast count (log cfu/g)
Control	6,46±0.03^{a}	0,756±0.01^{a}	32,22±0.06^{a}	1,87±0.11^{d}	2,27±0.05^{d}
0,5% C	6,19±0.02^{b}	0,735±0.01^{b}	32,05±0.18^{ab}	4,21±0.03^{c}	8,15±0.10^{c}
1.0% C	5,90±0.01^{c}	0,717±0.01^{c}	32,06±0.24^{ab}	5,29±0.01^{b}	8,44±0.01^{b}
1.5% C	5,74±0.02^{d}	0,710±0.01^{d}	31,35±0.46^{b}	5,80±0.01^{a}	8,61±0.01^{a}
P Value	<0.0001	<0.0001	0.109	<0.0001	<0.0001
r	-0.991**	-0.976**	-0.748*	0.951**	0.820*

a - c (↓): Values shown with the same lower case letters in the same column for each analysis are significantly different (P<0.05). p <0.0001: Statistically very significant, p < 0.01: Statistically very significant, p < 0.05: Statistically significant, p >0.05: Not statistically significant, ns: Not statistically significant,

The microorganisms in the culture mixture added to the ice cream mix metabolised the hexose sugars during fermentation, resulting in decreased aw and % dry matter values.

Adding kefir culture to ice cream samples increased the number of lactic acid bacteria and yeasts. This increase depended on the amount of culture added (Table 3; p<0.05). Before ripening, the mixture was cooled to 37 °C, inoculated with kefir culture, and then left to ripen at 4 °C for 24 hours. The number of microorganisms continued to increase until the temperature of the mixture dropped below the minimum limit for the growth of lactic acid bacteria and yeasts. However, some of these microorganisms were affected by the very low temperature and could not survive the ice cream production phase.

Adding kefir culture at different ratios increased the L* and b* values of the samples while decreasing the a* values (Fig. 2-4; p<0.05). The highest L* and b* values (89.14 and 5.93, respectively) were found in the sample to which 1.5% kefir culture was added, while the highest a* value (2.98) was found in the control.

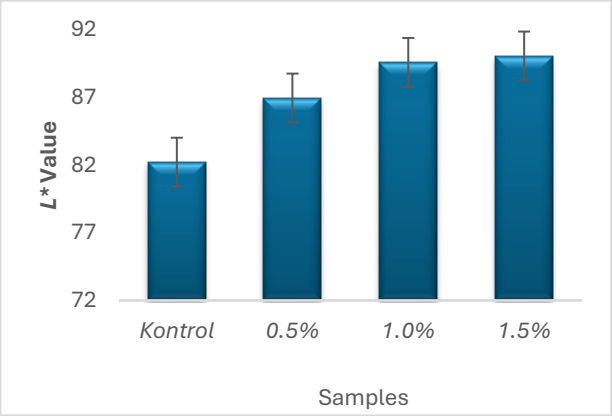

Figure 2. L* Values of Ice Cream Samples

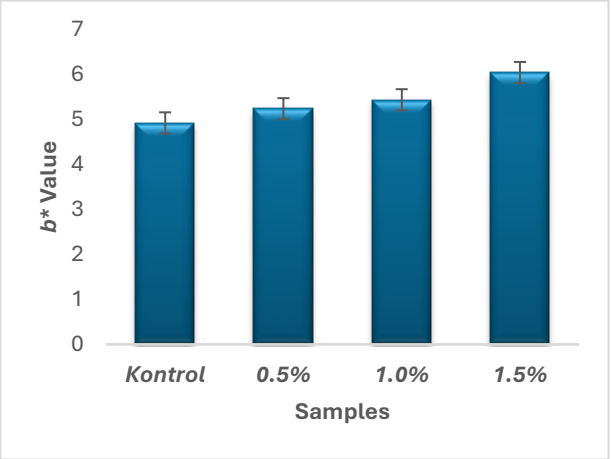

Figure 4. b* Values of Ice Cream Sample

The hardness, gumminess, chewiness and resilience interactions had a negative effect on sample diversity, while the adhesiveness interaction showed a positive correlation. Adding kefir culture decreased all TPA values (except springiness, p<0.05).

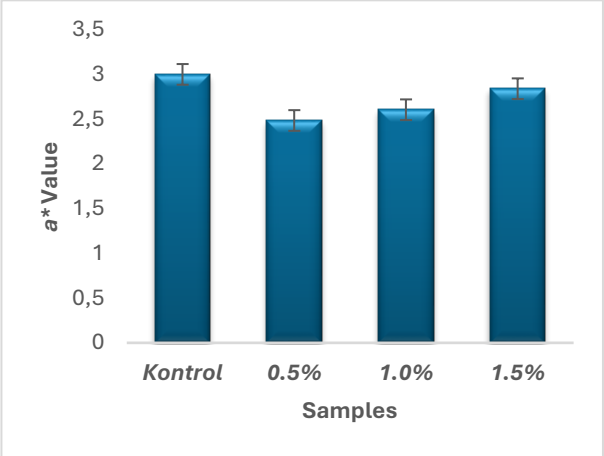

Figure 3. a* Values of Ice Cream Samples

This effect increased in line with the amount of culture added (see Table 4). The lowest values for chewiness, gumminess, adhesiveness, and hardness of the samples were determined to be 1759.32 N, -2.01 N, 742.42 N and 374.73 N, respectively, in samples to which 1.5 % kefir culture was added.

Table 4. Textural Analysis Results of Ice Cream Samples

Sample	Hardness (N)	Adhesiveness (g sec)	Springiness	Cohesiveness
Control	5386,35±10.15^{a}	-6,04±0.05^{c}	0,94±0.05^{a}	0,94±0.01^{a}
0,5% C	3175,51±330.82^{b}	-2,66±0.16^{b}	0,95±0.01^{a}	0,26±0.01^{b}
1.0% C	2459,03±30.70^{c}	-2,11±0.07^{a}	0,93±0.01^{a}	0,25±0.01^{bc}
1.5% C	1759,32±213.85^{d}	-2,01±0.01^{a}	0,90±0.01^{a}	0,23±0.01^{c}
P Value	<0.0001	<0.0001	0.519	<0.0001
r	-0.948**	0.853**	-0.535	-0.803*

a - d (↓): Values shown with the same lower case letters in the same column for each analysis are significantly different (P<0.05). p <0.0001: Statistically very significant, p < 0.01: Statistically very significant, p < 0.05: Statistically significant, p >0.05: Not statistically significant, ns: Not statistically significant,

Table 4. Textural Analysis Results of Ice Cream Samples (Continued).

Sample	Gumminess (N)	Chewiness (N)	Resilience
Control	815,27±11.10^{a}	736,04±10.90^{a}	0,959±0.02^{a}
0,5% C	785,95±6.52^{b}	626,54±8.76^{b}	0,871±0.02^{b}
1.0% C	760,45±8.93^{c}	472,66±10.26^{c}	0,741±0.01^{c}
1.5% C	742,42±6.07^{c}	374,73±9.03^{d}	0,698±0.01^{c}
P Value	0.004	<0.0001	<0.0001
r	-0.972**	-0.995**	-0.978**

a - d (↓): Values shown with the same lower case letters in the same column for each analysis are significantly different (P<0.05). p <0.0001: Statistically very significant, p < 0.01: Statistically very significant, p < 0.05: Statistically significant, p >0.05: Not statistically significant, ns: Not statistically significant,

DISCUSSION

The firmness and viscosity index values were found to be highly significant ($p<0.001$) in relation to sample variety. Additionally, the cohesiveness and viscosity index values exhibited a strong negative correlation with the sample variety interaction.

The addition of kefir culture to the mix during fermentation affected all the samples' viscosity values ($p<0.05$). Firmness and consistency values increased with the addition of kefir culture up to 1% addition level but decreased with the addition of more than 1% kefir culture ($p<0.05$). Akarca et al. (2024) stated that adding different lactic acid bacteria to ice cream mixes increases firmness and consistency values, similar to the results achieved in this study. Carbohydrates such as sucrose and lactose in the mix have a positive effect on its water-holding capacity. However, since the added lactic acid bacteria metabolized some of the carbohydrates in the mix, the water holding capacity decreased, as did the firmness and consistency values. In parallel with these results, Akarca et al. (2024) stated that lactic acid bacteria addition to mix samples decreased cohesiveness and viscosity index values.

Carbohydrates such as honey, sugar etc. have an increasing effect on stickiness and viscosity (Ozdemir et al., 2008). Yeast and lactic acid bacteria in the added kefir culture fermented the sugars in the mix and converted them into compounds such as organic acids with less density, causing a decrease in these two values of the mix. In parallel with the results achieved in this study, Alamprese et al. (2005) and Zhang et al. (2017) stated that *Lactobacillus plantarum* GG strains added to ice cream altered all textural properties of products. The complete melting time was strongly influenced by sample diversity ($p<0.0001$). Additionally, the interactions between the first drip time and complete melting time showed a very negative correlative effect on sample diversity. The first drip and complete melting times decreased with the addition of kefir culture ($p<0.05$), with the extent of the decrease depending on the amount of culture added. Consistent with these results, Akarca et al. (2024) found that adding lactic acid bacteria to mixes decreased the first drip and complete melting times. The melting rate is influenced by many factors, such as the mix composition, consistency coefficient, the amount of air included in the mix, and the structure of ice crystals, and the network of fat globules established during ice cream production.

Sugar and lactose addition increases the melting resistance due to their water-retention capacity and micro-viscosity-increasing properties (Bahramparvar and Mazaheri Tehrani, 2011; Muse and Hartel, 2004).

The addition of kefir culture containing yeast and lactic acid bacteria to the mix results in the metabolism of the carbohydrates in the mix. This increases the hydrolysis of milk proteins and the organic acid formation, such as lactic acid, decreasing the melting resistance.

Overrun values decreased in samples produced by adding kefir culture, with this decrease occurring in parallel with the amount of culture added ($P < 0.05$). Similar results were reported by Sarwar et al. (2021), Göktaş et al. (2022) and Akarca et al. (2024). The microorganisms (yeast and lactic acid bacteria) in the culture metabolized the sugars in the environment, yielding a decrease in viscosity and consequently in the overrun amounts.

The interactions between pH, a_w, lactic acid bacteria and yeast counts significantly affected sample diversity ($p<0.0001$). Similarly, the interactions between pH, a_w, and % dry matter had a negative effect on sample diversity, while the counts of lactic acid bacteria and yeast had a positive correlative effect (see Table 3). Adding kefir culture decreased the pH values, with a decrease in pH in parallel with the amount of culture added ($p<0.05$). The lower pH values in comparison to the control sample are due to the organic acids formed when the yeast and lactic acid bacteria in the added culture metabolize the hexose sugars in the mixture. Consistent with these findings, Zhang et al. (2014), Sarwar et al. (2021), and Göktaş et al. (2022) also reported that adding probiotic bacteria decreased the samples' pH values. Consistent with our research findings, Akarca et al. (2024) reported that the water activity (aw) values of ice cream samples decreased with the addition of lactic acid bacteria. Kılıç and Şevik (2021) reported L* values of 91.11, a* values of 1.39 and b* values of 8.07 for ice cream samples, which is consistent with the findings achieved in this study. The cohesiveness, resilience, chewiness, adhesiveness, and hardness had a significant effect on sample diversity ($p<0.0001$).

CONCLUSION

This study examined the alterations in the microbiological, physicochemical, and technological characteristics of ice creams produced using a mix ripened with kefir culture added at three different ratios (0.5%, 1% and 1.5%). Adding kefir culture to the mix decreased the firmness, cohesiveness, and viscosity index values, while increasing the consistency value. Additionally, the pH value, the time taken for the first drip, the time taken for complete melting, the overrun and all TPA values were lower in ice creams produced using these mixtures than in the control sample. Furthermore, these changes increased in parallel with the amount of culture added.

Ice cream is a popular dairy product consumed widely all over the world. It is also recognized as an important food thanks to its high nutritional value. The production of the mix after fermentation with kefir culture inoculation has also increased the functional and nutritional properties of the resulting products.

As well as being consumed as food, the fact that this valuable product is suitable for functional use reveals its potential for the natural treatment of intestinal and digestive diseases, especially food poisoning. Furthermore, consumer expectations were met by producing a functional and specialized food by enhancing the value of ice cream, which is already a valuable foodstuff.

Conflict of Interest: The authors have no conflicts of interest to report.

Authors' Contributions: MK contributed to the project idea, design and execution of the study, acquisition of data, analysed the data, drafted and wrote the manuscript, reviewed the manuscript critically.

Ethical Approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

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RESEARCH ARTICLE

Bibliometric Analysis of Postgraduate Theses on Eye and Their Diseases in Animals in Turkey

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ABSTRACT

The study aims to evaluate postgraduate theses on the eyes and diseases of different types of animals in our country, which have been conducted on an academic platform, using bibliometric analysis methods, and to contribute to new studies to be conducted in the future. The study employed the following tools and methods: searches were conducted on the official website of the National Thesis Center affiliated with the Higher Education Institution, in the postgraduate thesis section, by entering different combinations of keywords or phrases related to the research topic into the search box. Among the 4.886 postgraduate theses found using the keyword "Eye Diseases" in the search, 22 were found to be on eye diseases in animals. Of the findings obtained from the identified theses, 68. 2% were master's theses, 31.2% were doctoral theses, and 40.91% of the thesis studies were experimental in nature. When looking at the distribution of theses on eye diseases over the years, it was observed that there was an upward trend, partly due to the lack of sufficient research in the field. In conclusion, considering that the number of postgraduate studies on eye diseases is insufficient, an increase in the number of studies will enable the development of new treatment strategies for the prevention of diseases and will contribute to new researchers selecting suitable collaborators or projects for research topics related to ophthalmic diseases.

Keywords: Animal, Eye and Diseases, Bibliographic Analysis, Higher Education Institution, Postgraduate Thesis

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Türkiye'de Hayvanlarda Göz ve Hastalıkları Üzerine Yapılmış Lisansüstü Tezlerin Bibliyometrik Analizi

ÖZ

Yapılan çalışma; Ülkemizde farklı türdeki hayvanların göz ve hastalıkları üzerine akademik platformda yapılmış lisansüstü tezleri bibliyometrik analiz yöntemiyle değerlendirip sonrasında yapılacak yeni çalışmalara katkı sunmayı amaçlanmıştır. Çalışmada gereç ve yöntem olarak; internet üzerinden Yüksek Öğretim Kurumuna bağlı Ulusal Tez Merkezi'nin resmi sitesinde lisansüstü tezler bölümünde, araştırılacak konuya ait kelime veya kelimelerin yazıldığı tarama kutucuğuna farklı kombinasyonlarda anahtar kelime ya da kelimeler yazılarak aramaların yapılması biçiminde uygulanmıştır. Yapılan taramada yazılan farklı kelime kombinasyonları içinde en fazla "Göz Hastalıkları" anahtar kelimeleriyle ulaşılan 4886 lisansüstü tezden; 22'sinin hayvanlarda göz ve hastalıkları üzerine yapıldığı tespit edilmiştir. Tespit edilen tezlerden elde edilen bulgularda; araştırmaların %68.2'sinin yüksek lisans %31.2'sinin doktora tezleri olduğu, yapılmış tez çalışmalarında daha çok %40.91 deneysel türdeki araştırmaların tercih edildiği, göz ve hastalıkları üzerine yapılmış tezlerin yıllara göre dağılımına bakıldığında alanda yeterli çalışmanın olmamasının da etkisiyle yükselen bir trende sahip olduğu görülmüştür. Sonuç olarak bu tarz çalışmalar; göz ve hastalıkları üzerine yapılan lisansüstü araştırma sayılarının yeterli olmadığı göz önüne alındığında yapılacak araştırma sayılarındaki artışla hem oluşan hastalıkların önlenmesinde yeni tedavi stratejilerinin geliştirilmesine olanak sağlayacak hem de yeni araştırmacıların oftalmik hastalıklara yönelik araştırma konuları için uygun işbirlikçileri veya projeleri seçmelerine katkı sağlayacaktır.

Anahtar Kelimeler: Hayvan, Göz ve hastalıkları, Bibliyografik Analiz, Yüksek Öğretim Kurumu, Lisansüstü Tez

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INTRODUCTION

When evaluated from both a structural and functional perspective, the eye is an organ with a highly complex structure. It is a very sensitive structure whose function can be affected even by a slight disturbance in homeostasis due to direct trauma-related injury or local or systemic diseases. Ocular problems observed in domestic animals are becoming increasingly severe. Many of these problems are also difficult to diagnose with the naked eye in the early stages of the disease (Scountzou 2017). These diseases, which cause vision impairment in animals used for labor and domestic animals raised for production, result in greater economic losses for farmers and, indirectly, for society. In addition, treatment costs for pets also contribute to these losses. Therefore, detailed scientific studies are needed to diagnose and treat eye disorders (Reichmann et al. 2008).

Human physicians, veterinarians, scientific researchers, and other competent specialists in the field have made invaluable contributions to the current body of knowledge on eye diseases, eye health, and vision science. In response, medical treatment options for various disorders and pathologies continue to evolve, and new research has been conducted to provide practitioners with better tools to treat their patients (Gilger 2024).

Bibliometric analysis, also known as scientometrics, has been used continuously by researchers. It is known as a systematic analysis method used to quantitatively and visually reveal the characteristics of literature, such as institutions, countries/regions, journals, and authors (Lu et al. 2019; Xiao et al.2021). Bibliometrics can also be defined as the use of statistical methods to analyze a body of literature in order to reveal its historical development through the study of publications (Young and Belanger 1983). With its help, the discovery of existing knowledge in specific areas and the discovery of future research can be made more successful.

The trend of bibliometric analysis of current studies began with Garfield's study in JAMA and spread in subsequent years with other studies involving bibliometric article analyses conducted by many researchers in different fields of medicine (Petekkaya 2020). However, recent literature reviews have revealed that the number of bibliometric studies in the field of ophthalmology is low, and that most of these focus on ophthalmic epidemiology, the evaluation of ophthalmology journals, cataract surgery, and dry eye (Liu et al. 2018; Schargus et al. 2018). As more and more studies are published in the field of ophthalmology worldwide, it is clear that this will enable a more comprehensive bibliometric analysis of these studies on a global scale (Gu et al. 2017). Considering the limitations of bibliometric studies in the field of veterinary ophthalmology, this research is expected to make a significant contribution to the literature.

The study aims to evaluate postgraduate theses on the eyes and diseases of different types of animals in our country, which have been conducted on an academic platform, using bibliometric analysis methods, and to contribute to new studies to be conducted in the future

MATERIALS and METHODS

The research covers the scientific evaluation of postgraduate theses conducted in the field of veterinary surgery in Turkey between 1990 and 2025 on eye diseases observed in animals.

In this study, since no experimental or non-experimental intervention was performed, and since it involved a bibliometric analysis or citation analysis of existing published classical article studies and referenced published comparable studies, approval from an ethics committee was not required.

From a methodological perspective, the aim of the study was to investigate academic postgraduate theses related to the subject in the field of veterinary surgery in our country using bibliometric analysis techniques. In line with this objective, on June 18, 2025, searches were conducted on the official website of the National Thesis Center of the Higher Education Institution by entering the keywords "Eye and Eye Diseases in Animals," "Eye Diseases Observed in Animals," and "Eye Diseases" in the search box. However, the search did not yield a sufficient number of theses. Subsequently, a search was conducted using the keyword "eye," resulting in a total of 4.886 theses, of which n=22 postgraduate theses were found to have been conducted in the field of veterinary surgery between 1990 and 2025 and related to eye diseases in animals. Content analyses of the theses were conducted, and theses written in other fields were excluded from the study.

The theses obtained after the research were evaluated separately in terms of methodology, including the year they were conducted, content, institution where they were conducted, thesis level, scientific method, number of subjects, and the species and gender of the animals used in the research.

Statistical Analysis

The findings obtained from the postgraduate theses studied were statistically transferred to Microsoft Excel and visualized. The percentages and frequency values of the analyses to be converted into statistical data were calculated using the SPSS for Windows 21.0 (IBM, Inc., Chicago, IL, USA) program.

RESULTS

Following the research, data obtained from n=22 theses were loaded into the SPSS statistical program and analyzed. The results were presented in table and

figure form for evaluation. Within the scope of this study, it was considered appropriate to present the statistical expressions of the general distribution of the characteristics of the "n" variables in postgraduate theses on eye and eye diseases in our country in separate tables, as well as to present all data under a single heading. These data are shown in Table 1. The distribution by consultant title is shown in Table 2. The distribution of theses by university is shown in Table 3

Table 1. The general distributions of the variables in the theses used in "n" numbers

SERİAL NUMBER	CHE THESIS NO	ADVISOR	POSTGRADUATE THESIS	UNIVERSITY	YEAR	ANIMAL SPECIES	RESEARCH TYPE	EXPERIMENTAL NUMBER	PROJECT SUPPORT
1	022612	Dr. Lecturer	PhD	Uludağ University	1992	Dogs	Experimental	10 (5F+5M)	-
2	138232	Assoc. Prof. Dr	PhD	Ankara University	1993	Dogs	Retrospective	-	-
3	194352	Prof. Dr.	PhD	Fırat University	2005	Ruminants	Incidence	13873	-
4	192716	Dr. Lecturer	MD	Afyon Kocatepe University	2006	Dogs	Experimental	12 (6F+6M)	-
5	376829	Prof. Dr.	MD	Adnan Menderes University	2012	Rabbits	Invasive	12 (6F+6M)	-
6	352996	Assoc. Prof. Dr	PhD	Uludağ University	2013	Dogs	Retrospective	21	-
7	361582	Assoc. Prof. Dr	MD	Afyon Kocatepe University	2014	Cats and Dogs	Prevalence	-	-
8	462518	Prof. Dr.	MD	Selçuk Üniversitesi	2016	Dogs	Experimental	50 (33F+17M)	-
9	499244	Prof. Dr.	MD	Van Yüzüncü Yıl University	2018	Ruminants	Invasive	14720	-
10	518427	Prof. Dr.	MD	Ankara Üniversitesi	2018	Dogs	Experimental	20 (6F+14M)	-
11	519039	Prof. Dr.	PhD	Aydın Adnan Menderes University	2018	Rabbits	Experimental	36 (20F+16M)	-
12	569266	Prof. Dr.	MD	Van Yüzüncü Yıl University	2019	Ruminants	Incidence	13672 (10299F+3373M)	-
13	595026	Prof. Dr.	MD	Fırat University	2019	Rabbits	Experimental	14F	-
14	601464	Prof. Dr.	PhD	Adnan Menderes University	2019	Rabbits	Experimental	20	-
15	612495	Prof. Dr.	MD	Fırat University	2019	Sheep and Goats	Prevalence	30884 (25954F+4930M)	-
16	663102	Prof. Dr.	MD	Adnan Menderes University	2021	Cats and Dogs	Retrospective	45(17F+28M)	-
17	678955	Prof. Dr.	MD	Selçuk University	2021	Cats	Retrospective	50 (29F+21M)	-
18	681291	Prof. Dr.	PhD	Fırat University	2021	Mice	Experimental	28F	FU. SRPCO
19	715961	Prof. Dr.	MD	Afyon Kocatepe University	2022	Cats and Dogs	Prevalence	3387	-
20	855932	Prof. Dr.	MD	Fırat University	2024	Cats and Dogs	Incidence	1739	MAEU. SRPCO
21	890101	Dr. Lecturer	MD	SRPCO	2024	Rats	Experimental	28F	-
22	926510	Prof. Dr.	MD	Fırat University	2025	Cats and Dogs	Retrospective	199	-

Table 1. The general distributions of the variables in the theses used in "n" numbers (Continued)

SERİAL NUMBER	NUMBER OF THESIS PAGES	NUMBER OF SOURCES USED		THESIS ARTICLE		NUMBER OF CITATIONS FROM THE THESIS		NUMBER OF CITATIONS FROM THE ARTICLE	
		Domestic	Foreign	Domestic	Foreign	Domestic	Foreign	Domestic	Foreign
1	85	20	65	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
3	103	22	75	Fırat Uni. Health Sci. Vet. Journal	-	-	-	6	-
4	123	13	34	Kocatepe Veterinary Journal	-	-	-	-	-
5	70	7	48	-	-	-	-	-	-
6	150	1	173	-	-	-	-	-	-

7	49	16	4	-	-	1	-	-	-
8	66	15	64	-	Eurasian J Vet Sci	-	-	-	-
9	73	9	56	Fırat Uni. Health Sci. Vet. Journal	-	-	-	-	-
10	84	3	44	-	-	-	-	-	-
11	88	15	151	-	-	1	-	-	-
12	62	49	45	Van Health Sci. Journal	-	-	-	-	-
13	68	18	72	Fırat Uni. Health Sci. Med. Journal	-	-	-	-	-
14	94	4	140	Ankara Uni Vet. Faculty Journal	-	-	-	1	-
15	94	36	76	Fırat Uni. Health Sci. Vet. Journal	-	-	-	-	-
16	75	3	78	-	-	-	-	-	-
17	73	5	46	Bozok Vet. Sci.	-	-	-	-	-
18	130	3	298	-	Veterinaria México OA	-	-	-	-
19	67	4	55	-	-	-	-	-	-
20	58	19	55	-	-	-	-	-	-
21	90	7	60	-	-	-	-	-	-
22	53	8	56	-	-	-	-	-	-

*CHE: Conuncill of Higher Education, **MD: Master's Degree, PhD: Doctorate, ***F:Female, M:Male **** FU. SRPCO: Fırat University Scientific Research Projects Coordination Office, MAEU. SRPCO: Mehmet Akif Ersoy Univerity Scientific Research Projects Coordination Office.

Table 2. Distribution of theses according to advisor titles

Advisor Titles	**n**	**%**
Prof. Dr.	16	72.72
Associate Prof. Dr.	3	13.64
Dr. Lecturer	3	13.64

Table 3. Distribution of theses by university

University	**n**	**%**
Fırat University	6	27.27
Adnan Menderes University	4	18.18
Afyon Kocatepe University	3	13.63
Uludağ University	2	9.09
Ankara University	2	9.09
Van Yüzüncü Yıl University	2	9.09
Selçuk University	2	9.09
Mehmet Akif Ersoy University	1	4.54

When examining the distribution of postgraduate theses by year, it is observed that theses, which were rarely prepared in the 1990s, have seen a significant increase in number, especially after 2018. An average of two theses have been completed annually since 2021. This situation reveals that academic interest in veterinary ophthalmology has increased significantly in recent years (Figure 1). The distribution of animal species used in research on the eye and eye diseases in the theses examined is shown in Figure 2.

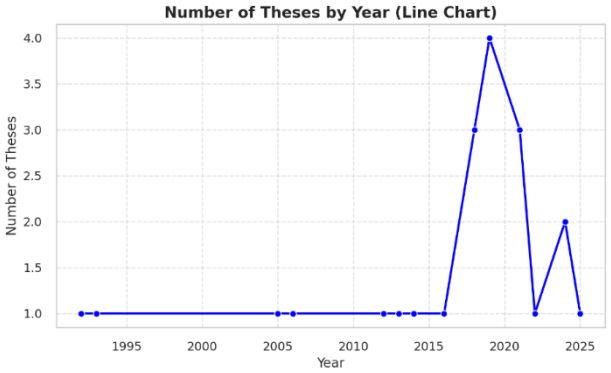

Figure 1: Distribution of theses by year

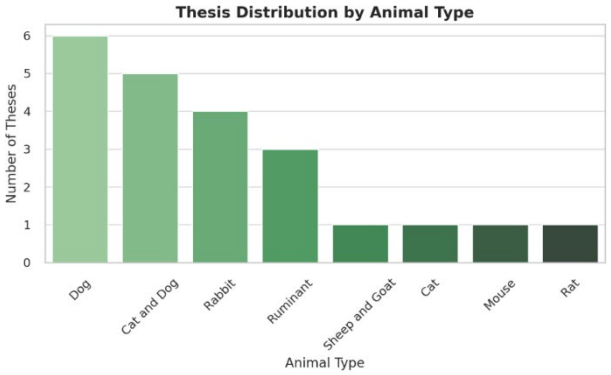

Figure 2: Distribution of theses by animal species

The postgraduate studies examined in the research are shown in Figure 3 in terms of thesis type.

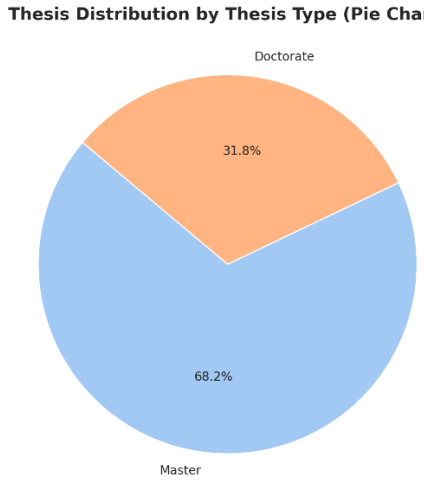

Figure 3: Distribution by thesis type

When evaluating postgraduate thesis studies in terms of research methods, it was determined that the most frequently used methods were experimental (n=9) and retrospective (n=5) approaches. In addition to these, the studies were found to be prevalence (n=3), incidence (n=3), and invasive (n=2) studies. It is thought that the reason for the frequent preference for experimental methods is that they reflect the need to evaluate effects under controlled conditions (Figure 4).

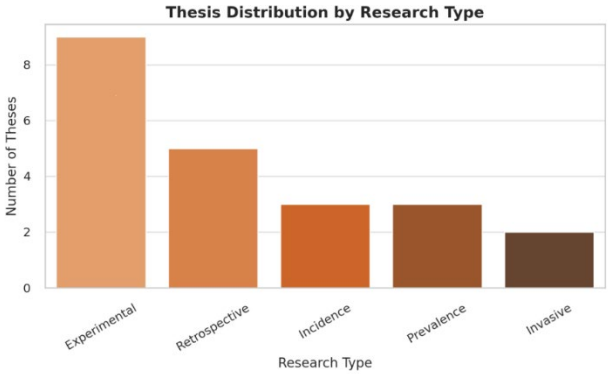

Figure 4: Distribution by research type

When examining the number of subjects used in the studies, it was observed that the sample size used in the theses ranged from 10 to 30.884. The average number of subjects was calculated to be approximately 3.941. The highest number of subjects was found to be from field-based incidence/prevalence studies (Table 1, Table 4).

The average number of pages in the theses is 83.6; the average number of domestic sources used is 13.2, while the average number of foreign sources used is 80.7. While a significant portion of the sources are from foreign studies, it is observed that national sources are also included. This indicates that the theses are at a reasonable level in terms of academic depth and validity (Table 1, Table 4).

Approximately 50% of the theses have been converted into scientific articles. The average number of citations per thesis is 0.09, while the average number of citations per article is 0.31.

Overall, it is observed that postgraduate theses on eye diseases in animals are increasing in terms of both quantity and quality, with experimental studies standing out, and that a portion of the theses can be converted into scientific publications.

Table 4. Minimum-maximum, mean, and standard deviations for certain characteristics of the theses

Features	Minimum	Maximum	Mean	Std. Deviation
Number of subjects	10.00	30884.00	3941.0000	8123.39427
Number of thesis pages	49.00	150.00	83.5714	25.82358
Number of sources used (domestic)	1.00	49.00	12.7727	11.74301
Number of sources used (foreign)	4.00	298.00	80.7143	63.55639
Quotations from thesis	1.00	1.00	1.0000	.00000
Quotations from articles	1.00	6.00	3.5000	3.53553

DISCUSSION

The numerical increase or decrease in publications in a specific academic field is an important criterion for assessing the general trend in that field (Peng et al. 2020). It has been stated that studies aimed at revealing data through analyses of research conducted in the field of eye and eye diseases are increasing every year and that this will be a focus point for future researchers. It has been emphasized that the global increase in this field is due to the increase in both human and animal populations and the growing awareness of eye health (Cheng et al. 2020; Arad et al. 2023). When we look at the distribution of postgraduate thesis studies on eye diseases in our country by year (Figure 1), we can say that there has been an upward trend every year.

One of the most frequently used parameters for the academic evaluation of research is the number of citations the research receives, which is an important indicator of the impact and contribution of the study to science (Shuaib et al. 2015; Doğan et al. 2018). In addition, citation counts, researchers' h-index, journal Impact Factor scores, and SCImago journal rankings are factors that influence the scientific metrics of both individual authors and journals. 3 Bibliometric analyses of citations to research provide a perspective on the historical development of specific disciplines, including fundamental literature and research areas, the contributions of scientists to the field, and the identification of institutions (Ramos et al. 2019; Bentley et al. 2019). A study on the conversion of postgraduate theses into research articles revealed that only 30% of all theses were published in journals as research articles related to the thesis. It was stated that 22% of these articles were published in SCIE journals and 8.1% in non-SCIE Turkish journals (Mahir et al. 2016). In our research, when the data obtained from Google Scholar and Wos searches of theses and articles related to postgraduate studies were evaluated, it was determined that they did not receive a large number of citations, and that some thesis studies were not converted into articles and remained only at the postgraduate education level (Table 1).

Analyses have revealed that universities are the institutions that produce the most academic work in the institutional sphere. A study on the subject reported that 15 of the top 20 institutions in terms of scientific research in the United States are universities (Lin et al. 2022). A study conducted in India emphasized that among 2,204 institutions in 63 countries conducting academic research, the highest scientific output was produced by researchers at universities (Zammarchi and Conversano 2021). Consistent with these studies, our study found that all postgraduate research related to the eye and eye diseases was conducted at universities in our country (Table 1, Table 3).

Some researchers have stated that the most important reason behind the preference for master's education over doctoral education in graduate education choices is that it offers a shorter education process, thesis topics require more superficial and narrow-scope research, and the education received is reflected in professional life in a shorter period of time (Neumann 2005; Vural and Başaran2021; Li et al. 2024). In our research, when we evaluated students' preferences according to their postgraduate education degrees, we found that the number of students who preferred master's education was much higher than those who preferred doctoral education (Table 1, Figure 3).

It has been reported that ophthalmological disorders seen in animals are generally common in all species, but cats and dogs have a genetic predisposition to these diseases (Djajadiningrat-Laanen et al. 2025). Scientific research published in ophthalmology journals and other related journals has reported that experiments on animals are preferred in articles that are clinically descriptive, basic science, case presentations, and studies of diseases (Xue et al. 2021; Kumaragurupari and Mishra 2022). Our research has found that cats and dogs are more commonly used in studies on diseases, while laboratory animals are preferred in experimental research (Table 1, Figure 2).

It has been reported that the number of scientific studies conducted in the field of ophthalmology has steadily increased, especially since the 2000s. In the studies conducted, it was noticeable that the number of articles published in the 2012s increased significantly, and it has been emphasized that its popularity has increased significantly since 2015. Additionally, it has been reported that this number reached its peak in 2021 with the highest number of studies, exceeding approximately twice the number from 2009 (Wang et al. 2023). When we examined postgraduate theses on eye diseases between 1990 and 2025, we found that studies on this topic increased significantly after 2021 (Table 1, Figure 1).

Experimental studies conducted on animals, particularly in laboratory settings, provide indispensable experimental models for interpreting fundamental mechanical discoveries and realizing their therapeutic potential in humans. On the other hand, advances in human medicine are often informed by clinical data. To this end, basic experimental model types are introduced in scientific research conducted specifically for this purpose. The role of laboratory and larger animal models has increased the tendency to select experimental study models in common ocular research areas such as intraocular neoplasia, corneal epithelial and stromal diseases, cataracts, uveitis, glaucoma, and retinal dystrophies (Zeiss 2013). According to the data obtained in our study, when we evaluated the studies conducted on the subject according to research types, we found that the largest group of studies focused on experimental studies (Table 1, Figure 4).

CONCLUSION

Future studies on this topic could provide a more comprehensive representation of the global research landscape by utilizing more advanced data collection and analysis methods. We believe that our study will contribute to identifying trends related to this topic in the future.

Conflict of interest: The authors have no conflicts of interest to report.

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

Ethical approval: This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules."

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CASE REPORT

Testicular Necrosis Following Orchitis in a Rabbit: A Case Report

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ABSTRACT

This case report presents a rare clinical presentation of testicular necrosis associated with orchitis and funiculitis in a 9-year-old intact male Holland Lop rabbit. The rabbit was presented to the Animal Hospital of Bursa Uludağ University with bilateral scrotal swelling, pain, erythema, and multiple perineal abscesses. Ultrasonographic examination revealed marked testicular enlargement, heterogeneity, and hydrocele. Despite initial antimicrobial and anti-inflammatory therapy, inflammation extended to the spermatic cord. Bilateral orchiectomy was performed using the closed castration (scrotal ablation) method after stabilization. Histopathology confirmed necrosis in the left testis, chronic orchitis in the right testis, and severe funiculitis. The rabbit recovered uneventfully and remained asymptomatic during five months of follow-up. The animal died 15 months postoperatively due to multiple organ failure secondary to age-related complications. This case emphasizes the importance of timely surgical intervention in progressive testicular infections to prevent irreversible tissue damage.

Key Words: Funiculitis, Orchiectomy, Orchitis, Rabbit, Testicular necrosis

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Bir Tavşanda Orchitis Sonrası Testiküler Nekroz: Olgu Sunumu

ÖZ

Bu olguda, 9 yaşında, kısırlaştırılmamış erkek Holland Lop ırkı bir tavşanda görülen orchitis ve funiculitis' e bağlı nadir bir testiküler nekroz vakası sunulmaktadır. Tavşan, Bursa Uludağ Üniversitesi Hayvan Hastanesi'ne bilateral skrotal şişlik, ağrı, eritem ve çok sayıda perineal apse şikayetleriyle getirildi. Ultrasonografik incelemede belirgin testiküler büyüme, heterojenite ve hidrosel tespit edildi. Başlangıçta uygulanan antimikrobiyal ve antiinflamatuar tedaviye rağmen inflamasyon spermatik kordu tutacak şekilde ilerleme gösterdi. Stabilizasyonun ardından, kapalı kastrasyon (skrotal ablasyon) yöntemi ile bilateral orşiektomi uygulandı. Histopatolojik incelemede sol testiste nekroz, sağ testiste kronik orşitis ve şiddetli funikülit saptandı. Tavşan, ameliyat sonrası dönemde komplikasyonsuz şekilde iyileşti ve beş aylık takip sürecinde herhangi bir klinik belirti göstermedi. Operasyondan 15 ay sonra, yaşa bağlı gelişen çoklu organ yetmezliği nedeniyle hayatını kaybetti. Bu olgu, ilerleyici testiküler enfeksiyonlarda geri dönüşümsüz doku hasarını önlemek adına zamanında cerrahi müdahalenin önemini vurgulamaktadır.

Anahtar Kelimeler: Funiculitis, Orşiektomi, Orchitis, Tavşan, Testiküler nekroz

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INTRODUCTION

Specific reports of testicular inflammation and necrosis in rabbits are scarce. Nevertheless, infectious diseases remain a significant cause of morbidity and mortality in pet rabbits. Recent comprehensive studies have identified various infectious agents, including *Encephalitozoon cuniculi*, as major contributors to mortality in this species (Doboşi et al. 2024; Espinosa García-San Román et al. 2024). These findings underscore the clinical relevance of infectious diseases in rabbits and the critical importance of early recognition and management of systemic infections. Testicular inflammation and necrosis are rarely reported in rabbits but can pose significant clinical challenges when they occur (Maxie 2016). Orchitis and funiculitis may develop as sequelae to bacterial infections or trauma, and if left untreated, can progress to ischemic testicular necrosis (Sarierler and Kılıç 2003; Suciu et al. 2017). Among lagomorphs, interstitial cell tumors are the most commonly reported testicular disorders, while inflammatory conditions are less frequent but potentially life-threatening (Irizarry-Rovira et al. 2008; Reineking et al. 2019). Delayed diagnosis and intervention in such cases can result in irreversible tissue damage, systemic illness, and sepsis (Maxie 2016). However, documented clinical reports detailing the progression and surgical management of severe testicular infections in rabbits remain scarce in the veterinary literature (Bertram et al. 2021; Reineking et al. 2019). This case report aims to describe a rare presentation of testicular necrosis associated with orchitis and funiculitis in a rabbit, highlighting the importance of early diagnosis and timely surgical intervention to prevent life-threatening complications.

CASE HISTORY

A 9-year-old intact male Holland Lop rabbit (*Oryctolagus cuniculus*), weighing 3 kg, was presented to the Veterinary Hospital of Bursa Uludağ University with a history of hindlimb lameness, chronic diarrhea, and swelling in the scrotal and perineal regions. General clinical examination revealed poor body condition and hyperemic mucous membranes, with no other abnormalities noted. On clinical examination, both testicles were markedly enlarged, warm, erythematous, and painful on palpation. The scrotum appeared diffusely reddened. The left testis measured approximately 7 × 3 cm, while the right was 2 × 1 cm. Multiple abscesses were detected in the perineal area and around the testes (Figure 1). Inguinal lymph nodes were bilaterally enlarged. Pathogen isolation and identification were also not performed, as the owner declined these additional diagnostic procedures due to financial limitations.

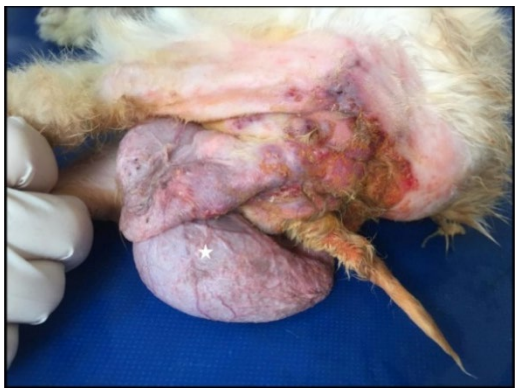

Figure 1: Bilateral testicular enlargement, predominantly on the left side (white star), accompanied by multiple abscess foci in the perineal region and around the testes.

Ultrasonographic evaluation of both testicles was performed using a high-frequency (7.5 MHz) linear probe (Figure 2). The left testis was markedly enlarged, displayed a heterogeneous echotexture with multiple anechoic regions, and lacked a visible mediastinum testis—no echogenic linear structure was identified within the testicular parenchyma. Additionally, a hydrocele was detected as an anechoic fluid accumulation in the intrascrotal space. Transverse and sagittal sonographic images confirmed loss of normal architecture in the left testis. The epididymis appeared enlarged and exhibited mixed echogenicity.

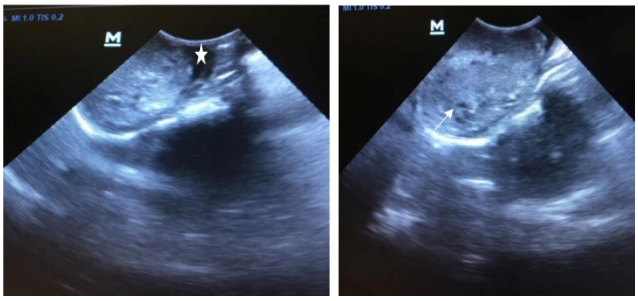

Figure 2: Ultrasonographic image of the left testicle showing marked enlargement, heterogeneous echotexture, and multiple anechoic areas (white arrows). The mediastinum testis is not visualized. A hydrocele is present, evident as an intrascrotal anechoic fluid accumulation (white star).

The affected area was cleaned and compressed using warm povidone-iodine solution (Batticon®, Adeka, Samsun, Türkiye). Enrofloxacin (Baytril-K®, Bayer, Istanbul, Türkiye; 10 mg/kg, s.c.) and cefazolin sodium (Iespor®, I.E. Ulagay Co., Istanbul, Türkiye; 20 mg/kg, s.c.) were administered once daily for 10 consecutive days. As an anti-inflammatory agent, flunixin meglumine (Flumeglin®, Teknovet, Istanbul, Türkiye; 1.1 mg/kg, s.c.) was administered once daily for three days. After the abscesses matured, they were drained via puncture and irrigated. Once the local

inflammation and testicular swelling regressed, a surgical appointment was scheduled. However, due to a delay in scheduling by the owners, the inflammation extended to the spermatic cord (funiculus spermaticus). The rabbit returned with worsening general condition and lameness. Antibiotic and anti-inflammatory treatments were repeated, and after one week, clinical improvement was observed. Bilateral orchiectomy was then scheduled under general anesthesia.

Premedication was performed with xylazine (Basilazin® 2%, Bavet, Istanbul, Türkiye; 4 mg/kg, i.m.). Ten minutes later, ketamine hydrochloride (Alfamine® 10%, Alfasan, Woerden, The Netherlands; 35 mg/kg, i.m.) was administered for induction. An intravenous catheter was placed into the lateral auricular vein, and preventive antibiotherapy with enrofloxacin (Baytril-K®, Bayer, Istanbul, Türkiye; 10 mg/kg, i.v.) and preemptive analgesia with meloxicam (Metacam®, Boehringer Ingelheim, Ridgefield, CT, USA; 1 mg/kg, i.v.) were administered. Anesthesia was maintained with a combination of diazepam and ketamine administered intravenously. The rabbit was positioned in dorsal recumbency, and the surgical site was shaved and aseptically prepared. Bilateral orchiectomy was performed using the closed castration (scrotal ablation) method. The inflamed spermatic cord was excised as extensively as possible. The subcutaneous tissue was closed with continuous simple sutures, and the skin was closed with interrupted simple sutures using 2-0 Vicryl absorbable suture material. Both testes were submitted for histopathological examination, which confirmed the presence of severe testicular necrosis.

Gross examination showed the left testis to be enlarged, firm, and discolored. The spermatic cord appeared thickened and congested (Figure 3). Histopathological evaluation revealed: Left testis: widespread coagulative necrosis, hemorrhage, tubular degeneration, and absence of active spermatogenesis. Right testis: chronic interstitial orchitis with infiltration of lymphocytes and macrophages. No evidence of neoplasia was found in either testis.

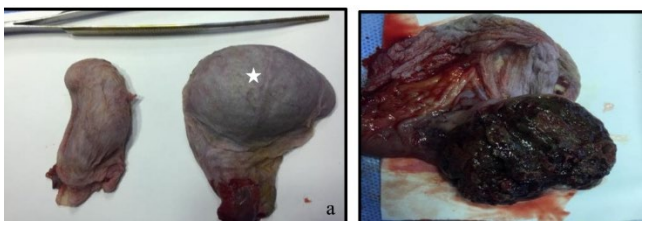

Figure 3: Macroscopic postoperative view of the excised testes (a-b). The left testis appears markedly enlarged (white star), firm, and discolored, while the right testis is smaller but inflamed.

Postoperative recovery was uneventful. Within two weeks, the rabbit regained normal appetite, mobility, and coat condition. During the first five months of follow-up, no recurrence or systemic signs were observed, and the animal maintained stable clinical health. Periodic abdominal ultrasonography was recommended. Unfortunately, the rabbit died 15 months after the surgery due to multiple organ failure associated with age-related complications. No signs of recurrence or metastasis related to the previous testicular pathology were observed prior to death.

DISCUSSION

Pet rabbits, increasingly popular worldwide as companion animals, can harbor various zoonotic pathogens—including parasites (e.g., *Encephalitozoon cuniculi*), viruses (hepatitis E), bacteria (e.g., *Bartonella spp*., *Pasteurella spp.*), and fungi. In rabbits, systemic bacterial infections such as pasteurellosis are known to predispose to abscess formation and reproductive tract inflammation, including orchitis and funiculitis, which may progress if not promptly managed (Fernández et al. 2023; College of Veterinary Medicine, University of Missouri n.d.). In the present case, however, pathogen isolation was not performed as the owner declined additional diagnostic tests. While the initial signs—such as perineal abscesses, scrotal erythema, and testicular swelling—often indicate localized infection, the ascending spread of inflammation to the spermatic cord (funiculitis) can lead to vascular compromise and subsequent ischemic necrosis. Similar mechanisms of inflammation-induced ischemia have been documented in other species, where prolonged or inadequately treated infections result in coagulative necrosis and irreversible parenchymal damage (Zachary and McGavin 2012; Varga 2014). In the present case, a severe bilateral inflammatory condition evolved into unilateral testicular necrosis due to a delay in surgical intervention. Despite initial antimicrobial and anti-inflammatory treatment, the infection extended from the perineal region to the spermatic cord, compromising blood flow to the left testis. This progression underscores the critical importance of early recognition and timely surgical management in preventing irreversible testicular damage in lagomorphs.

Ultrasonographic findings in the present case supported the clinical suspicion of severe testicular pathology. The absence of mediastinum testis, heterogeneous parenchymal echotexture, and hydrocele indicated significant tissue disruption. Such ultrasonographic features are considered consistent with testicular degeneration or necrosis, and in this case, histopathological confirmation revealed widespread necrosis, tubular degeneration, and a complete absence of spermatogenesis in the affected testis. The concurrent diagnosis of chronic orchitis and funiculitis in the contralateral testis and spermatic cord, respectively, reflects the extent and chronicity of the inflammatory process.

Interestingly, while testicular neoplasms remain the most frequently reported testicular pathologies in rabbits, particularly in aging, intact males (Heatley and Smith 2004; Reineking et al. 2019), this case demonstrated a purely inflammatory etiology without any evidence of neoplastic transformation. This distinction is crucial, as early clinical signs in such cases can mimic testicular tumors, leading to delayed or inappropriate therapeutic approaches.

The outcome of this case underlines the importance of timely diagnosis and definitive surgical intervention. The rabbit exhibited full recovery following bilateral orchiectomy and remained clinically stable for five months but died 15 months postoperatively due to multiple organ failure secondary to age-related complications. The absence of recurrence or systemic illness supports the effectiveness of surgical castration in resolving advanced testicular infections when performed promptly.

This case contributes to the limited literature on inflammatory testicular disease in rabbits and emphasizes the need for early therapeutic intervention in similar clinical scenarios. It also highlights the diagnostic value of ultrasonography in differentiating between neoplastic and non-neoplastic testicular conditions in lagomorphs, aiding timely and appropriate clinical decision-making.

CONCLUSION

This case report highlights an unusual but clinically significant progression of testicular inflammation in a pet rabbit, resulting in unilateral testicular necrosis and funiculitis. The findings support the necessity of timely surgical management in severe scrotal infections, and the five-month disease-free interval followed by natural death at 15 months postoperatively suggests successful resolution.

A limitation of the present case is that pathogen isolation and hematological examination could not be performed because the owner declined additional diagnostic tests, limiting both the identification of the causative agent and the assessment of systemic involvement.

Conflict of interest: The author have no conflicts of interest to report.

Authors' Contributions: The author conceived the study, designed and conducted the experiments, collected and analyzed the data, and wrote and critically revised the manuscript. The author approved the final version of the manuscript.

Ethics Committee Information: This study does not fall under the scope requiring HADYEK approval, as outlined in Article 8(k) of the "Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees." All data, findings, and materials presented in this report were obtained in compliance with academic integrity and ethical standards.

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