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Journal of Faculty of Veterinary Medicine, Erciyes University

20(2): 76-155, 2023

İÇİNDEKİLER / CONTENTS

Sayfa / Page

ARAŞTIRMA MAKALELERİ / RESEARCH ARTICLES

DERLEMELER / REVIEW ARTICLES

OLGU SUNUMU/ CASE REPORTS

Journal of Faculty of Veterinary Medicine, Erciyes University
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Investigation of the Protective Effects of Diosmin Against Emamectin Benzoate Induced Oxidative Damage in Rats*,**

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Abstract: This study was aimed to investigate the effects of diosmin (DIO) in rats exposed to emamectin benzoate (EB). For this purpose, a total of 60 Wistar Albino male rats, aged 6 to 8 weeks and weighing 180 to 250 g, were used, 10 in each group. The groups were determined, respectively, control, EB (10 mg kg^{-1}), DIO (50 mg kg^{-1}), DIO (100 mg kg^{-1}), EB (10 mg kg^{-1}) + DIO (50 mg kg^{-1}), EB (10 mg kg^{-1}) + DIO (100 mg kg^{-1}), and the indicated doses were applied by gavage for 21 days. At the end of the trial period, a heart puncture was performed under anesthesia and blood samples were taken into tubes with heparinized and anticoagulant-free properties. The liver, kidney, brain, testis, heart and lung tissues were removed after cervical dislocation. While some biochemical markers, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), triglyceride, cholesterol, total protein, albumin, uric acid, blood urea nitrogen (BUN) and, creatinine were assessed in the serum, oxidative stress indicators, such as MDA, NO, SOD, CAT, GSH-Px, GR, GST, and GSH, were determined in tissue samples. At the end of the investigation, no changes between the parameters of the DIO groups and the control group were discovered. Comparing the EB group to the control group, it was found that the levels of MDA and NO in tissues/erythrocytes had significantly increased. The activity of the GSH, GR, GST, SOD, and CAT enzymes, as well as GSH levels, was found to have decreased. Furthermore, serum total protein and albumin levels were reduced, although AST, ALT, ALP, LDH, cholesterol, triglyceride, BUN, uric acid, and creatinine levels/activities were elevated. Depending on the dose, it was found that the values of the groups coadministered with EMB and DIO were close to those of the control group. In conclusion, it is proposed that DIO could provide protection against EMB-induced toxicity in rats.
Keywords: Emamectin benzoate, diosmin, oxidative damage, rat

Sıçanlarda Emamektin Benzoat Kaynaklı Oksidatif Hasara Karşı Diosminin Koruyucu Etkilerinin Araştırılması

Öz: Bu çalışma ile sıçanlarda emamektin benzoat (EB) maruziyetine karşı diosminin (DİO) etkilerinin araştırılması amaçlandı. Çalışmada her grupta 10 tane olacak şekilde toplam 60 adet 6-8 haftalık 180-250 g ağırlığında Wistar Albino ırkı erkek sıçan kullanıldı. Gruplar sırasıyla kontrol, EB (10 mg kg^{-1}), DİO (50 mg kg^{-1}), DİO (100 mg kg^{-1}), EB (10 mg kg^{-1}) + DİO (50 mg kg^{-1}), EB (10 mg kg^{-1}) + DİO (100 mg kg^{-1}) olarak belirlenerek belirtilen miktarlarda 21 gün boyunca gavajla uygulama yapıldı. Deneme süresinin sonunda anestezi altında kalbe punksiyon yapılarak heparinize ve antikoagulansız tüplere kan örneği alındı. Servikal dislokasyon sonrası sıçanların karaciğer, böbrek, beyin, testis, kalp ve akciğer dokuları çıkarıldı. Serumda bazı biyokimyasal parametreler (aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), laktat dehidrogenaz (LDH), alkalin fosfataz (ALP), trigliserit, kolesterol, total protein, albümin, ürik asit, kan üre azotu (BUN) ve kreatinin) ölçülürken doku örneklerinde ise oksidatif stres parametreleri (MDA, NO, SOD, CAT, GSH-Px, GR, GST ve GSH) analiz edildi. Çalışma sonunda kontrol grubu ile karşılaştırıldığında, DİO uygulanan grubun parametrelerinde herhangi bir değişiklik tespit edilmedi. EMB uygulanan grupta kontrole kıyasla tüm dokularda MDA ile NO seviyelerinde anlamlı bir artış kaydedildi. GSH düzeyleri ile GSH, GR, GST, SOD ve CAT enzim aktivitelerinde ise azalma olduğu belirlendi. Ayrıca serum AST, ALT, ALP, LDH, kolesterol, trigliserit, BUN, ürik asit ve kreatinin düzey/aktivitelerinde artış görülürken serum total protein ve albümin düzeylerinde azalma görüldü. EB ile DİO'nun birlikte uygulandığı grupların değerlerinin doza bağlı olarak kısmen ya da tamamen kontrol grubunun değerlerine yaklaştığı tespit edildi. Sonuç olarak, DİO'nun sıçanlarda EMB kaynaklı toksisiteye karşı koruma sağlayabileceği düşünülmektedir.
Anahtar kelimeler: Emamektin benzoat, diosmin, oksidatif hasar, rat

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Introduction

Pesticides are chemical compounds that are commonly used in agriculture or public health protection programs to protect plants from pests, weeds, dis-

eases, livestock from pest infestations, and people from vector-borne diseases (e.g., Lyme disease, malaria, West Nile virus). Despite their beneficial effects, pesticides are not selective, and as a result, they pose severe risks to people, the environment, or non-target organisms such as beneficial soil bacteria, insects, plants, fish, and birds (Aktar et al., 2009).

Avermectins, a class of 16-membered macrocyclic lactones, were discovered at the Kitasato Institute in Japan in 1967 as a natural fermentation product of the soil actinomycete *Streptomyces avermitilis* (Bai and Ogbourne, 2016). Emamectin benzoate (EB), a semi-synthetic avermectin derivative, is used to treat sea lice in salmon and to control lepidopteran pests in leafy vegetables such as lettuce, broccoli, cabbage, and cauliflower (JECFA, 2013). EB, a neurotoxin, disrupts the normal function of gamma-aminobutyric acid (GABA) like other avermectins. The opening of GABA-gated chloride channels, triggered by EB, causes increased permeability of chloride ions, resulting in a signal blockade at neuromuscular junctions. Previous studies have demonstrated the toxic effects of EB on different organs, including the liver (Khaldoun Oularbi et al., 2017), kidney (Abou-Zeid et al., 2018), testis (Zhang et al., 2020), and brain (Madkour et al., 2021) due to oxidative stress.

Since ancient times, it has been common to use plants and plant-derived phytochemicals for the treatment or prevention of health issues (Patel et al., 2013). Diosmin (DIO) is a naturally occurring flavone glycoside that can be produced by dehydrating hesperidin, a flavanone glycoside, or by extraction from a variety of plant sources, including *Teucrium gnaphalodes*. DIO, which was isolated from *Scrophularia nodosa* in 1925, was introduced as a therapeutic agent in 1969 (Mustafa et al., 2022). Due to its vascular protective function, it is used to treat varicose veins, lymphedema, and chronic venous insufficiency. Studies have also reported that it has many beneficial effects, such as antioxidant, anti-apoptotic, anti-ulcer, hepatoprotective, and neuroprotective activity (Gerges et al., 2022).

A number of studies on the effectiveness of several natural products, including vitamin C (Khaldoun Oularbi et al., 2017), pumpkin seed oil (Abou-Zeid et al., 2018), and Nigella sativa oil (Madkour et al., 2021), against oxidative stress and lipid peroxidation caused by EB are available in the literature. It has been demonstrated that diosmin protects against a variety of substances, including methotrexate (Abdel-Daim ve ark., 2017), aflatoxin (Eraslan et al., 2017), cadmium (Ağır and Eraslan, 2019), lead (Bozdağ and Eraslan, 2020), and deltamethrin (Tekeli et al., 2021). However, there was no mention of any scientific report on the efficacy of DIO against oxidative stress and lipid peroxidation induced by EB. Therefore, the purpose of this investigation was to evaluate the beneficial role of DIO on lipid peroxidation and enzymatic/non-enzymatic antioxidant status in rats exposed to EB.

Materials and Methods

Animals and experimental design

A total of 60 Wistar albino male rats, weighing 180-250 g and aged 6-8 weeks, were utilized in the study. Throughout the experiment, water and commercial pellet feed (protein 24%, fats 5.09%, cellulose 3.2%, and a total of 3100 kcal/kg metabolic energy) were provided to all rats ad libitum in cages made of polyethylene, which were housed in accordance with standard laboratory conditions (12-hour light/dark cycle at 22±2 °C and relative humidity of 45–55%). Protocols for the experiments were approved by the ERU Animal Research Ethics Committee and conducted in compliance with global standards (Report No. 19/051). On the basis of prior research, the EB (Khaldoun-Oularbi et al., 2017) and DIO (Abdel-Daim et al., 2017) dose administered to rats were determined. Corn oil, a commercially available product that is not an analytical standard, was utilized as a vehicle substance. Rats were administered suspensions of EB and DIO in corn oil to construct identical circumstances. A total of six groups of ten rats each were designed at random. The first group was identified as a control, and 1 ml kg^{-1} body weight (BW) of the corn oil was administered orally for 21 days. The other groups received continuous oral administration of EB (10 mg kg^{-1}.BW day^{-1}), DIO (50 mg kg^{-1}.BW day^{-1}), DIO (100 mg kg^{-1}.BW day^{-1}), DIO (50 mg kg^{-1}.BW day^{-1}) with EB (10 mg kg^{-1}.BW day^{-1}), and DIO (100 mg kg^{-1}.BW day^{-1}) with EB (10 mg kg^{-1}.BW day^{-1}), respectively, for a total of 21 days.

Sampling and preparation for laboratory measurements

At the end of the trial period, the rats in all groups were anesthetized with a combination of ketamine-xylazine intraperitoneally under the same conditions. Subsequently, blood samples were taken into tubes with heparinized and anticoagulant-free properties by puncturing the heart. After that, cervical dislocation was applied to sacrifice. The serum samples were separated from the blood samples in the anticoagulant-free tubes by centrifugation at 3000 rpm for 10 minutes at 4°C and analyzed on the same day. The plasma-containing supernatant was carefully separated after centrifuging blood samples in heparinized test tubes at 3000 rpm for 10 minutes (min) at 4°C. The buffy coat was removed from the remaining phase, and the erythrocytes were washed three times in normal saline (0.9% NaCl). For this process, the mixture was centrifuged at 2000 g for 5 min, and the supernatant was discarded. The erythrocytes were

diluted with an equal volume of normal saline before being stored at -80°C. Before analysis, erythrocytes were hemolyzed 1:5 with ice-cold distilled water. The brain, heart, kidney, liver, lung, and testis tissues were quickly dissected, and blood clots were eliminated by washing the organs in 0.9% NaCl solution. Using a homogenizer (Silent Crusher M, Heidolph), tissues were homogenized 1:5 in cold phosphate buffer (pH 7.4) on ice before being centrifuged for 60 minutes at 10000 rpm at 4°C. In order to measure tissue enzymatic or non-enzymatic antioxidants and lipid peroxidation, supernatants were transferred to Eppendorf tubes and stored in a deep freezer (-80° C).

Measurement of serum biochemical parameters

A Roche Cobas C 8000 autoanalyzer and the same brand kits were used for the determination of serum triglyceride, cholesterol, albumin, total protein, BUN, uric acid and creatinine levels, and LDH, AST, ALT and ALP enzyme activities.

Measurement of lipid peroxidation and enzymatic/ non-enzymatic antioxidant parameters

The spectrophotometric method described by Lowry et al. (1951) was applied to measure the protein levels in tissue homogenates. According to the procedures of Habig et al. (1957), Paglia and Valentine (1967), and Carlberg and Mannervik (1985), glutathione S transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR) activity in tissues/esrythrocyte was analyzed, respectively. In terms of nmol/min/g protein or mg hemoglobin, enzyme activity was expressed. By using the Sun et al. (1988) technique, the enzyme activity of superoxide dismutase (SOD) in tissues/esrythrocyte was quantified and expressed as U/g protein or mg hemoglobin. The Luck (1965) method was utilized to determine the catalase (CAT) enzyme activity in tissues/ esrythrocyte, and was presented as katal/g protein or mg hemoglobin. The amounts of reduced glutathione (GSH), malondialdehyde (MDA), and nitric oxide (NO) in tissues/esrythrocyte/plasma were tested by Sedlak and Lindsay (1968), Ohkawa et al. (1979), and Tracey et al. (1995), respectively. These data were stated in nmol per g protein or mg hemoglobin.

Statistical analysis

The research data were statistically analyzed utilizing SPSS 21.0 statistical program. The data were expressed as arithmetic mean and standard deviation. The conformity of the variables to the normal distribution was evaluated with the Kolmogrov-Smirnov test. The homogeneity of variances was assessed by Levene's test. One-way analysis of variance (one-way ANOVA) was utilized to assess the statistical difference. The Welch test was utilized to assess the difference between the groups in situations when the homogeneity of variance assumption was rejected. Statistical differences between the groups were evaluated with the Tukey test in situations where there was homogeneity of variance and with the Games Howell test in situations where the homogeneity of variance assumption was not maintained. Data were regarded significant at $P<0.05$.

Results

The biochemical and oxidative stress parameters assessed in all samples were compared between the control and diosmin-treated groups, but no statistically significant difference was found ($P>0.05$) (Table 1-3).

When compared to the control group, the group exposed to EB showed a significant reduction in tissue/ esrythrocyte GSH levels and GPx, GR, GST, SOD, and CAT enzyme activity, as well as a significant increase in plasma/tissue MDA and NO levels ($P<0.05$). In comparison to the control group, additionally, it was also observed that serum total protein and albumin levels were lower, but AST, ALT, ALP, LDH, cholesterol, triglycerides, BUN, uric acid, and creatinine activities/levels were higher ($P<0.05$) (Table 1-3).

It was discovered that the group co-administered with EMB and DIO (50 mg kg^{-1}) had lower plasma and tissue MDA levels than the group exposed to EB ($P<0.05$). Similarly, all tissues other than plasma showed a significant reduction in NO levels ($P<0.05$). The GSH level was higher in the other tissues than in the EB group, with the exception of the heart, lung, and plasma tissues ($P<0.05$). Compared to the EB group, a significant increase was observed in GPX activity in plasma and tissues other than heart tissue ($P<0.05$). Comparatively to the group exposed to EB, CAT and GR activity increased in all tissues ($P<0.05$). Except for the liver, all tissues displayed a considerable increase in GST activity ($P<0.05$). Except for cardiac tissue, SOD activity increased in tissues and erythrocytes ($P<0.05$). Serum total protein and albumin levels increased in comparison to the EB group, but AST, ALT, ALP, LDH, cholesterol, triglyceride, BUN, and uric acid levels and activities decreased ($P<0.05$). Along with this, it was determined that the CAT, SOD, GPX, and GR enzyme activities in liver tissue, the SOD and GR enzyme activities in kidney tissue, and the LDH, AST, and ALT enzyme activities in serum were close to those of the control group ($P>0.05$) (Table 1-3).

All oxidative stress and biochemical stress parameters altered by EB exposure were either completely or partially reversed in the group co-administered with EMB and DIO (50 mg kg^{-1}). Additionally, it was determined that the levels/activities of GSH, MDA, NO GPX, GR, GST, SOD, and CAT in liver tissue, the SOD MDA, NO, GPX, GR, and GST in kidney

tissue, NO, GSH, GPX, GST, and SOD in heart tissue, GSH, GR, and GST in testis tissue, NO, GST, and CAT in plasma/esrythrocyte and the albumin, creatinine, uric acid, LDH, AST, and ALT in serum were close to those of the control group (P>0.05) (Table 1-3).

The changes in all biochemical and oxidative stress parameters investigated in the combination groups were dose-dependent.

Table 1. Effects of DIO treatment on the levels/activities of AST, ALT, LDH, ALP, triglyceride, cholesterol, total protein, albumin, uric acid, BUN and, creatinine in serum of EB-exposed rats

Parameters	Groups Control	EB	DIO50	DIO100	EB+DIO50	EB+DIO100
BUN (mg/dl)	16.5±1.6^{a}	21.6±1.2^{d}	17.0±0.8^{ab}	16.5±0.9^{a}	19.5±1.0^{c}	18.1±1.5^{bc}
		P<0.001	P=0.898	P=1.000	P<0.001	P=0.031
Creatinine (mg/dl)	0.31±0.03^{ab}	0.37±0.03^{d}	0.31±0.02^{a}	0.31±0.01^{a}	0.34±0.02^{cd}	0.33±0.02^{ab}
		P<0.001	P=0.997	P=1.000	P<0.001	P<0.001
Uric Acid (mg/dl)	0.94±0.19^{a}	1.74±0.27^{c}	1.00±0.24^{ab}	1.22±0.25^{ab}	1.32±0.36^{b}	0.99±0.25^{ab}
		P<0.001	P=0.996	P=0.181	P=0.024	P=0.998
Triglyceride (mg/dl)	98.8±17.2^{a}	172.4±29.2^{d}	104.3±16.6^{ab}	117.9±29.9^{abc}	142.2±17.9^{c}	130.8±13.0^{bc}
		P<0.001	P=0.993	P=0.371	P=0.001	P=0.020
Cholesterol (mg/dl)	59.5±5.5^{a}	98.6±7.8^{d}	64.3±4.06^{a}	61.0±3.94^{a}	82.9±5.74^{c}	75.4±5.66^{b}
		P<0.001	P=0.404	P=0.991	P<0.001	P<0.001
LDH (U/L)	1514.8±115.9^{ab}	1966.9±98.7^{c}	1456.9±96.1^{a}	1441.2±100.8^{a}	1622.9±125.5^{b}	1574.2±127.2^{ab}
		P<0.001	P=0.853	P=0.680	P=0.269	P=0.839
AST (U/L)	106.8±6.5^{abc}	129.9±10.2^{d}	102.5±5.0^{ab}	99.3±9.3^{a}	116.1±8.2^{c}	111.8±9.8^{bc}
		P<0.001	P=0.860	P=0.356	P=0.149	P=0.766
ALT (U/L)	41.2±4.7^{ab}	52.0±3.0^{d}	39.4±2.8^{a}	40.4±3.5^{ab}	47.4±3.2^{b}	44.8±2.4^{bc}
		P<0.001	P=0.835	P=0.995	P=0.002	P=0.175
ALP (U/L)	253±13.4^{a}	346±22.5^{c}	264±19.1^{a}	257±17.5^{a}	297±22.6^{b}	290±13.4^{b}
		P<0.001	P=0.744	P=0.993	P<0.001	P=0.001
Total Protein (mg/dl)	7.38±0.15^{a}	5.67±0.10^{d}	7.36±0.27^{a}	7.19±0.12^{a}	6.30±0.10^{c}	6.55±0.19^{b}
		P<0.001	P=1.000	P=0.165	P<0.001	P<0.001
Albumin (mg/dl)	4.85±0.36^{ab}	3.67±0.20^{d}	4.95±0.30^{a}	5.02±0.25^{a}	4.30±0.27^{c}	4.45±0.42^{bc}
		P<0.001	P=0.972	P=0.808	P=0.003	P=0.060

The mean ± standard deviation is used to express the data. A statistically significant difference (P<0.05) between groups is indicated by different superscripts (a, b, and c) in the same row.
Groups: Control, corn oil; EB, emamectin benzoate (10 mg kg^{-1}.BW day^{-1}); DIO50, diosmin (50 mg kg^{-1}.BW day^{-1}); DIO100, diosmin (100 mg kg^{-1}.BW day^{-1}); EB+DIO50, emamectin benzoate plus diosmin (50 mg kg^{-1}.BW day^{-1}); EB+DIO100, emamectin benzoate plus diosmin (100 mg kg^{-1}.BW day^{-1}).

Table 2. Effects of DIO treatment on the levels/activities of MDA, NO, CAT, and SOD in liver, kidney, brain, testis, heart and lung tissues, and erythrocytes/plasma of EB-exposed rats

Sample	Groups	Parameters MDA nmol/mg P	NO nmol/mg P	SOD U/g P	CAT k/g P or mg Hb
Liver	Kontrol	2.04±0.31^{a}	3.54±0.53^{a}	0.14±0.02^{a}	601.6±24.5^{abc}
	EB	3.83±0.58^{c}	5.23±0.55^{c}	0.07±0.02^{b}	524.5±18.0^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	2.09±0.41^{a}	3.48±0.61^{a}	0.12±0.05^{a}	611.0±29.8^{ab}
		P=1.000	P=1.000	P=0.223	P=0.923
	DIO100	1.99±0.52^{a}	3.40±0.53^{a}	0.13±0.02^{a}	616.2±18.9^{a}
		P=1.000	P=0.989	P=0.803	P=0.662
	EB+DIO50	2.83±0.55^{b}	4.31±0.45^{b}	0.11±0.01^{a}	575.3±14.7^{c}
		P=0.006	P=0.014	P=0.137	P=0.104
	EB+DIO100	2.45±0.43^{ab}	4.05±0.32^{ab}	0.11±0.02^{a}	584.2±24.2^{bc}
		P=0.380	P=0.234	P=0.155	P=0.496
Kidney	Kontrol	1.21±0.28^{a}	4.38±0.38^{ab}	0.24±0.02^{a}	730.7±90.6^{a}
	EB	2.40±0.31^{c}	6.04±0.55^{c}	0.19±0.02^{b}	316.6±41.6^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	1.26±0.21^{a}	4.34±0.70^{ab}	0.25±0.03^{a}	712.6±118.9^{ab}
		P=0.999	P=1.000	P=1.000	P=0.998
	DIO100	1.17±0.27^{a}	4.26±0.37^{a}	0.24±0.02^{a}	752.3±97.1^{a}
		P=1.000	P=0.997	P=0.950	P=0.994
	EB+DIO50	1.76±0.31^{b}	5.10±0.76^{b}	0.22±0.02^{a}	538.6±88.8^{c}
		P<0.001	P=0.066	P=0.337	P<0.001
	EB+DIO100	1.48±0.25^{ab}	4.94±0.56^{ab}	0.23±0.02^{a}	600.9±76.9^{bc}
		P=0.219	P=0.260	P=0.588	P=0.022
Brain	Kontrol	1.67±0.20^{a}	2.14±0.29^{a}	0.29±0.02^{a}	355.1±51.3^{a}
	EB	3.72±0.16^{d}	3.75±0.32^{c}	0.13±0.03^{d}	142.3±33.8^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	1.64±0.18^{a}	2.10±0.37^{a}	0.27±0.03^{ab}	361.0±51.9^{a}
		P=0.998	P=1.000	P=0.410	P=1.000
	DIO100	1.84±0.17^{a}	2.12±0.22^{a}	0.30±0.03^{a}	337.4±49.9^{a}
		P=0.397	P=1.000	P=1.000	P=0.938
	EB+DIO50	2.87±0.27^{c}	3.19±0.18^{b}	0.19±0.03^{c}	230.4±28.1^{b}
		P<0.001	P<0.001	P<0.001	P<0.001
	EB+DIO100	2.43±0.17^{b}	2.87±0.29^{b}	0.24±0.03^{b}	268.5±36.0^{b}
		P<0.001	P<0.001	P<0.001	P<0.001
Testis	Kontrol	3.45±0.24^{a}	2.18±0.36^{a}	0.11±0.03^{a}	116.7±12.9^{a}
	EB	5.25±0.32^{d}	3.56±0.15^{c}	0.06±0.02^{c}	60.6±11.8^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	3.54±0.31^{a}	1.88±0.25^{a}	0.11±0.01^{a}	108.9±5.7^{a}
		P=0.988	P=0.052	P=0.998	P=0.705
	DIO100	3.72±0.38^{ab}	1.89±0.13^{a}	0.13±0.02^{a}	119.6±13.9^{a}
		P=0.452	P=0.078	P=0.682	P=0.994
	EB+DIO50	4.14±0.40^{bc}	3.29±0.17^{bc}	0.08±0.01^{b}	81.2±12.3^{b}
		P<0.001	P<0.001	P=0.003	P<0.001
	EB+DIO100	4.22±0.30^{c}	2.99±0.25^{b}	0.09±0.01^{b}	91.0±14.4^{b}
		P<0.001	P<0.001	P=0.011	P<0.001
Heart	Kontrol	2.17±0.28^{a}	3.11±0.21^{a}	0.17±0.02^{a}	338.2±32.0^{ab}
	EB	4.30±0.31^{c}	4.41±0.38^{c}	0.10±0.01^{d}	217.3±28.0^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	2.27±0.31^{a}	3.21±0.40^{ab}	0.16±0.02^{a}	334.7±23.7^{ab}
		P=0.098	P=0.988	P=0.825	P=1.000
	DIO100	2.02±0.24^{a}	3.10±0.34^{a}	0.16±0.02^{ab}	351.2±43.6^{a}
		P=0.866	P=1.000	P=0.458	P=0.932
	EB+DIO50	3.21±0.36^{b}	3.63±0.25^{b}	0.12±0.02^{cd}	264.1±25.4^{c}
		P<0.001	P=0.010	P<0.001	P<0.001
	EB+DIO100	2.83±0.28^{b}	3.43±0.30^{ab}	0.13±0.02^{bc}	298.2±27.8^{bc}
		P<0.001	P=0.265	P<0.001	P=0.057
Lung	Kontrol	3.34±0.36^{a}	3.84±0.67^{a}	0.36±0.02^{a}	100.6±7.6^{a}
	EB	5.18±0.42^{d}	6.35±0.34^{c}	0.28±0.01^{c}	59.9±4.4^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	3.49±0.39^{a}	3.81±0.39^{a}	0.35±0.01^{a}	97.2±8.7^{a}
		P=0.908	P=1.000	P=0.867	P=0.960
	DIO100	3.36±0.28^{a}	3.78±0.37^{a}	0.36±0.02^{a}	103.8±12.8^{a}
		P=1.000	P=0.999	P=0.999	P=0.971
	EB+DIO50	4.48±0.32^{c}	5.59±0.52^{b}	0.31±0.01^{b}	73.8±10.6^{b}
		P<0.001	P<0.001	P=0.001	P<0.001
	EB+DIO100	3.95±0.27^{b}	5.04±0.33^{b}	0.33±0.01^{b}	78.7±8.2^{b}
		P=0.003	P<0.001	P=0.027	P<0.001
Esryth rocyte s/ plas- ma	Kontrol	11.27±0.76^{a}	18.00±7.40^{a}	0.71±0.04^{a}	2039.4±139.7^{ab}
	EB	16.12±0.79^{c}	53.26±11.27^{c}	0.51±0.03^{d}	1469.8±145.9^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	11.68±0.85^{a}	26.07±7.50^{ab}	0.72±0.03^{a}	2095.6±211.1^{a}
		P=0.851	P=0.200	P=0.778	P=0.969
	DIO100	11.12±0.80^{a}	19.71±3.73^{a}	0.72±0.04^{a}	1949.2±162.5^{ab}
		P=0.998	P=0.984	P=0.834	P=0.804
	EB+DIO50	14.01±0.76^{b}	37.81±13.16^{bc}	0.58±0.03^{c}	1715.6±107.3^{c}
		P<0.001	P=0.010	P<0.001	P<0.001
	EB+DIO100	13.65±0.66^{b}	34.64±14.14^{ab}	0.63±0.02^{b}	1850.6±173.1^{bc}
		P<0.001	P=0.050	P<0.001	P=0.104

The mean ± standard deviation is used to express the data. A statistically significant difference (P<0.05) between groups is indicated by different superscripts (a, b, and c) in the same column.

Groups: Control, corn oil; EB, emamectin benzoate (10 mg kg^{-1}.BW day^{-1}); DIO50, diosmin (50 mg kg^{-1}.BW day^{-1}); DIO100, diosmin (100 mg kg^{-1}.BW day^{-1}); EB+DIO50, emamectin benzoate plus diosmin (50 mg kg^{-1}.BW day^{-1}); EB+DIO100, emamectin benzoate plus diosmin (100 mg kg^{-1}.BW day^{-1}).

Table 3. Effects of DIO treatment on the levels/activities of GSH, GPx, GR, and GST in liver, kidney, brain, testis, heart and lung tissues, and erythrocytes/plasma of EB-exposed rats

Sample	Groups	Parameters			
		GSH nmol/mg P or	GPX nmol/min/g P	GR nmol/min/g P	GST nmol/min/g P
Liver	Kontrol	98.19±7.15^{a}	19.25±2.30^{a}	29.41±2.52^{a}	135.17±17.66^{ab}
	EB	73.88±8.18^{c}	14.67±1.32^{b}	21.08±4.06^{b}	96.97±14.88^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	95.81±7.45^{a}	19.30±1.61^{a}	29.81±2.53^{a}	134.68±15.38^{ab}
		P=0.977	P=1.000	P=1.000	P=1.000
	DIO100	99.81±6.45^{a}	19.09±2.01^{a}	29.67±3.21^{a}	141.50±16.67^{a}
		P=0.996	P=1.000	P=1.000	P=0.952
	EB+DIO50	85.15±7.74^{b}	17.45±2.07^{a}	27.95±1.95^{a}	114.74±19.91^{bc}
		P=0.002	P=0.316	P=0.871	P=0.072
	EB+DIO100	91.31±6.20^{ab}	18.26±2.13^{a}	26.13±2.88^{a}	128.44±12.17^{ab}
		P=0.289	P=0.862	P=0.142	P=0.939
Kidney	Kontrol	90.70±7.38^{a}	41.54±2.93^{a}	43.15±2.65^{ab}	148.38±18.22^{a}
	EB	55.66±7.39^{c}	27.84±2.74^{c}	30.29±6.39^{c}	102.81±9.50^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	91.86±7.94^{a}	39.57±5.74^{ab}	42.94±4.33^{ab}	144.24±13.81^{a}
		P=1.000	P=0.901	P=1.000	P=0.980
	DIO100	90.25±9.87^{a}	41.26±4.60^{a}	44.00±6.10^{a}	147.17±9.72^{a}
		P=1.000	P=1.000	P=0.999	P=1.000
	EB+DIO50	75.35±9.19^{b}	35.28±3.55^{b}	37.07±4.36^{b}	125.36±13.52^{b}
		P=0.001	P=0.019	P=0.068	P=0.003
	EB+DIO100	77.53±6.57^{b}	37.61±4.90^{ab}	40.88±3.97^{ab}	131.60±11.30^{ab}
		P=0.008	P=0.312	P=0.897	P=0.060
Brain	Kontrol	57.34±2.11^{a}	18.74±1.74^{a}	25.22±1.59^{a}	48.71±3.67^{a}
	EB	38.70±2.69^{d}	11.16±2.13^{d}	15.27±1.79^{c}	35.01±2.42^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	56.30±3.33^{a}	17.39±0.63^{ab}	23.81±1.98^{a}	49.08±1.95^{a}
		P=0.960	P=0.444	P=0.464	P=1.000
	DIO100	58.52±3.11^{a}	18.17±1.44^{ab}	25.16±1.88^{a}	47.93±3.92^{a}
		P=0.930	P=0.970	P=1.000	P=0.992
	EB+DIO50	45.27±2.79^{c}	13.71±1.08^{c}	18.92±1.81^{b}	41.43±3.28^{b}
		P<0.001	P<0.001	P<0.001	P<0.001
	EB+DIO100	49.67±2.41^{b}	16.36±2.19^{b}	19.72±1.37^{b}	43.43±2.47^{b}
		P<0.001	P=0.023	P<0.001	P=0.004
Testis	Kontrol	38.43±4.51^{a}	9.94±1.00^{a}	12.14±1.64^{a}	318.87±23.67^{ab}
	EB	23.54±5.65^{c}	6.24±0.63^{c}	8.21±0.45^{c}	163.90±18.43^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	37.20±2.00^{ab}	10.49±1.25^{a}	12.40±1.28^{a}	306.95±18.62^{ab}
		P=0.969	P=0.824	P=0.997	P=0.806
	DIO100	38.34±3.75^{a}	10.34±1.25^{a}	12.68±1.61^{a}	325.88±8.37^{a}
		P=1.000	P=0.945	P=0.922	P=0.943
	EB+DIO50	33.69±1.40^{b}	7.97±1.11^{b}	10.24±0.79^{b}	221.95±33.65^{c}
		P=0.042	P=0.001	P=0.011	P<0.001
	EB+DIO100	34.53±1.30^{ab}	8.17±0.48^{b}	11.15±1.04^{ab}	282.62±29.95^{b}
		P=0.147	P=0.003	P=0.459	P=0.072
Heart	Kontrol	72.68±11.01^{a}	15.41±1.45^{a}	7.12±0.86^{a}	24.93±2.24^{ab}
	EB	43.68±13.15^{c}	11.78±1.17^{c}	3.33±0.65^{c}	11.51±3.05^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	67.07±12.63^{ab}	14.69±1.65^{ab}	7.34±0.43^{a}	25.06±4.14^{ab}
		P=0.860	P=0.797	P=0.984	P=1.000
	DIO100	71.54±9.10^{a}	15.51±1.27^{a}	7.04±0.79^{a}	26.89±3.58^{a}
		P=1.000	P=1.000	P=1.000	P=0.760
	EB+DIO50	54.81±7.99^{bc}	13.43±0.91^{bc}	4.78±0.65^{b}	17.84±3.86^{c}
		P=0.007	P=0.011	P<0.001	P<0.001
	EB+DIO100	61.28±10.90^{ab}	14.44±0.95^{ab}	5.32±0.87^{b}	21.08±2.01^{bc}
		P=0.201	P=0.529	P<0.001	P=0.102
Lung	Kontrol	103.72±11.25^{a}	28.48±1.31^{a}	37.52±1.42^{a}	89.84±7.65^{a}
	EB	72.38±7.47^{d}	20.97±1.68^{d}	25.21±2.16^{d}	53.43±4.53^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	97.47±6.83^{ab}	27.77±1.53^{ab}	36.47±3.92^{ab}	83.30±4.58^{a}
		P=0.578	P=0.872	P=0.963	P=0.140
	DIO100	98.82±4.62^{a}	28.11±1.14^{ab}	37.03±3.35^{a}	86.98±5.33^{a}
		P=0.792	P=0.984	P=0.998	P=0.882
	EB+DIO50	83.30±9.84^{cd}	24.10±1.45^{c}	31.32±3.16^{c}	64.75±5.92^{b}
		P<0.001	P<0.001	P=0.001	P<0.001
	EB+DIO100	87.44±9.52^{bc}	25.48±2.27^{bc}	32.63±2.36^{bc}	71.25±6.39^{b}
		P=0.001	P=0.026	P=0.001	P<0.001
Esryth-rocytes/ plasma	Kontrol	39.18±3.46^{a}	31.62±1.60^{a}		32.56±2.43^{ab}
	EB	24.32±2.79^{c}	20.94±1.45^{c}		22.27±1.89^{d}
		P<0.001	P<0.001		P<0.001
	DIO50	37.74±2.37^{a}	32.01±1.52^{a}		34.03±2.66^{a}
		P=0.940	P=0.995		P=0.790
	DIO100	38.46±5.38^{a}	31.55±2.01^{a}		35.92±3.11^{a}
		P=0.997	P=1.000		P=0.052
	EB+DIO50	28.96±3.05^{bc}	25.69±1.93^{b}		27.40±2.40^{c}
		P<0.001	P<0.001		P<0.001
	EB+DIO100	31.68±3.23^{b}	26.42±1.52^{b}		29.47±2.68^{bc}
		P<0.001	P<0.001		P=0.091

The mean ± standard deviation is used to express the data. A statistically significant difference (P<0.05) between groups is indicated by different superscripts (a, b, and c) in the same column.

Groups: Control, corn oil; EB, emamectin benzoate (10 mg kg^{-1}.BW day^{-1}); DIO50, diosmin (50 mg kg^{-1}.BW day^{-1}); DIO100, diosmin (100 mg kg^{-1}.BW day^{-1}); EB+DIO50, emamectin benzoate plus diosmin (50 mg kg^{-1}.BW day^{-1}); EB+DIO100, emamectin benzoate plus diosmin (100 mg kg^{-1}.BW day^{-1}).

Discussion and Conclusion

One of the primary mechanisms by which pesticides can damage cells and tissues is through oxidative stress processes, which include the production of free radicals, lipid peroxides, oxidized proteins, and oxidized carbohydrates (Jabłońska–Trypuć and Wiater, 2022). As is well known, oxidative stress is described as an imbalance between the generation of free radicals and reactive metabolites known as oxidants or reactive oxygen species (ROS) and their elimination by defense systems known as antioxidants (Pizzino et al., 2017). ROS are generated by cells as a byproduct of their metabolic processes. When ROS levels are too high, they interact negatively with DNA, lipids, and proteins in the body (Birben et al., 2012). Excessive formation of hydroxyl radicals and peroxynitrite causes lipid peroxidation, which damages cell membranes and lipoproteins (Pizzino et al., 2017). MDA, a lipid peroxidation marker, is one of the secondary oxidation products of lipid peroxidation (LPO). NO, another lipid peroxidation marker and reactive nitrogen species (RNS), interacts with the superoxide (O_2^-) radical to form peroxynitrite ($ONOO^-$), which is responsible for its toxic effect (Birben et al., 2012). The increase in MDA and NO levels in all tissues of rats exposed to EB in the study indicates lipid peroxidation triggered by excessive ROS and RNS production. Enzymatic and non-enzymatic antioxidants are responsible for protecting the cell from ROS-induced damage (Lü et al., 2010). GSH is an essential part of metabolic defensive processes such as free radical quenching, hydroperoxide reduction, and xenobiotic detoxification. The GSH -dependent antioxidant system consists of GSH and enzymes with similar functions, including GST, GSH-Px, and GR.GSH-Px reduces hydroperoxides and H_2O_2, whereas GSH is oxidized to GSSG. Then, GR regenerates GSH from GSSG (Nimse and Pal, 2015). The study revealed that GSHPx activity and GSH levels were decreased in all tissues of rats exposed to EB. The reduction in GSH levels promotes lipid peroxidation and oxidative stress (Jabłońska-Trypuć, 2017). Due to the antioxidant role of GSH in inhibiting free radical reactions in the tissues, it is possible that the decrease in GPx activity seen in the study was caused by GSH depletion. The initial lines of defense against reactive intermediates are SOD and CAT. Superoxide radicals are scavenged by SOD, while hydrogen peroxide radicals are neutralized by CAT (Nimse and Pal, 2015). The reduction of CAT activity in all tissues in this study can be attributed to excessive production of H_2O_2, which indicates EB-induced oxidative stress. The decreased SOD activity of rats exposed to EB may be the result of its increased degradation and decreased production as a result of increased oxidative stress. The findings of our study are in parallel with those of previous studies. Similarly, Madkour et al. (2021) reported that EB exposure (orally 9 mg kg^{-1} BW for 6 weeks) increased MDA levels in the brain tissue of rats while decreasing GPx, CAT, and SOD activities. In the study published by El-Sheikh and Galal (2015), the researchers noted that liver tissue MDA dramatically increased while SOD activity decreased when EB (2.5 mg EB kg^{-1} BW) was administered orally for 28 days. Abou-Zeid et al. (2018) reported that 75 ppm EB in the diet of mice induces oxidative stress by increasing MDA levels and suppressing GSH, CAT, and SOD levels and activities in liver and kidney tissue, as well as inhibiting SOD activity and increasing MDA levels in brain tissue. In the testes of mice exposed to EB in their study, Zhang et al. (2020) found that GPx and SOD activity decreased and MDA levels elevated. In the liver and kidney tissues of rats exposed to abamectin, another avermectin derivative, Abdel-Daim and Abdellatief (2018) discovered an elevation in MDA concentrations and a reduction in GSH levels and antioxidant enzyme activity (GPX, CAT, and SOD).

Our results, which demonstrate decreased total protein and albumin, are in line with earlier research (El-Sheikh and Galal, 2015; Madkour et al., 2021). Their concentration may have decreased due to an imbalance between the rates of protein synthesis and degradation. The liver is the organ where albumin is intensively synthesized. Therefore, pesticide-induced hepatocyte damage may reduce the capacity for albumin synthesis. It is thought that liver damage due to the toxic effects of EB also causes a decrease in albumin levels. The serum AST, ALT, ALP, LDH, cholesterol, and triglyceride levels/activities that increased as a result of EB administration were observed in our findings to be consistent with other research (El-Sheikh and Galal, 2015; Abdel-Daim and Abdellatief, 2018; Khaldoun Oularbi et al., 2017). The enhanced plasma membrane permeability caused by EB toxicity in this study may be the cause of the elevated ALT and AST levels. The increase in serum total cholesterol level can be attributed to decreased secretion into the duodenum due to obstruction of the liver bile ducts. Triglyceride accumulation is the result of an imbalance between the synthesis and release of triglycerides into the systemic circulation by parenchymal cells. The increase in these parameters suggests potential liver tissue damage (Hamed and Abdel-Razik, 2015; Meligi and Hassan, 2017). In line with prior investigations, our study also found increased BUN, uric acid, and creatinine levels in serum (Meligi and Hassan, 2017; Abdel-Daim and Abdellatief, 2018; Madkour et al., 2021). The elevation in these measurements could be evidence of renal tissue damage associated with oxidative stress triggered by EB. Increased levels of uric acid and creatinine in the serum can be linked to decreased renal glomerular filtration and urine excretion capacity, which reflect renal tubular dysfunction (Madkour et

al., 2021). As the kidney is the organ that primarily secretes urea into the urine, a high uric acid level is an indicator of reduced renal function. Due to the fact that urea is the end product of protein catabolism, elevated creatinine levels are linked to increased protein catabolism. Our study, which states that EB decreases the total protein level due to increased protein catabolism, is compatible with the literature (Meligi and Hassan, 2017).

Investigating the ability of natural antioxidants to mitigate oxidative stress induced by pesticides is gaining more attention. Flavonoids, which are members of the polyphenol family, have strong antioxidant properties (Zeng et al., 2021). The main molecular modes of action of flavonoids include their capacity to chelate metals, inhibit different types of oxidases including lipoxygenases, and cyclooxygenases, and promote the activity of antioxidant enzymes including SOD, CAT, and GPX (Jabłońska–Trypuć and Wiater, 2022). Furthermore, they can reduce free radical levels in the cell by inhibiting the activities or expression of free radical-producing enzymes like NAD(P)H oxidase and xanthine oxidase (XO) (Lü et al., 2010). Due to the fact that diosmin is a flavonoid, it shares similar effects with other flavonoids. All tissues in the groups in the current study that only received DIO were similar to those in the control group; therefore, it can be concluded that DIO had no negative effects. Similar findings were reported in previous studies (Rehman et al., 2013; Eraslan et al., 2017; Ağır and Eraslan, 2019). Depending on the dose, it caused the effects of EB at both doses to be completely or almost completely reversed in the groups that received EB plus DIO. This effect may be due to either the inhibition of XO enzyme activity, which causes free radical formation, or the reduction of the cellular level of free radicals as a result of its radical scavenging activity. Previous investigations found similar results. Tekeli et al. (2021) reported that DIO can protect against deltamethrin in rats by increasing GSH levels and GPx, SOD, and CAT activities while decreasing MDA and NO levels in the liver, kidney, brain, testis, heart, and erythrocyte/plasma. According to Bozdağ and Eraslan (2020), DIO reverses the effects of lead by increasing total protein and albumin levels while decreasing AST, ALT, ALP, LDH, cholesterol, triglyceride, BUN, uric acid, and creatinine activity/levels in serum. Additionally, they demonstrated that decreasing MDA and NO levels and increasing GSH, GPX, SOD, and CAT levels/activities can alleviate lead-induced damage to the blood, liver, kidneys, testes, brain, and heart. As said by Abdel-Daim et al. (2017), the treatment of DIO (50 and 100 mg kg-1) either partially or completely restored dose-dependently the elevated AST, ALT, ALP, LDH, urea, and creatinine levels/activities in the serum in mice exposed to methotrexate. Moreover, depending on the dose, DIO treatment improved the levels and activities of MDA, NO, GSH, GST, GR, GPx, SOD, and CAT in the liver, kidney, and heart tissue closer to those of the control group.

In conclusion, it was demonstrated that EB administered orally might decrease the antioxidant defenses of rats and increase their susceptibility to oxidative stress. However, the severity of oxidative damage induced by EB regressed with DIO treatment in a dose-dependent manner, especially at a dose of 100 mg/kg. As a result of its antioxidant effect, it is expected that the aforementioned flavonoid may be used as a preservative and help to prevent pesticide toxicity. However, more investigation is required to completely understand the precise mechanisms of action and the application of DIO to mitigate pesticide -induced toxicities.

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Evaluation of Blood and Cerebrospinal Fluid Biochemistry, Cytology and Haematological Parameters in Head-and-Eye Form of Malignant Catarrhal Fever in Cattle

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Abstract: In this study, it was aimed to examine the biochemical changes, hematological changes and cerebrospinal fluid (CSF) cytology and blood serum of cattle with head-eye form of Malignant Catarrhal Fever (MCF). For this purpose, 22 cattle diagnosed with "head-eye form" of MCF and clinically healthy 10 cattle were evaluated. Blood and cerebrospinal fluid (CSF) were collected from all cattle. In sera, AST, urea, glucose, CK (P<0.05), LDH levels (P<0.01) were found be high, ALT, ALP, cholesterol (P<0.05), Ca, total protein (P<0.01) and Mg, albumine and Fe levels (P<0.001) were found to be low in MCF group when compared to the control group. In CSF, Ca (P<0.01) and total protein levels (P<0.001) were found high glucose level (P<0.05) was found low in MCF group when compared to the control group. In haematology, some parameters were determined to be different between the groups. In cytological results of CSF in MCF group, polymorphonuclear leucocytes, lymphocytes, erytrocytes, macrophages and plasma cells were determined. In conclusion, since there were a limited number of studies examining biochemical, cytologic and hematological results of MCF especially in CSF, the results from our study were thought to be important for future studies in which viral diseases affects the nervous system of cattles.
Keywords: Biochemistry, cattle, CSF, cytology, haematology, malignant catarrhal fever

Coryza Gangrenosa Bovum'un Baş-Göz Formu Belirlenen Sığırlarda Kan ve Beyin Omurilik Sıvısı Biyokimyası, Sitolojisi ve Hematolojik Parametrelerin Değerlendirilmesi

Öz: Bu çalışmada, coryza gangrenosa bovum'un (CGB) baş-göz formu belirlenen sığırların beyin omurilik sıvısı (BOS) ve kan serumlardaki biyokimyasal değişiklikler, hematolojik değişiklikler ve BOS'un sitolojik olarak incelenmesi amaçlanmıştır. Bu amaçla CGB'nin baş-göz formu belirlenen 22 adet sığır ve klinik olarak sağlıklı 10 adet sığırdan kan ve beyin omurilik sıvısı (BOS) alınmıştır. CGB grubunun kan serumlardaki AST, üre, glukoz, CK (P<0.05), LDH düzeyleri (P<0.01), ALT, ALP, kolesterol (P<0.05), Ca, total protein (P<0.01) ve Mg değerleri kontrol grubuna göre yüksek, albümin ve Fe düzeyleri (P<0.001) ise düşük bulunmuştur. CGB gurubundaki sığırların BOS'larında Ca (P<0.01) ve total protein düzeyleri (P<0.001) yüksek, glukoz düzeyleri (P<0.05) ise kontrol grubuna göre düşük olarak belirlenmiştir. Hematolojik bazı parametrelerde de gruplar arasında farklılık görülmüştür. CGB gurubundaki sığırların BOS'larında yapılan sitolojik incelemelerde, sitolojik lamlarda polimorfonükleer lökositler, lenfositler, eritrositler, makrofajlar ve plazma hücreleri belirlenmiştir.Sonuç olarakCGB'da, özellikle BOS'ta sitolojik ve biyokimyasal, ayrıca kanda biyokimyasal ve hematolojik sonuçları bir arada inceleyen sınırlı sayıda çalışma olduğundan, çalışmamızdan elde edilen sonuçların sığırların sinir sistemini etkileyebilenviral hastalıklarda gelecekte yapılacak çalışmalar için önemli bir veri oluşturabileceği düşünülmüştür.
Anahtar kelimeler: Biyokimya, BOS, coryza gangrenosa bovum, hematoloji, sığır, sitoloji

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Introduction

Malignant Catarrhal Fever (MCF) is a disease in which two factors may be involved in the etiology ofclinically distinct disease. "*Alcelaphine herpesvirus* (AHV1-2)/*Bovid-Bovine herpesvirüs 3*" plays a role in cases in wild ruminants in Africa (wildebeest-associated) *ovine herpesvirus-2* (OvHV-2) plays a role in cases in Europe, North America and Asia (sheep-associated). It is known that in the cases seen in Africa, theagent is transmitted to cattle by an antelope of *Connochaetes taurinus* breed (Blue wildebeest), so domestic ruminants may be at environmental risk worldwide. In other continents, sheepare commonly responsible for transportation of the agent and therefore the disease. Sheep have been reported to be able to infect cattle at short-to-medium distancesviaaerosols and oculer or nasal fluids. MCF can also be caused by ingestion of contaminated food, direct contact with caretakers and even from birds (Crawford et al., 1999; Andrews, 2004; Radostits et al., 2007). MCF has a very high mortality rate but cattle, one of the last hosts, do not spread the virus when they die because their secretions do not contain virus (Metzler, 1991; Smith, 1996; Andrews, 2004; Radostits et al., 2007).

The disease occurs several forms in cattle: peracute, digestive system and the"*Head-Eye*" form which is the most common. Typical signs of the head-eye form of MCF are weakness, loss of appetite, fever up to 41°C, increased pulsation (100-120/bpm), redness of the buccal mucosa, erosion and necrosis in the mouth, labial papillae and anterior nasal mucosa, congestion of the scleral vessels, edema oft he eyelids, photophobia, blepharospasm, centripetal corneal opacity starting at the edge of the sclera and discharge causing narrowing of the nasal cavity. Centripetaly corneal opacity can be considered as pathognomonic and is almost always present invarying degrees. Sick animals often have neurological manifestations especially in the terminal phase (Metzler, 1991; Smith, 1996; Radostits et al., 2007). A significant or moderate leucopenia associated with agranulocytosis is observed in the "early stages of infection" of the disease but this can easily be missed (Liggit and DeMartini, 1980a; Liggit and DeMartini, 1980b; Dewals and Vanderplasschen, 2011).

The aim of this study was to assess possible changes in routine hematological and biochemical values, as well as to determine biochemical and cytological findings obtained from cerebrospinal fluid (CSF) and to evaluate their clinical significance in the head-eye form of MCF in cattle. Although CSF is a body fluid that can be directly affected by many brain inflammations and diseases, the changes that can occur in this fluid in many large or small animal diseases, including MCF disease, have not been adequately studied to date.

Material and Methods

Animals

The study involved a total of 32 cattle, including 22 cattle with head-eye form of MCF diagnosed at the Teaching Hospital of the Faculty of Veterinary Medicine on admission by the owners, 10 healthy cattle on routine health checks in Research Farm of the Faculty.

A routine full physical examination was performed on all animals. Blood samples (n=32) were properly collected from juguler vein from all animals into plain tubes and transported immediately to laboratory. Sera were obtained by centrifugation at 3000 rpm for 10 minutes at room temperature and were kept frozen (-20°C) until the analysis were performed.

CSF samples (n=17) were collected properly in sterile micro tubes after the animal was sacrified, and routine biochemical analyzes were performed on fresh material. Cytological evaluation of CSF was performed in the Department of Pathology of the Faculty of Medicine.

Hematologic and biochemical analysis

Blood samples from MCF and healthy cattle were analyzed on (VG-Ms4e) automated hematology analyzer and serum and CSF samples on (Mindray BS 120) fully automated biochemistry analyzer.

Cytological analysis

Cytological examinations were carried out on specimen prepared by direct smear and cytospin methods from CSF samples obtained in the study. Hematoxylin and Eosin (H&E), May-Grünwald-Giemsa (MGG) and Papanicolaou (PAP) stains were used in the preparations. Cases were scanned at 200x magnification and detailed cellular assessments were made at 400x magnification in cellular areas.

PCR analysis

Total DNA was extracted from whole blood using phenol-chloroform method (Sambrook et al., 1989). Atwo-step PCR amplification was performed as described previously (Dabak and Bulut, 2003). PCR conditions were as follows: a preliminary denaturation at 99°C/5 min followed by 39 cycles at 94°C/20 sec, 60°C/30sec and 72°C/30 sec and a final extension at 72°C/1 min with primers set 556 (5'-AGTCTGGGTATATGAATCCAGATGGCTCTC-3') and 775 (5'-AAGATAAGCACCAGTTATGCATCT-GATAAA-3') to obtain a PCR product of 422 bp. A nested PCR was also conducted, with exactly the same conditions as detailed above, the primers were 556 and 555 (5'-TTCTGGGGTAGTGGCGAGC-GAAGGCTTC-3') to amplify a sequence of 238 bp.

Products were visualized by electrophoresis in 2% agarose gels stained with ethidium bromide. We used SA-MCF positive samples of previous study in our department (Erkılıç et al., 2017) as a positive control and a SA-MCF negative cattle whole blood sample as a negative control.

Biochemical results

The serum level of ALT, AST, GGT, ALP, creatine, urea, calcium, magnesium, glucose, phosphor, total protein, albumin, lipase, creatine kinase (CK) and creatine kinase-myocardial band are shown in Table 1, and the same parameters in the CSF are shown in Table 2 without cholesterol, Fe and LDH.

Table 1. Biochemical results obtained from the sera of control and MCF groups

PARAMETERS	CONTROL (mean±std.err.)	MCF (mean±std.err.)	P
ALT (U/L)	35.7±1.53	22.06±3.42	P<0.05*
AST (U/L)	82.89±4.21	117.69±10.32	P<0.05*
GGT (U/L)	20.56±1.31	24.08±1.70	P>0.05
ALP (U/L)	64.79±3.77	46.06±4.82	P<0.05*
Crea (mg/dL)	1.96±0.08	1.82±0.13	P>0.05
Urea(mg/dL)	8.01±0.67	11.06±0.72	P<0.05*
Ca (mg/dL)	9.15±0.11	7.55±0.32	P<0.01**
Mg (mEq/L)	2.73±0.12	1.85±0.11	P<0.001***
Glu (mg/dL)	59.04±2.86	70.12±2.67	P<0.05*
P (mg/dL)	5.42±0.34	4.62±0.26	P>0.05
TP (g/dL)	7.06±0.15	5.75±0.23	P<0.01**
Alb (g/dL)	3.08±0.13	2.42±0.7	P<0.001***
Lipaz (U/L)	2.79±0.19	2.57±0.19	P>0.05
CK (U/L)	231.88±39	781.22±143.84	P<0.05*
CK-MB (U/L)	84.03±10.01	62.43±6.84	P>0.05
Chol (mg/dL)	84.89±3.88	67.47±4.99	P<0.05*
LDH (U/L)	433.16±20.66	730.32±51.51	P<0.01**
Fe (µmol/L)	19.45±1.26	9.04±0.77	P<0.001***

Statistical analysis

Related with the assumptions of central limit theory, by increasing sample size (over 30) the distribution approaches to normal. Because of the sample size in this study is over 30, to compare the differences between the control and the study group, independent samples t test is applied. All the statistical analyses are done at 95% confidence level. P values of tests are given in relevant tables. The data obtained in this study were evaluated by SPSS®software.

Results

Clinical examination results

Typical clinical symptoms of the head-eye form of MCF have been identified in clinical examinations of infected cattle which were housed with sheep as stated by the owners. These typical symptoms consist of high fever (39.5-41ºC), keratoconjunctivitis, resulting in excessive mucopurulent lacrimation and photophobia, centripetal keratitis, corneal opacity, disphagie, redness of the mouth and nasal mucosa, nasal discharge, necrotic and erosive lesions in the mouth and buccal papillae, enlargement of lymph nodes. In addition, findings such as a tendency to sleep, indifference to the environment, a tendency to tilt the head to one side and changes in gait were observed, which were identified by researchers and thought to be caused by encephalitis.

Biochemical evaluations of the sera from both groups revealed statistically significant higher levels of AST, urea, glucose, CK (P<0.05) and LDH (P<0.01) levels, and statistically significant lower levels of ALT, ALP, cholesterol (P<0.05), Ca, TP (P<0.01), Mg, Albumin and Fe (P<0.001) levels in the MCF-diagnosed group of cattle than the healthy group.

Table 2. Biochemical results obtained from control and MCF groups CSF's

PARAMETERS	CONTROL (mean±std.err.)	MCF (mean±std.err.)	P
ALT (U/L)	5.45±0.45	6.01±0.78	P>0.05
AST (U/L)	16.75±0.59	22.82±2.52	P>0.05
GGT (U/L)	0.18±0.07	0.55±0.15	P>0.05
ALP (U/L)	3.93±0.31	4.66±0.52	P>0.05
Crea (mg/dL)	0.33±0.06	0.36±0.06	P>0.05
Urea (mg/dL)	16.09±3.6	20.23±3.09	P>0.05
Ca(mg/dL)	1.7±0.6	2.8±0.22	P<0.01**
Mg (mEq/L)	2.07±0.22	2.32±0.3	P>0.05
Glu(mg/dL)	49.21±2.10	34.60±3.63	P<0.05*
P (mg/dL)	4.24±0.88	4.61±0.89	P>0.05
TP (g/dL)	0.16±0.02	0.39±0.04	P<0.001*
Alb(g/dL)	0.1±0.01	0.11±0.01	P>0.05
Lipaz(U/L)	4.78±0.18	4.98±0.24	P>0.05
CK(U/L)	7.23±0.35	7.32±0.27	P>0.05
CK-MB(U/L)	2.01±0.59	2.66±0.30	P>0.05

Biochemical evaluations of CSF obtained from both groups resulted statistically significant lower levels of glucose (P<0.05) and statistically significant higher levels of Ca (P<0.01) and total protein levels (P<0.001) in the MCF-diagnosed group than the healthy group.

Hematological results

Results of hematological parameter levels are shown in Table 3.

Table 3. Hematological results obtained from Control and MCF groups

PARAMETERS	CONTROL (mean±std.err.)	MCF (mean±std.err.)	P
WBC	11.5±0.7	15.04±2.52	P<0.05*
LYM %	51.06±2.15	55.33±1 .94	P<0.05*
MON%	3.96±0.3	4.01±0.59	P>0.05
GRA%	44.98±3.34	45.62±4.10	P>0.05
LYM	5.45±0.21	7.16±1.2	P<0.01**
MON	0.42±0.02	0.36±0.05	P>0.05
GRA	4.18±0.7	4.71±0.4	P>0.05
RBC	5.68±0.43	8.43±0.43	P<0.001***
MCV	50.15±1.33	45.32±2.05	P>0.05
MCH	18.63±0.5	12.48±0.8	P<0.001***
MCHC	37.16±0.55	27.72±1.53	P<0.001***
Hct	28.21±1.89	37.05±1.35	P<0.001***
RDW	13.22±0.23	13.71±0.36	P>0.05
Hb	10.43±0.56	10.17±0.60	P>0.05
MPV	6.83±0.16	7.18±0.18	P>0.05
PCT	0.18±0.04	0.37±0.06	P<0.05*
PLT	229,2±36.57	467.26±62.63	P<0.01**
PDW	5.05±0.85	7.61±0.45	P<0.01**

Hematological evaluations of whole blood obtained from both groups showed statistically significant higher levels of WBC, LYM%, PCT (P<0.05), LYM, PDW, PLT (P<0.01), RBC and Hct (P<0.001) in the MCF-diagnosed group than the healthy group while statistically significant lower levels of MCH and MCHC (P<0.001) were observed.

Cytological results

The results of direct and cytospin slides of CSF are shown in Table 4.

Table 4. Cytologic slide results obtained from MCF group CSFs (n=10)

Case No	Results
CSF--0	A small number of lymphocytes and polymorphonuclear leukocytes (PNL)
CSF--1	A small number of lymphocytes and a small number of macrophages
CSF--2	Squamous epithelial cells compatible with contamination and a small number of macrophages
CSF--3	Blood components (erythrocytes and PNL), a small number of lymphocytes and plasma cells
CSF--4	A small number of PNL, lymphocytes and macrophages ***(Fig. 1)***
CSF--5	A large number of PNL and lymphocytes
CSF--6	Squamous epithelial cells compatible with contamination and PNL's, lymphocytes and macrophages
CSF--7	Hypocellular slides and a small number of PNL and lymphocytes
CSF--8	Acellular slides
CSF--9	Hypocellular slides and lymphocytes

Direct smear and cytospin preparations were scanned at 200x magnification and evaluated at 400x magnification in cellular areas (Figure 1). The presence of epithelial cells was not observed in any of the preparations examined to assess cytopathic effect. The blood elements detected in the slides were thought to be secondary to the traumatization caused by the process, whereas the squamous epithelium was thought to be due to contamination during passage through the skin.

In general evaluation of the preparations, it was considered that the existing numbers and proportions of inflammatory cells present were not too high for CSF according to the "human meningoencephalitis assessment". As the central nervous system findings in cattle in the study group were limited or mild, the cytological findings were evaluated according to the cases investigated.

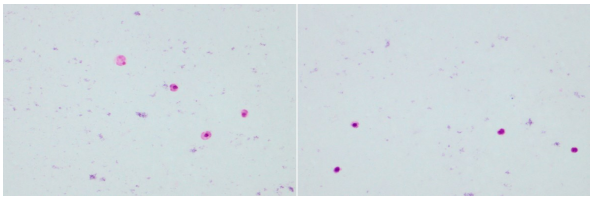

Figure 1. Degenerated macrophages and lymphocytes in CSF in MCF group (H&E, 400x).

PCR results

All of the 22 samples were detected as positive by nested-PCR (Figure 2) in the MCF group. A targeted 238 bp of PCR product was visualized from the positive control DNA extract.

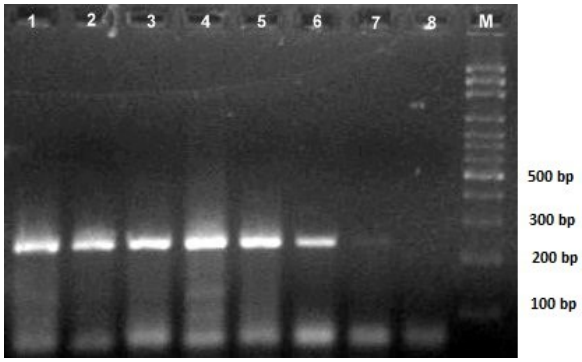

Figure 2. Nested-PCR product samplified from whole blood samples.
Lane 1**:** positive control sample**,** ***Lane 2-7*****:**nested-PCR products (238 bp), ***Lane 8*****:** negative control sample, M: 100 bp molecular weight marker (SolisBiodyne).

Discussion and Conclusion

In the aim of this study was to evaluate some physiological and biochemical parameters of blood, serum and CSF, as well as to investigate cytological changes that may occur in CSF and to evaluatetheir clinical significance in cattle clinically diagnosed with the head-eye form of MCF. To understand the pathogenesis of MCF, investigations based mainly on inflammation, immune mediated organ failure, toxins and effects of virus are frequently evaluated in natural cases. However, it is very important to reveal all the organs, tissues and related markers influenced by the forms investigated in the researches in order to reveal the pathogenesis and effective mechanisms that are quite complicated due to the affected organs and systems in different forms of the disease.

We determined specific clinical symptoms such as; high fever (39.5-41ºC), keratoconjunctivitis, resulting in excessive mucopurulent lacrimation and photophobia, centripedal keratitis, corneal opacity, disphagia, redness of the mouth and nasal mucosa, nasal discharge, necrotic and erosive lesions in the mouth and

buccal papillae, enlargement in lymph nodes in all infected animals in this study. We have also detected the finding that can be seen secondary to encephalitis such as; somnolence, keeping the head on one side, anxious and shaky gait, indifference to the environment etc. which are compatible with the reports for MCF (Masters et al., 2003; Radostits et al., 2007; Russel et al., 2009; Cunha et al., 2012; Headley et al., 2015; Lankester et al., 2016). Furthermore, the case history revealed that all the infected cattle werekept with sheep, which is a well-known source of infection in cattle (Roizman et al., 1992; Muller-Doblies et al., 1998; Masters et al., 2003; Erkılıç et al., 2017).

In the study, biochemical evaluations of the sera revealed statistically higher levels of AST, urea, glucose, CK ($P<0.05$) and LDH ($P<0.01$), and statistically lower levels of ALT, ALP, cholesterol ($P<0.05$), Ca, TP ($P<0.01$), Mg, albumin and Fe ($P<0.001$) in the group diagnosed with MCF-compared to healthy cattle. These findings were found to be consistent with those previously reported (Hill et al., 1993; Dettwiler et al., 2011; Dabak et al., 2012). As albumin is a negative acute phase protein, it is considered normal for it to decrease during inflammatory events. It is known that this decrease is also common for Fe. The decrease in albumin and Fe obtained in our study was considered as compatible with the disease. In a study on ruminants with septicemia, it is suggested that low values of Ca, Mg, and P are due to anorexia and malabsorption (Çitil et al., 2004). Similar values obtained in our study are probably due to the same clinical symptoms.

Biochemical evaluation of CSF from cattle diagnosed with MCF revealed that Ca ($P<0.01$) and total protein levels ($P<0.001$) were higher than in healthy cattle in the control group, and glucose ($P<0.05$) level was lower. The normal CSF protein level is less than 30 mg/dL. This value was found by some investigators to be 36-98 mg/dL in MCF. Damage to the blood-brain barrier causes the amount of protein in CSFto increase. Although this is usually explained by the amount of albumin in the blood, in some diseases it may also be caused by γ-globulin, which is over expressed by B lymphocytes in the nervous system. The CSF protein value obtained in our study is consistent with those reported by researchers (Abate et al., 1998; Di Terlizzi and Platt, 2006; Stokol et al., 2009; Scott, 2010; Pandey et al., 2015). Normal Ca values in CSF have been reported by researchers to be 1-1.5 mmol / L. Calcium is released from the choroid plexus and its level is provided by an active system. Plasma Ca level has no significant effect on Ca level in CSF. Some investigators have considered increased Ca in CSF as a marker of blood-brain barrier damage, which in various studies is thought to reflect the increased CSF protein concentrations (Rutter and Smales, 1976; Di Terlizzi and Platt, 2006; Stokol et al., 2009; Scott, 2010; Pandey and al., 2015).

In our study, CSF glucose levels are lower in healthy animals. Normally, CSF glucose levels are directly related to blood glucose levels. The fact that the CSF glucose is 60-80% of the blood glucose levels indicates that the functions of the central nervous system are normal. In our study, the values obtained from the CSF of MCF animals are 50% of the glucose values obtained from the sera of these animals. The low glucose level in CSF is also an important parameter for distinguishing bacterial/supportive meningitis from aseptic meningitis. Researchers have also reported that the low glucose level obtained from CSF is due to changes in the physiological function of the choroidal epithelium and the consumption of pathogenic elements or leukocytes in the system (Abate et al., 1998; Di Terlizzi and Platt, 2006; Pandey et al., 2015; Sri Rekha et al., 2015).

Increased CK is usually a sign of muscle damage, but may also be elevated in the CSF in diseases of the nervous system. Although there is no direct relationship between serum level and CSF levels, increases in CSF are considered to be indicative of poor prognosis in neurological diseases. In our study, serum CK levels were determined to be statistically significantly increased in MCF cases, but this increase was not at levels that would make a statistical difference in the CSF of the same group. It has also been reported that increases in AST and CK in CSF may be an important indicator of myelin degeneration (Hill et al., 1993; Di Terlizzi and Platt, 2006). Although the CSF AST and CK values in our study were not statistically significant, the relative increases were present.

In the present study, hematological evaluations of whole blood obtained from both groups showed higher LYM%, PCT ($P<0.05$), LYM, PDW, PLT ($P<0.01$), RBC and Hct ($P<0.001$) and lower MCH and MCHC ($P<0.001$) in the MCF group than in the healthy group. Although it was determined that the LYM, LYM%, RBC and Hct values obtained in the study were high in statistically different ratios compared to the healthy group, it was determined that these increases obtained from the MCF group were within the reference values and that the relative increase in the WBC value was not statistically significant. It was found that the low values obtained at the MCH and MCHC levels are compatible with the values found by the researchers (Hill et al., 1993; Detwiller et al., 2011; Kırbaş et al., 2013).

In our study, the presence of epithelial cell was not observed in the cytological evaluation of CSF. Blood elements and squamous epithelium, which were rarely detected in the slides, were thought to be related to traumatization during the procedure and contamina-

tion through the skin. A total of nine cases were examined; in four of the cases macrophages, six of the cases polymorphonuclear leukocytes (PNL) and eight of the cases lymphocytes were detected cytologically. Normal CSF consists of smaller number than 10 cells/µL of predominantly lymphocytes and neutrophils. In animals with encephalopathy, pleostosis is an important sign. In general, the lymphocytic mononuclear response is more prominent in viral infections, whereas PNLs predominate in acute bacterial CNS disease. In a study of calves infected by bovine herpes infested calves, the increase in CSF mononuclear cells was also found to be more pronounced after day 21. It has been stated that the presence of macrophages in CSF may occur after destruction of cerebral tissue or cerebral hemorrhage and/or may be due to protein-energy malnutrition (Stokol et al., 2009; Scott, 2010; Insemhagen et al., 2011).

In this study, it was aimed to reveal the changes in cattle diagnosed with the head-eye form of MCF by physical examination and laboratory tests, which were brought to Teaching Hospital of Faculty of Veterinary Medicine, as a whole by making CSF and blood biochemical, hematological and CSF cytological examinations and it was considered that these values, which have been studied in this disease very limited other viral diseases effecting the nervous system of cattle, would be the reference for future studies. As a result, in this study, although there were statistically significant differences in many biochemical parameters in blood serum, it was determined that especially ALB, Fe and Mg values showed greater differences, while TP, Glu and Ca values in CSF were statistically different. Many haematological parameters were found to be statistically different.

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Entansif Süt Sığırcılığında Buzağı Hastalıkları ve Ölümlerine Bağlı Ekonomik Kayıpların Belirlenmesi*,**,***

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Öz: Bu çalışmada, entansif süt sığırcılığında buzağı hastalıkları ve ölümlerine bağlı ekonomik kayıpların belirlenmesi amaçlanmıştır. Çalışma, Ekim 2020 ile Ekim 2021 tarihleri arasında Kayseri-Develi'de bulunan özel bir damızlık süt sığırı işletmesinde toplam 1147 buzağı verisi kullanılarak yapılmıştır. Çalışma boyunca incelenen buzağılar D1 (sağlıklı), D2 (hastalanıp iyileşen) ve D3 (hastalanıp ölen) şeklinde üç farklı duruma ayrılmıştır. Çalışmada, sağlıklı buzağılar için sadece büyütme maliyeti hesaplanırken, hasta buzağılar için ilave işçilik, veteriner hekim, altlık ve ilaç/tedavi masrafları hesaplanmıştır. Ölen buzağılar için ise ilave işçilik, veteriner hekim, ilaç/tedavi masrafı ile ölen buzağı bedeli hesaplamalara dâhil edilmiştir. Ölen buzağı bedeli belirlenirken TİGEM tarafından ırklara ve cinsiyete göre belirlenen buzağı fiyatları dikkate alınmıştır. Çalışma bulgularına göre, hasta buzağı sayısının 626 (%54.6), hastalık (vaka) sayısının ise 809 (%70.5) ve buzağı ölüm oranının %13.3 olduğu belirlenmiştir. Buzağı hastalık ve ölümleri üzerinde anne yaşının, annenin laktasyon sayısının, doğum mevsiminin, babanın (boğa), buzağı ırkının, kolostrum alma durumunun etkili olduğu tespit edilmiştir (P<0.05). Çalışmada sütten kesime kadar sağlıklı, hastalanıp iyileşen ve ölen buzağıların ortalama büyütme maliyetleri sırasıyla 6295.3TL ($340.2), 6508.6TL ($351.8) ve 2547.3TL ($137.7) olarak hesaplanmıştır. Çalışmada en çok görülen hastalık grubu sindirim sistemi hastalıkları olup 175.4-186.5TL/vaka ($9.5-10.1) arasında ve solunum sistemi hastalıkları 114.0-138.1TL/vaka ($6.2-7.5) arasında ekonomik kayba neden olmaktadır. Sindirim sistemi hastalıkları kaynaklı buzağı ölümü 8199.0-10190.5TL ($443.2-550.8) arasında ve solunum sistemi hastalıkları kaynaklı buzağı ölümü 7642.6-11860.1TL ($413.1-641.1) arasında ekonomik kayba neden olmaktadır. Buzağı hastalıklarının işletmeye toplam maliyeti 122650.8TL/yıl (6629.8 $/yıl) olurken, ölümlerin toplam ekonomik kaybı 1462618.8TL/yıl (79060.5 $/yıl) olmuştur. Süt sığırcılığı işletmelerinde buzağılardaki hastalık ve ölümlerin azaltılabilmesi için; buzağı sağlığını etkileyen risk faktörlerinin (genel olarak işletmelerin uygulamaları, anneye ait ve buzağıya ait risk faktörleri) iyi bilinmesi ve gerekli tedbirlerin alınması gerekmektedir. Mevcut çalışma ile Türkiye şartlarında buzağıların hastalıklarına ve ölümlerine bağlı ekonomik kayıplar hesaplanarak hayvansal üretimin daha karlı yapılabilmesi için karar desteği oluşturulması sağlanmıştır.
Anahtar kelimeler: Buzağı, ekonomik kayıp, hastalık, ölüm, süt sığırcılığı

Determination of Economic Losses Related to Calf Diseases and Mortalities in Intensive Dairy Cattle

Abstract: This study aimed to determine the economic losses due to calf diseases and deaths in intensive dairy cattle. The study was conducted using data from a total of 1147 calves on a private dairy farm in Kayseri-Develi between October 2020 and October 2021. The calves examined throughout the study were divided into three different status: D1 (healthy), D2 (recovered), and D3 (death). For the death calves, additional labour, veterinarian, medicine/treatment costs, and the cost of the decreased calf are included in the calculations. While determining the price of the death calves, the calf prices determined by TİGEM according to race and gender were taken into account. In the study, it was determined that the number of sick calves was 626 (54.6%), the number of diseases (case) was 809 (70.5), and the calf mortality rate was 13.3%. It was determined that maternal age, lactation number of the cow, birth season, father (bull), calf race, and colostrum intake were effective on calf morbidity and mortality (P<0.05). In the study, it was determined that the rearing costs of healthy, sick, and death calves up to weaning were 6295.3TL ($340.2), 6508.6TL ($351.8) and 2547.3TL ($137.7), respectively. The costs of most common digestive system diseases in the study calculated between 175.4-186.5TL/case (9.5-10.1 $/case) and respiratory system diseases between 114.0-138.1TL/case (6.2-7.5 $/case).The cost of calf death due to digestive system diseases calculated 8199.0-10190.5TL ($443.2-550.8) and calf death due to respiratory system diseases calculated 7642.6-11860.1TL ($413.1-641.1). While the total cost of calf diseases was 122650.8TL/year (6629.8 $/year), the total economic losses of deaths was 1462618.8TL/year (79060.5 $/year). In dairy cattle farming, to reduce diseases and deaths in calves, is necessary to know the risk factors affecting the calf health (generally the practices of the farms, the risk factors of the cow and the calf) and to take the necessary precautions. In this study, the economic losses of calves due to disease and death in Turkish conditions were calculated and decision support was provided to make more profitable livestock production.
Keywords: Calf, dairy cattle, disease, economic losses, mortality

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Giriş

Ekonomik bir faaliyet olan süt sığırcılığında ana gelir süt üretiminden elde edilirken, buzağılar da en önemli tali geliri oluşturmaktadır. O nedenle daha fazla karlılık için bir taraftan hayvanlardan yüksek süt verimi alınması hedeflenmekte, diğer taraftan da sağlıklı buzağıların doğması sürünün geleceği ve işletmenin sürdürülebilirliği açısından önem arz etmektedir. İşletmelerde buzağıların elde edilmesi zor olduğu kadar (kızgınlık, tohumlama, gebelik, doğum) sağlıklı bir şekilde yaşatılması için de ayrı bir özen ve çaba gerektirmektedir. Aksi halde, hasta olan buzağıları iyileştirme çabaları sonuçsuz kalırsa ölüm şekillenebilmektedir.

Türkiye'de süt sığırcılığı işletmelerinde buzağı hastalıkları az ya da çok hemen her işletmede görülmekte ve yaklaşık %5-20 arasında ölümle sonuçlanmaktadır. Hatta aile tipi işletmelerde bu oran daha da (% 50'ye kadar) artabilmekte ve önemli ekonomik kayıplara neden olmaktadır (Demir ve ark., 2019). Bu durumda, azami kar amacıyla üretimde bulunan süt sığırcılığı işletmelerinde yaşanan buzağı hastalıkları ve kayıpları, gerek işletme düzeyinde gerekse ulusal düzeyde ekonomik yönden optimumdan uzaklaşılmasına, genetik potansiyelin gerilemesine ve böylece üreticilerin daha düşük karlılıkla hatta bazen zararla üretim yapmasına yol açmaktadır. Ayrıca Türkiye'de artan nüfusun kırmızı et ihtiyacının sağlanabilmesi ve sosyo-ekonomik refah seviyesi yükselebilmesi için buzağıların hayatta kalması gerekmektedir. Çünkü Türkiye'de kırmızı etin tamamı büyükbaş ve küçükbaş hayvanlardan sağlanmakla birlikte mevcut talebin %88'i sığır etinden karşılanmaktadır (Akın ve ark., 2020).

Süt sığırcılığı işletmelerinde görülen buzağı hastalıkları ve kayıplarının önlenmesi için; hastalık ve kayıplara neden olan faktörlerin (etiyoloji) belirlenmesi ve işletme ya da ülke ekonomisine bunların yansımalarının ortaya konulması gerekmektedir. Bu sayede karar desteği sağlanabilir, risk faktörleri doğru belirlenebilir, hastalık kontrol tedbirleri alınabilir ve kayıpların azaltılması yoluna gidilebilir.

Bu düşüncelerden hareketle mevcut çalışma ile süt sığırcılığı işletmelerinin en önemli tali gelir kaynağı olan ve ülkenin canlı büyükbaş hayvan stokunu oluşturacak buzağıların, hastalıklarına ve ölümlerine bağlı ekonomik kayıplarının ortaya konulması, elde edilen sonuçların gelecekte hastalık kontrol kararlarında yol gösterici olarak kullanılması ve böylece hayvancılığın daha karlı yapılmasının önünün açılmasına katkı sağlanması düşünülmektedir.

Materyal ve Metod

Çalışma gereci

Çalışmada, Ekim 2020 ile Ekim 2021 tarihleri arasında Kayseri-Develi'de bulunan özel bir damızlık süt sığırı işletmesinde 1147 baş gebe sığırdanelde edilen buzağılardan temin edilmiş, ancak 48 buzağı ölü doğduğu için analizler toplam 1099 buzağı verisi üzerinde yapılmıştır. İşletmede bulunan buzağıların doğumdan itibaren sütten kesim yaşına kadar (0-70 gün) verileri bireysel olarak kaydedilmiştir.

Çalışmada incelenen buzağılar D1 (sütten kesime kadar hiç hastalık geçirmeyen), D2 (en az bir kez hastalık geçirip iyileşen) ve D3 (en az bir hastalık geçirip ölen) şeklinde üç duruma ayrılmıştır (Tablo 1).

Ekonomik analiz metodu

Çalışmada uygulanan ekonomik analiz metodu Tablo 1'de verilmiştir.

Tablo 1. Ekonomik analiz için oluşturulan metod

Durumlar	Açıklama	Ekonomik Analiz Metodu (Masraflar)
Durum 1	Sağlıklı	**D1=** [Beslenme maliyeti + İşçilik + Veteriner Hekim + Kontrol Harcaması+ Amortisman + Bakım/Onarım + Elektrik/Su+ Genel İdare Gideri + Altlık Maliyeti]
Durum 2	Hastalanıp İyileşen	**D2=** [D1 + (İlave İşçilik + Veteriner Hekim + Tedavi Gideri)]
Durum 3	Hastalanıp Ölen	**D3=** [D2 + (Ölen Buzağı Bedeli)]

D: Durum

Hastalık ve ölüm durumunda (D2 ve D3) yeni ve nüks eden hastalıklar olması durumunda işçilik, veteriner ve tedavi ücretleri her bir vaka için yeniden ayrı ayrı hesaplamalara dâhil edilmiştir (Tablo 1).

İşletme Giderleri

a. Beslenme gideri: Çalışma dönemi boyunca bir buzağının sütten kesim yaşına kadar süt ve yem [kaba ve buzağı başlangıç yemi (BBY)] tüketim miktarının cari süt ve yem fiyatı ile çarpımı sonucu elde edilmiştir. Buzağıların ilk 30 günlük dönemde ortalama 5.8 lt/gün, 31-70 günlük dönemde ise 7.4 lt/gün süt tükettikleri tespit edilmiş olup, süt fiyatı olarak sütün işletmede üretim maliyeti (8.5TL/lt=0.5$/lt) dikkate alınmıştır. Buzağılar ilk 15 günlük yaşa kadar sadece süt tüketirken, 15 günlük yaştan sonra (55 gün) ortalama 45 kg yem (kaba+BBY) tükettiği ve toplam tüketimin %80'inin BBY, %20'sinin kaba yem olduğu tespit edilmiş olup, ekonomik analizlerde bu

tüketim miktarı dikkate alınmıştır. BBY'nin fiyatı 6.4TL/kg ($0.3), kaba yem (yonca) fiyatı 3TL/kg ($0.2) olarak hesaplanmıştır.

b. Veteriner hekim ve işçilik maliyeti: Veteriner hekim ve işçilik maliyeti hesaplanırken maaş+sigorta+yemek ücreti dikkate alınmıştır. Hastalanan veya ölen buzağılarda tedavi için harcanan ilave süre (dk) ile veteriner hekim ücreti (fırsat maliyeti) ve işçilik ücreti hesaplamalara dâhil edilmiştir.

c. Koruyucu hekimlik gideri: Bir buzağıya hastalıklardan korunması için doğumdan itibaren sütten kesim yaşına kadar uygulanan aşı, ilaç vb. giderler cari fiyatlarına göre dikkate alınmıştır.

d. Amortisman maliyeti: Buzağı büyütme bölümünde bulunan bina, alet ve ekipmanların yıllık amortismanı (yıpranma payı);

Elde ediliş değer –Hurda değeri / Ekonomik ömür formülü yardımıyla hesaplanmıştır (Capital, 2002).

e. Elektrik/su gideri: Buzağı büyütme bölümünde* tüketilen elektrik ve su giderleri hesaplanmıştır (*buzağının bu bölmede kaldığı süre boyunca).

f. Altlık Maliyeti: Sağlıklı buzağılarda altlık değişimi beş gün arayla, hastalarda ve ölenlerde ise iki gün arayla olacak şekilde altlık değişimi yapılmıştır. Hesaplamalarda altlık fiyatı 1.6TL/kg ($0.1) alınmıştır.

g.Genel idare gideri: Buzağıların sütten kesim yaşına kadar oluşan genel idare giderleri (kırtasiye, telefon, yönetici ücretleri, yakıt vs.) hesaplamalara dahil edilmiştir.

h. Bakım/onarım gideri: Bakım ve onarım giderleri buzağı büyütme bölümünün elde ediliş değerlerinin % 3 (bakım=%1; onarım=%2) şeklinde alınmıştır (Günlü ve Sakarya, 2001).

i. Ölen buzağı gideri: Ölen buzağı maliyetinin hesaplanmasında büyütme giderleri (beslenme, işçilik, Veteriner Hekim, kontrol harcaması, amortisman, bakım/onarım, elektrik/su, genel idare ve altlık) ile ilave giderler (işçilik, Veteriner Hekim ve tedavi) ve ölüm yaşındaki (gün) bir buzağının TİGEM tarafından ırklara ve cinsiyete göre belirlenen buzağı bedelleri dikkate alınarak yapılmıştır (TİGEM, 2022).

İstatistiksel analizler

Verilerin parametrik test varsayımlarına uygunluğu normal dağılım için Kolmogorov-Smirnov testi, varyansların homojenliği Levene testi ile kontrol edildi. Anneye ait bilgiler kısmında anne yaşı; buzağıya ait bilgiler kısmında doğum mevsimi, buzağı ırkı ve kolostrum alma durumu kategorik bağımsız değişken olarak alınarak bağımlı değişken (buzağıların sağlık durumu) ile aralarındaki ilişki beklenen gözlem sayıları dikkate alınarak Pearsonki-kare istatistiği ile incelendi. Buzağıların babalarıalgomeratif hiyerarşik bir yöntem olaniki adımlı (TwoStep) kümeleme analizi ileikidoğal ayrılarak ki-kare analizine dâhil edildi. Buzağıların sağlık durumları, maliyet ve gelir yönünden tek örneklem t test ile karşılaştırıldı. Verilerin özetlenmesindeki-kare analiziiçin frekans ve yüzdelikler, tek örneklem t test için aritmetik ortalama ve standart sapma kullanılmıştır. İstatistiksel analizlerde IBM SPSS 14 paket programı kullanıldı. Anlamlılık düzeyi P<0.05 olarak belirlendi.

Bulgular

Çalışma bulguları; anneye ait, buzağıya ait, hastalık ve ekonomik analiz bulguları şeklinde dört kategoride incelenmiştir.

Anneye ait bulgular

Anne yaşı ve laktasyon sayısının buzağı hastalıkları ve ölümü üzerine etkisi incelenmiş ve Tablo 2'de verilmiştir.

Anne yaşı ile buzağı hastalık ve ölümleri arasında istatistiksel fark olduğu tespit edilmiştir (P<0.05). En düşük hastalık ve ölüm oranı 5 ve üzeri yaştaki anne-

Tablo 2. Anne yaşı ve laktasyon sayısına göre buzağı hastalık ve ölüm sayıları

Anne Yaşı	Durum			Toplam (n=1099)	P Değeri
	D1 (n=473)	D2 (n=521)	D3 (n=105)		
2	158 (%46.7)	153 (%45.3)	27 (%8.0)	338 (%100.0)	X^2=11.814
3-4	**229[a] (%38.8)**	**294[b] (%49.8)**	**67[b] (%11.4)**	**590 (%100.0)**	Sd=4
5 ve üzeri	86 (%50.0)	75 (%43.6)	11 (%6.4)	172 (%100.0)	P< 0.05
Laktasyon Sayısı	**Durum**			**Toplam (n=1099)**	**P Değeri**
	D1 (n=473)	**D2 (n=521)**	**D3 (n=105)**		
1	158 (%46.7)	153 (%45.3)	27 (%8.0)	338 (%100.0)	X^2=10.070
2-3	**235[a] (%39.2)**	**298[ab] (%49.7)**	**67[b] (%11.2)**	**600 (%100.0)**	Sd=4
4 ve üzeri	80 (%49.7)	70 (%43.5)	11 (%6.8)	161 (%100.0)	**P<0.05**

[a,b]: aynı satırda farklı harfler ile gösterilen durumlar arasındaki farklılık istatistiksel olarak önemlidir.

lerden doğan buzağılarda görülürken, en yüksek hastalık ve ölüm oranı 3-4 yaşındaki annelerin buzağılarında görülmüştür. Yani en sağlıklı buzağılar 5 ve üzeri yaştaki annelerin buzağıları olmuştur (Tablo 2).

Laktasyon sayısı ile buzağı hastalık ve ölümleri arasında istatistiksel fark olduğu tespit edilmiştir (P<0.05). En düşük hastalık ve ölüm oranı, 4 ve üzeri laktasyona sahip annelerden doğan buzağılarda görülürken, en yüksek hastalık ve ölüm oranı 2. ve 3. laktasyondaki annelerin buzağılarında görülmüştür. Ayrıca 2. laktasyondan sonra buzağılarda ölüm oranı giderek azalmıştır (Tablo 2).

Buzağıya ait bulgular

Doğum mevsimi ve babanın buzağı hastalık ve ölümleri üzerine etkisi incelenmiş ve Tablo 3'de verilmiştir.

yavruları hastalıklara daha dirençli olurken, bazılarının daha hassas olduğu görülmüştür (Tablo 3).

Buzağı ırkının hastalıklar ve ölüm üzerine etkisi

Buzağı ırkı ve kolostrum alma durumununhastalıklar ve ölüm üzerine etkisi incelenmiş ve Tablo 4'de verilmiştir.

İncelenen buzağı ırkı ile hastalık ve ölüm oranları arasında istatistiksel bir ilişki tespit edilmiştir (P<0.001). Simental ırkı buzağıların %31.4'ü sütten kesilene kadar (70 gün) hiç hastalanmazken, % 68.6'sı en az bir kez hastalanmış, bunların %54.9'u hastalığı atlatmış ve geriye kalan 54 buzağı (%13.7) ise ölmüştür. Çalışma boyunca 704 baş Holstein buzağı dünyaya gelmiştir. Bunların 349'u (%49.6) sağlıklı kalırken, 355 buzağı (%50.4) en az bir kez hasta-

Tablo 3. Doğum mevsimi ve babanın buzağı hastalık ve ölümlerine etkisi

Doğum Mevsimi	Durum			Toplam	P Değeri
	D1 (n=473)	D2 (n=521)	D3 (n=105)	(n=1099)	
Kış	**148[a] (%59.0)**	**77[b] (%30.7)**	**26[a] (%10.4)**	**251 (%100.0)**	X^2=49.07 Sd=6**P<0. 001**
İlkbahar	126 (%43.8)	133(%46.2)	29 (%10.1)	288 (% 100.0)	
Yaz	**109[a] (%32.9)**	**195[b] (%58.9)**	**27[ab] (%8.2)**	**331 (%100.0)**	
Sonbahar	90 (%39.3)	116 (%50.7)	23 (%10.0)	229 (%100.0)	
Baba/Grup	**Durum**			**Toplam**	**P Değeri**
	D1 (n=473)	**D2 (n=521)**	**D3 (n=105)**	**(n=1099)**	
1	**161[a] (%31.9)**	**284[b] (%56.3)**	**59[b] (%11.7)**	**504 (%100.0)**	X^2=46.841 Sd=2 **P<0.001**
2	**312[a] (%52.4)**	**237[b](%39.8)**	**46[b] (%7.7)**	**595 (%100.0)**	

[a,b]: aynı satırda farklı harfler ile gösterilen durumlar arasındaki farklılık istatistiksel olarak önemlidir.

Çalışma bulgularına göre, mevsim ile buzağı hastalıkları ve ölüm oranları arasında istatistiksel fark olduğu tespit edilmiştir (P<0.001). Mevsimler itibariyle en fazla buzağının yazın (%30.1) ve ilkbaharda (%26.2), en az ise sonbaharda (%20.8) doğduğu belirlenmiştir. En fazla hastalık oranı yaz mevsiminde daha sonra sonbahar, ilkbahar ve en az kış mevsiminde olmuştur. Mevsimlere göre en fazla ölüm oranı kış (%10.4) mevsiminde olurken, en az ölüm oranı ise yaz (%8.2) mevsiminde olmuştur. İşletmedeki toplam hastalık oranı %73.6 olurken, ortalama ölüm oranı ise ölü doğumlar dikkate alındığında %13.3; alınmadığında ise %9.6 olmuştur (Tablo 3).

Çalışmada işletme tarafından kullanılan 15 farklı boğanın buzağı hastalık ve ölümlerine etkisi incelenmiştir. Bu 15 farklı boğa yapılan kümeleme analizinde program tarafından 2 gruba ayrılmıştır. Elde edilen bulgulara göre boğanın buzağı hastalık ve ölümleri üzerinde etkili olduğu tespit edilmiştir (P<0.001). Birinci grupta bulunan babaların buzağılarında hastalık ve ölüm oranı, ikinci gruptaki babaların buzağılarından daha yüksek bulunmuştur. Yani bazı babaların

lanmış ve 304'ü (%43.2) tedaviye cevap vermiş, geriye kalan 51 buzağı (%7.2) ise tedaviye cevap vermeyip ölmüştür. Simental buzağıların hastalık ve ölüm oranları Holstein buzağılara göre daha fazla olduğu görülmüştür. Simental buzağılarda ölüm oranı %5.5 daha fazla gerçekleşmiştir (Tablo 4).

Buzağıların kolostrum alma durumu ile hastalık ve ölüm oranı arasında istatistiksel bir ilişki olduğu tespit edilmiştir (P<0.001). Ayrıca yetersiz kolostrum alan toplam 130 buzağının 37'si (%28.5) hiç hastalanmamış olup, 93 (%71.5) buzağı en az bir kez hastalanmış ve 23 (%17.7) buzağı ölmüştür. Yeterli miktarda kolostrum alan 969 buzağının 436'sı (%45.0) sütten kesim boyunca sağlıklı olup, hastalanan 533 (%55.0) buzağının 82'sinin (%8.5) ise öldüğü görülmüştür. Buna göre yeterli miktarda kolostrum almayan buzağılarda hastalık ve ölüm oranının yeterli miktarda kolostrum alanlara göre daha fazla olduğu tespit edilmiştir. Ölüm oranında %9.2'lik fark oluşmuştur (Tablo 4).

Tablo 4. Buzağı ırkı ve kolostrumalma durumunun hastalıklar ve ölüm üzerine etkisi

Buzağı Irkı	Durum D1 (n=473)	D2 (n=521)	D3 (n=105)	Toplam (n=1099)	P Değeri
Simental	**124[a] (%31.4)**	**217[b] (%54.9)**	**54[b] (%13.7)**	**398 (%100.0)**	X^2=37.747 Sd=2
Holstein	**349[a] (%49.6)**	**304[b] (%43.2)**	**51[b] (%7.2)**	**701 (%100.0)**	**P<0.001**
Kolostrum Alma Durumu	**Durum D1 (n=473)**	**D2 (n=521)**	**D3 (n=105)**	**Toplam (n=1099)**	**P Değeri**
Yetersiz*	**37[a] (%28.5)**	**70[b] (%53.8)**	**23[b] (%17.7)**	**130 (%100.0)**	X^2=18.790 Sd=2
Yeterli**	**436[a] (%45.0)**	**451[b] (%46.5)**	**82[b] (%8.5)**	**969 (%100.0)**	**P<0.001**

[a,b;] aynı satırda farklı harfler ile gösterilen durumlar arasındaki farklılık istatistiksel olarak önemlidir. . *:<canlı ağırlığın %10'undan az/günlük, **; ≥canlı ağırlığın %10'undan fazla/günlük

Ekonomik analiz

Çalışmada 1099 buzağıya ait büyütme (beslenme + işçilik + veteriner hekim + amortisman + bakım/ onarım + elektrik + su + altlık + genel idare) maliyetleri ile birlikte hasta ve ölen buzağılara ait ilave (işçilik + veteriner hekim + ilaç + ölen buzağı bedeli) maliyetler ayrı ayrı hesaplanmıştır.

1- Buzağı büyütme, hastalık ve ölüm maliyetleri

Buzağıların sütten kesime kadar (0-70 gün) büyütme, hastalık ve ölüm maliyetleri Tablo 5'de verilmiştir.

Çalışmada elde edilen bulgulara göre, toplam büyütme maliyeti sağlıklı buzağılarda 6295.3TL (=$340.3 ve 740.6 lt süt eşdeğeri) olurken, hastalanarak iyileşen bir buzağının büyütme maliyeti %3.4 (hastalık maliyeti=213.3TL=$11.5) artarak 6508.6TL (=$351.8 ve 765.7 lt süt eşdeğeri) olmuştur. Ölen buzağılarda ise büyütme maliyetlerine (2547.3TL= $137.7 ve 299.7 lt süt eşdeğeri) 357.3TL (=$19.3 ve 42.0 lt süt eşdeğeri) hastalık maliyeti dâhil edilmiştir (ortalama ölüm günü= 26) (Tablo 5).

Tablo 5. Sütten kesim döneminde buzağı büyütme (D1), hastalık (D2) ve ölüm (D3) maliyetleri (0-70 gün)

Maliyet Unsuru	D1		D2		D3	
	TL	%	TL	%	TL	%
A. Buzağı Büyütme Maliyeti (TL/Buzağı)						
A1. Buzağı Besleme Maliyeti	4252.3	67.5	4252.3	65.3	1415.2	55.5
-Süt Maliyeti	3995.0	63.4	3995.0	61.4	1354.8	53.2
-BBY	230.3	3.7	230.3	3.5	54.1	2.1
-Kaba Yem	27.0	0.4	27.0	0.4	6.3	0.2
A2. İşçilik	836.7	13.3	854.3	13.1	339.3	13.4
A3. Veteriner Hekim	310.5	5.0	340.8	5.3	162.9	6.4
A4. Amortisman	71.9	1.1	71.9	1.1	16.2	0.6
A5. Bakım/Onarım	2.2	0.04	2.2	0.03	0.5	0.02
A6. Elektrik	72.7	1.2	72.7	1.1	28.6	1.1
A7. Su	26.7	0.4	26.7	0.4	9.1	0.4
A8. Altlık	370.3	5.9	434.3	6.7	146.8	5.8
A9. Genel İdare	156.0	2.5	156.0	2.4	57.7	2.3
A10. Koruyucu Hekimlik	196.0	3.1	196.0	3.0	143.7	5.6
A11. İlaç Maliyeti	-	-	101.4	1.6	227.3	8.9
A12. Ölen Buzağı Bedeli*	-	-	-	-	*	-
Toplam Maliyet	6295.3	100.0	6508.6	100.0	2547.3*	100.0

**Ölen buzağıların işletmeye olan toplam maliyetinde; ırk, yaş ve cinsiyete göre buzağı bedeli eklenecektir. $1=18.5 TL*

2- Hastalık ve ölümlerin işletmeye olan toplam maliyeti

Sütten kesim yaşına kadar incelenen 1099 buzağının toplam hastalık ve ölüm maliyetleri Tablo 6'da verilmiştir.

Buzağı hastalıklarının işletmeye toplam maliyeti 122650.8 (6629.8 $/yıl) TL olurken, ölümlerin maliyeti 1003760.5 (54257.3 $/yıl) TL olmuştur. Eğer ölü doğumlara bağlı ekonomik kayıplar da dikkate alınırsa (458858.3 TL= $24803.2) toplam ölümlere bağlı ekonomik kayıp 1462618.8 (79060.5 $/yıl) TL olacaktır.İncelenen işletmede bir yılda toplam 1147 buzağı doğmuş, 48 buzağı ölü doğmuş ve 105 buzağı da doğduktan sonra olmak üzere toplam 153 buzağı (% 13.3) ölmüştür (Tablo 6).

Tablo 6. Toplam hastalık ve ölüm maliyetleri (TL)

Hastalık	HS	THM	OHM	ÖBS	TÖBM*	OÖBM*
Sindirim S.	522	95810.3	185.5	52	502321.5	9660.0
Solunum S.	151	19540.0	129.4	29	284066.6	9795.4
Miks	29	7053.7	243.2	20	186235.7	9311.8
Diğer	2	246.8	123.4	4	31136.7	7784.1
Toplam Hastalık	704	122650.8	174.2	105	1003760.5	9559.6
İncelenen Hastalıklı/ Ölen Buzağı	521	122650.8	235.4	105	1003760.5	9559.6

** ilaç+veteriner hekim+işçilik+ölen buzağı*

Çalışmada elde edilen bulgulara göre, buzağılarda sütten kesim döneminde en fazla hastalık ve ölümler sindirim sistemi kaynaklı olmuştur. Hastalık başına ortalama maliyet en yüksek miks hastalıklarında (243.2TL/vaka) hesaplanmıştır. Ortalama ölen buzağı maliyeti en yüksek solunum sistemi hastalığında (9795.4TL/baş) tespit edilmiştir. Tüm dönemlerde hastalanan buzağıların ortalama maliyeti 174.2TL/vaka (235.4/baş), ölen buzağıların ise ortalama 9559.6TL/baş ($516.7) olarak hesaplanmıştır (Tablo 6).

3- Hastalık ve ölümlerin maliyet, gelir ve karlılığa etkisi

Buzağılardaki hastalık ve ölümlerin maliyet, gelir ve karlılığa etkisi buzağının ırkına ve cinsiyetine göre incelenmiş ve Tablo 7'de verilmiştir.

Tablo 7. Buzağının ırkına ve cinsiyetine göre hastalık ve ölümlerin maliyet, gelir ve karlılığa etkisi (TL)

Holstein Erkek	**D1** $\bar{X} \pm S$	**D2** $\bar{X} \pm S$	**D3** $\bar{X} \pm S$	**D2-D1**	**D3-D1**	**P Değeri** (Tek Örneklem t Test)
Maliyet	6295.3	6492.4±119.1	8305.3±1926.8	197.1	2010.0	**<0.001**
Gelir*	7201.6	6840.9	0.0	-360.7	-7201.6	-
Kar	906.3	348.5±119.1	-8305.3±1926.8	-557.8	-9211.6	**<0.001**
Holstein Dişi	**D1** $\bar{X} \pm S$	**D2** $\bar{X} \pm S$	**D3** $\bar{X} \pm S$	**D2-D1**	**D3-D1**	**P Değeri** (Tek Örneklem t Test)
Maliyet	6295.3	6475.7±69.0	10795.2±2563.2	180.4	4499.9	**<0.001**
Gelir*	9801.0	9311.6	0.0	-489.4	-9801.0	-
Kar	3505.7	2835.9±69.0	-10795.2±2563.2	-6698	14300.9	**<0.001**
Simental Erkek	**D1** $\bar{X} \pm S$	**D2** $\bar{X} \pm S$	**D3** $\bar{X} \pm S$	**D2-D1**	**D3-D1**	**P Değeri** (Tek Örneklem t Test)
Maliyet	6295.3	6539.2±124.6	12568.3±2323.1	243.9	6273.0	**<0.001**
Gelir*	10701.0	10165.9	0.0	-535.1	-10701.0	-
Kar	4405.7	3626.7±124.6	-12568.3±2323.1	-779.0	-16974.0	**<0.001**
Simental Dişi	**D1** $\bar{X} \pm S$	**D2** $\bar{X} \pm S$	**D3** $\bar{X} \pm S$	**D2-D1**	**D3-D1**	**P Değeri** (Tek Örneklem t Test)
Maliyet	6295.3	6541.2±161.3	16625.3±1578.6	245.9	10330.0	**<0.001**
Gelir*	15211.0	14450.5	0.0	-760.5	-15211.0	-
Kar	8915.7	7909.3±161.3	-16625.3±1578.6	1006.4	25541.0	**<0.001**

** Durumlar kendi içerisinde gelir yönünden sabit olduğundan istatistiksel test yapılmamıştır.*

Çalışmada, hiç hastalık geçirmeyen Holstein erkek buzağılar işletmeye sütten kesim dönemi sonunda 906.3TL/baş ($49.0), hastalanıp iyileşenler 348.5TL/baş ($18.8) işletmeye kar sağlarken, hastalanıp ölen buzağılar 8305.3TL/baş ($448.9) zarar oluşturmuştur (Tablo 7).

Hiç hastalık geçirmeyen Holstein dişi buzağılar işletmeye sütten kesim dönemi sonunda 3505.7TL/baş ($189.5), hastalanıp iyileşenler 2835.9TL/baş ($153.3) kar sağlarken, hastalanıp ölen buzağılar 10795.2TL/baş ($583.5) zarar oluşturmuştur (Tablo 7).

Çalışmada, hiç hastalık geçirmeyen Simental erkek buzağılar işletmeye sütten kesim dönemi sonunda 4405.7TL/baş ($238.1), hastalanıp iyileşenler 3626.7TL/baş ($196.0) kar sağlarken, hastalanıp ölen buzağılar 12568.3TL/baş ($679.4) zarar oluşturmuştur (Tablo 7).

Çalışmada, hiç hastalık geçirmeyen Simental dişi buzağılar işletmeye sütten kesim dönemi sonunda 8915.7TL/baş ($481.9), hastalanıp iyileşenler 7909.3TL/baş ($427.5) işletmeye kar sağlarken, hastalanıp ölen buzağılar 16625.3TL/baş ($898.7) zarar oluşturmuştur (Tablo 7).

Tartışma ve Sonuç

Ekonomik bir faaliyet olan süt sığırcılığında ana gelir süt üretiminden elde edilirken, buzağılar da en önemli tali geliri oluşturmaktadır. O nedenle daha fazla karlılık için bir taraftan hayvanlardan yüksek süt verimi alınması hedeflenirken, diğer taraftan da sağlıklı buzağıların elde edilmesi sürünün geleceği ve sürdürülebilirlik açısından önem arz etmektedir. Ayrıca buzağıların hayatta kalması ve sağlıklı bir şekilde büyüyerek üretime katkı sağlamaları süt işletmelerinin dolayısıyla ülkenin süt endüstrisinin geleceği açısından önemli bir faktördür (Akın, 2020; Akın ve ark., 2020). Buzağı hastalık ve ölümleri, işletmelerin ekonomilerini etkilediği gibi sektör ve ülke ekonomilerini de ağır kayıplara uğratmaktadır.

Anneye ait faktörler incelendiğinde; mevcut çalışmada hastalık ve ölüm oranı genç yaştaki (≤4 yaş) annelerin buzağılarında daha fazla görülmüş, anne yaşının ilerlemesi ile birlikte (≥5 yaş), buzağı hastalık ve ölüm oranlarının azaldığı belirlenmiştir. Anne yaşı ile benzer sonuçlar laktasyon sayısında da elde edilmiştir. Literatürde genç yaştaki annnelerin buzağılarında hastalık ve ölüm oranının yüksek olduğunu bildiren çok sayıda çalışma vardır (Cornaglia ve ark., 1992; Fink, 1980; Johanson ve ark., 2011; John ve ark., 2019; Raboisson ve ark., 2013; Sieber ve ark., 1989). İleri yaştaki annelerin buzağılarının hastalık ve ölüm oranının düşük olmasının nedenleri olarak; kolostrumlarındaki immun sistem elemanlarının (IG'ler) yüksekliği ve güç doğum probleminin daha az görülmesi gösterilebilir.

Buzağıya ait faktörler incelendiğinde; buzağı hastalık ve ölümleri üzerinde mevsimin önemli bir faktör olduğu görülmektedir. Çünkü iklim değişiklikleri (yağışlar, şiddetli soğuklar ve aşırı sıcaklıklar) immun sistem yetersizliği olan buzağılarda strese neden olmakta, hastalık ve ölüm riskini artırmaktadır. Mevcut çalışmada, hastalıkların en yüksek (%58.9) yaz mevsiminde görülmesine rağmen, buzağı ölümünün en yüksek (%10.4) kış mevsiminde olduğu tespit edilmiştir. Ancak yaz aylarında hastalık sayısı fazla olmasına karşılık, en düşük ölüm oranı (%8.2) bu mevsimde olmuştur. Almanya (Fink, 1980), ABD (Martin ve ark., 1975), Belçika (Massip ve Pordant, 1975), Danimarka (Reiten ve ark., 2018), Fransa (Raboisson ve ark., 2013), İngiltere (Hyde ve ark., 2020), İsviçre (Busoto ve ark., 1997), Kanada (Renaud ve ark., 2018), Kore (Hur ve ark., 2013), Norveç (Gulliksen ve ark., 2009) ve Türkiye'de (Kozat, 2019; Hızlı ve ark., 2017) yapılan önceki çalışmalarda da benzer şekilde kış mevsiminde buzağı ölüm oranının daha yüksek olduğu bildirilmiştir. ABD'de Godden (2008) ve Mısır'da Al ve ark. (2019) tarafından yapılan çalışmalarda da yazın hastalık oranının yüksek olması çalışma bulgularını desteklemektedir. Çalışmada buzağı hastalık oranının yaz mevsiminde daha fazla olmasında bu mevsimde işletmede doğan buzağı sayısının fazlalığına bağlı olarak hastalıkların hızlı yayılması, buzağı başına düşen işçi sayısının azalması ve predispoze faktörlerin (yüksek sıcaklığa bağlı stres durumu, hastalığı taşıyıcı sinek ve kuş sayısının artması) etkili olabileceği düşünülmektedir (Boyer Douglas ve Kuczynska, 2010; Rhoades ve ark., 2009). Kış mevsiminde ölüm oranlarının yüksek olmasının nedeni olarak; hava sıcaklıklarının düşük olması sonucu buzağının üretmiş olduğu enerjiyi hem hastalıklara karşı savunma hem de vücut ısının sağlanmasında kullanması, termoregülasyon sağlanmaması, ayrıca bazı çalışmalarda belirtildiği gibi kış aylarında immunglobulin seviyesinin daha düşük olması ve bağışıklığın olumsuz etkilenmesi gösterilebilir (Kozat, 2019).

Çalışmada boğaların (baba) buzağı hastalık ve ölümleri üzerinde etkili olduğu tespit edilmiştir. Buzağılarda babalarından dolayı genetik bazı hastalıklar görülebileceği gibi, hastalıklara karşı dirençte geçebileceğinden bazı babaların buzağıları hastalıklara diğerlerinden daha dayanıklı olmuştur. Ayrıca boğalar, buzağı doğum ağırlığı üzerinde etkili olduğundan güç doğuma da neden olacağı için dolaylı bir şekilde buzağılarda hastalık ve ölümlerin görülme olasılığı artmaktadır. O nedenle, baba adaylarının iyi belirlenmesi ve takibi gerekmektedir.

Diğer taraftan, çalışmada Simental ırkı buzağılarda hastalık ve ölüm oranları önemli düzeyde Holstein buzağılara göre sırasıyla %11.7 ve %6.5 daha fazla görülmüştür. Bu çalışmaya benzer şekilde Yıldırım ve Koçak (2019) da Simental buzağılarda ölüm oranı (% 12.6), Holstein buzağılardan (%8.2) yüksek bulmuştur. Mevcut çalışma şartlarında Simental buzağıların

Holstein ırkı buzağılara göre daha hassas olduğu, kolay hastalandığı ve öldüğü görülmüştür. Türkiye'de özellikle son yıllarda süt sığırcılığında et yönü de dikkate alınarak Simental ırkının tercih edildiği dikkat çekmektedir. Fakat buna rağmen bu ırktaki hastalık ve ölüm oranının daha çok görülmesi, işletmelerin ve karar mekanizmalarının değerlendirmesi gereken önemli bir veri olarak karşımıza çıkmaktadır.

Buzağıların hastalanmasına ve ölmesine neden olan risk faktörlerinin bilinmesinin yanında bunların işletmelere olan maliyetleri de oldukça önemlidir. Öncelikle buzağıların bir büyütme maliyeti bulunmaktadır. Çalışmada ölen buzağılar dikkate alınmazsa, büyütme maliyetleri içerisinde en büyük payı besleme maliyeti (%55.5-67.5) oluşturmaktadır. Bunu sırasıyla işçilik (%13.1-13.4), altlık (%5.8-6.7), veteriner hekim (%5.0-6.4), koruyucu hekimlik (%3.0-5.6), genel idare (%2.3-2.5), ilaç/tedavi maliyeti (hasta buzağılarda=% 1.7), elektrik+su (%1.5-1.6), amortisman (%0.6-1.1) ve bakım/onarım giderleri (%0.02-0.04) takip etmektedir. Çalışmaya benzer şekilde Tandoğan (2006) yaptığı çalışmada da en büyük giderin besleme gideri (%47.8) olduğunu sonra işçilik giderinin (%26.9) geldiğini bildirmiştir. Aynı şekilde Günlü ve ark. (2001) Afyon'da süt sığırı işletmelerinde yaptıkları çalışmada en büyük giderlerinin beslenme (%58.5) ve işçilik (% 15.7) gideri olduğunu bildirmişlerdir.

Sağlıklı buzağılarda sadece yukarıda bahsedilen büyütme giderleri yer alırken, hasta buzağılarda bunlara ilave olarak işçilik, veteriner hekim, ilaç ve altlık masrafları yer almış ve ölen buzağılarda ise ilave işçilik, veteriner hekim, ilaç ve ölen buzağı bedeli yer almıştır. Çalışmada hasta buzağılarda ilave olarak ortalama 213.3TL (=$11.5 ve 25.1 lt süt eşdeğeri) tedavi maliyeti oluşurken, ölen buzağılarda buzağı bedeli hariç ortalama 357.3TL'lik (=$19.3 ve 42.0 lt süt eşdeğeri) tedavi maliyeti oluşmuştur. Demir ve ark. (2019) Kars'ta yaptıkları çalışmada hasta buzağının ilave maliyetinin 156.3TL ($29.4) olduğunu, ölen buzağı başına ortalama kaybın ise 4597TL ($867.4) olduğunu bildirmişlerdir. Vittum ve ark. (1993) Amerika'da yaptıkları çalışmada ölen buzağı başına kaybın $216 (buzağı bedeli=$208, işçilik veteriner, ilaç ve karkas kaybı= $8) olduğunu bildirmişlerdir. Kossabati ve Esslemont (1997) yaptıkları çalışmada ölen buzağı başına £310 kayıp olduğunu bildirmişlerdir. Uza ve ark. (2005) Nijerya'daki işletmelerde buzağı ölümlerinde, hayvan başına toplam maliyetinin ~$18.64 (~$17.75 buzağı bedeli, ~$0.89 ise veteriner hizmetleri, işçilik ve karkasta meydana gelen kayıp) olduğunu bildirmişlerdir.

Mevcut çalışmada sindirim sistemi hastalığında ortalama 185.5TL/vaka ($10.0), solunum sistemi hastalığında 129.4TL/vaka ($6.9), miks hastalıklarda 243.2TL/vaka ($13.1) diğer hastalıklar da ise 123.4TL ($6.7) ilave maliyet oluşmaktadır. Kaneene ve Hurd (1990) sindirim sistemi hastalığına bağlı kaybın $33.5, solunum sistemine bağlı kaybın ise $14.7 olduğunu bildirmişlerdir. Van der Fels-Klerx ve ark. (2001) solunum sistemi hastalıklarına bağlı ilave maliyetin €31.2, Chirase ve ark. (2001) $15.6 ve Dubroviski ve ark. (2020) $42.2 olduğunu bildirmişlerdir. Busato ve ark. (1997) sindirim sistemi hastalığına bağlı kaybın 19.5 İsviçre Frangı, solunum sistemine bağlı kaybın ise 18.2 İsviçre Frangı olduğunu bildirmişlerdir. Mevcut çalışmada olduğu gibi literatürde de sindirim sistemi hastalıklarına bağlı ilave maliyetlerin solunum sisteminden daha fazla olduğu görülmüştür. Bunun muhtemel nedeni olarak, solunum sistemi hastalıklarında hastalıklı gün sayısının ve kullanılan ilaç miktarının sindirim sistemi hastalıklarından daha az olması gösterilebilir (Busato ve ark., 1997; Kaneene ve Hurd, 1990). Mevcut çalışmada hastalıklar başına ilave maliyetlerin diğer bazı çalışmalardan (Busato ve ark., 1997; Chirase ve ark., 2001; Dubroviski ve ark., 2020; Van der Fels-Klerx ve ark., 2001) daha düşük bulunmasının nedenleri olarak; çalışmanın entansif işletmede yürütülmüş olması buzağı başına düşen işçi ve veteriner hekim ücretlerinin düşük olması (kısmi verimliliğin yüksekliği) ve tedavide kullanılan ilaçların sadece verilen dozu kadar (ml) hesaplama yapılmasından kaynaklı olabileceği ve diğer çalışmalardaki hesaplama yöntemleri, hastalık süresi ve şiddeti ile incelenen işletmelerin yapılarının farklılığından kaynaklandığı söylenebilir.

Mevcut çalışmada, sindirim sistemi kaynaklı ölen bir buzağının işletmeye ortalama maliyeti 9660.0TL ($522.1), solunum sistemi kaynaklı 9795.4TL ($529.5), miks hastalıklarda 9311.8TL ($503.3) ve diğer hastalıklarda 7784.1TL ($420.8) bulunmuştur. Engelken ve ark, (1997) ABD'de yaptıkları çalışmada sindirim sistemi hastalıklarına bağlı buzağı ölümlerinde $215, solunum sistemi hastalıklarında $263 kayıp olduğu bildirilmiştir. Hastalıklarda oluşan ilave kayıplar miks hastalıklarda en yüksek iken, ölümlerde en yüksek ekonomik kaybın solunum sistemi hastalıklarında olmasının nedeni, solunum sistemi hastalıklarının bu çalışmada olduğu gibi genellikle ileri yaşlarda görülmesinden dolayı, ölen buzağının yaşı arttıkça maliyetinin de artmasıdır.

Sonuç olarak, Türkiye'de entansif bir işletmede yürütülen çalışmada görülen buzağı ölüm oranları (%9.6) hedeflenen değerden (≤%5) yüksek bulunmuştur. Ayrıca hastalıkların da hayvan başına ilave maliyetinin yüksek olmasından [sindirim sistemi hastalığında ortalama 185.5TL/vaka ($10.0= 21.8 lt süt eşdeğeri), solunum sistemi hastalığında 129.4TL/vaka ($6.9= 15.2 lt süt eşdeğeri), miks hastalıklarda 243.2TL/vaka ($13.1= 28.6 lt süt eşdeğeri) diğer hastalıklar da ise 123.4TL ($6.6= 14.5 lt süt eşdeğeri)] dolayı hayvancılığın lokomotifi olan süt sığırcılığında, hem süt hem de besiciliğin geleceği niteliğinde olan buzağılardaki hastalık ve ölümlerin azaltılabilmesi için;

-buzağı sağlığını etkileyen risk faktörlerinin (genel

olarak işletmelerin uygulamaları, anneye ait ve buzağıya ait risk faktörleri) işletmeler ve karar vericiler tarafından iyi bilinmesi ve gerekli tedbirlerin alınması,

-kolostrumun zamanında, yeterli miktarda ve kalitede içirilmesinin sağlanması,

-çalışmada görüldüğü üzere Holstein ırkının, Simental ırkına göre hastalıklara ve ölüme daha dirençli olması nedeniyle, işletmelerin ırk seçiminde daha fazla dikkat etmesi gerektiği, babaların da buzağı sağlığını etkilemesinden dolayı kullanılan boğa ya da spermalarının kayıt altına alınıp takip edilmesi gerektiği,-mevsimlere bağlı oluşabilecek (olumsuz hava şartı, predispoze faktörler vb.) olumsuzlukların işletmeler tarafından iyi analiz edilerek gerekli önlemlerin alınması,

-ileri yaşlı annelerde buzağı hastalık ve ölüm oranının genç yaştaki annelere göre daha az görülmesinden dolayı ileri yaşlı annelerin işletme karlılığını olumsuz etkileyecek bir sağlık problemi olmadığı sürece damızlık olarak kullanılması gerekmektedir. Bu bahsedilen risk faktörlerinin elimine edilmesi halinde buzağı hastalık ve ölümleri hedeflenen değerlere kademeli olarak çekilebilir. Aksi takdirde buzağı hastalık ve ölümleri hem üreticilerde hem de ulusal ölçekte önemli ekonomik kayıplara neden olmakta ve sürdürülebilir hayvancılık için risk oluşturarak karlılığı ve verimliliği düşürmektedir.

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Effect of Almond (*Prunus dulcis*) Hull Addition to Alfalfa Silage on Silage Quality and *In Vitro* Digestibility

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Abstract: In this study, it was aimed to determine the effects of the addition of almond hull, which has no economic value and causes environmental pollution, to alfalfa silage as an easily soluble carbohydrate source on silage quality, fermentation characteristics and *in vitro* organic matter digestion (IVOMD). The groups were designed to contain 0% (control), 1%, 2%, 4% and 6% almond hull, respectively. When the IVOMD and metabolizable energy (ME) values of the silages were examined, increases were observed in all additive groups compared to the control group. When the pH, NH_3-N/TN and carbon dioxide (CO_2) values of the silages were examined, the highest values were determined in the control group, while the lowest pH, NH_3-N/TN and CO_2 values were determined in the silage group with 6% almond hull added. Although an increase was determined in all trial groups in terms of lactic acid (LA) and acetic acid (AA) values of silages compared to the control group, the highest values were determined in the group with 6% almond hull addition, and a decrease was determined due to the increase in almond hull in terms of butyric acid (BA) values. Yeast and mold values of the silages decreased due to the increase in almond hull compared to the control group. As a result, it was determined that the addition of 6% almond hull as an easily soluble carbohydrate source had a positive effect on alfalfa silage quality and fermentation characteristics.
Keywords: Almond hull, silage, silage additives, silage fermentation

Yonca Silajına Badem (*Prunus dulcis*) Kabuğu İlavesinin Silaj Kalitesi ve *İn Vitro* Sindirilebilirlik Üzerine Etkisi

Öz: Bu çalışmada ekonomik değeri bulunmayan ve çevre kirliliğine neden olan badem kabuklarının kolay çözünebilir karbonhidrat kaynağı olarak yonca silajına ilavesinin silaj kalitesi, fermantasyon özellikleri ve *in vitro*organik madde sindirimi üzerine etkilerinin belirlenmesi amaçlanmıştır. Gruplar sırasıyla %0 (kontrol), %1, %2, %4 ve %6 badem kabuğu içerecek şekilde tasarlanmıştır. Silajların *in vitro* organik madde sindirimi (IVOMS) ve metabolik enerji (ME) değerleri incelendiğinde kontrol grubuna kıyasla tüm katkılı gruplarda artışlar gözlemlenmiştir. Silajların pH, amonyak azotu (NH_3 -N/TN) ve karbondioksit (CO_2) değerleri incelendiğinde, en yüksek değerler kontrol grubunda tespit edilirken, en düşük %6 badem kabuğu ilave edilen silaj grubunda tespit edilmiştir. Silajların laktik asit (LA) ve asetik asit (AA) değerleri kontrol grubuna kıyasla tüm deneme gruplarında artış göstermiş, en yüksek %6 badem kabuğu ilavesinin olduğu grupta belirlenmiştir. Bütirik asit (BA) değerleri bakımından badem kabuğu artışına bağlı olarak azalma tespit edilmiştir. Silajların maya ve küf değerleri kontrol grubuna kıyasla badem kabuğu artışına bağlı olarak azalmıştır. Sonuç olarak kolay eriyebilir karbonhidrat kaynağı olarak %6 badem kabuğu ilavesinin yonca silaj kalitesi ve fermantasyon özellikleri üzerine olumlu etkisinin olduğu belirlenmiştir.
Anahtar kelimeler: Badem kabuğu, silaj, silaj katkıları, silaj fermantasyonu

Introduction

Alfalfa is a perennial and multiform plant among leguminous forage crops, and it is a delicious forage plant rich in nutrients, especially crude protein (Gökkaya and Orak, 2021). Additives are needed in case of silage making, because the alfalfa plant is insufficient in terms of easily soluble carbohydrates and its buffering capacity is high (Kurtoğlu, 2011). In order to eliminate these negativities, the use of easily soluble carbohydrate sources such as molasses, whey and cereal grains to alfalfa silage has found a widespread application area (Topçuoğlu and Ersan 2020). Almond, one of the easily soluble carbohydrate sources, is a stone fruit seed belonging to the

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"Amygdalus" genus of the "Prunoideae" subfamily of the "Rosaceae" family of the "Rosales" team. Almond cultivation is carried out in Turkey, mostly in the Aegean Region (especially the Datça peninsula), and in the Mediterranean, Central Anatolia and Marmara Regions. As a result of breeding studies carried out in recent years, the emergence of new varieties with high efficiency and high economic return increases the interest in almond cultivation. For this reason, it is observed that almond production is increasing rapidly in Southeastern Anatolia and other regions. Almonds contain approximately 50% fat, 16.9% starch and 100 g has an energy content of approximately 575 kcal. Almonds contain high levels of monounsaturated fatty acids (MUFA) (62%) and are known to have the lowest saturated fat content (24%) among all nuts (Topçuoğlu and Ersan, 2020). The yield part of almond consists of 23% hazelnut kernel, 13% waste material, 14% shell and 50% hull. Almond hulls contain 10-30% moisture, 10-17% fiber, 1-4% oil, 2-5% protein and 50-60% nitrogen-free extract. This nitrogen-free extract contains sugars and other carbohydrates (EPA 1995; Holtman and ark., 2015).

This study was carried out to determine whether the addition of almond hull, which has no economic value and causes environmental pollution, to alfalfa silage as an easily soluble carbohydrate source has a positive effect on silage quality, fermentation properties and *in vitro* organic matter digestion.

Material and Method

The silage material of the research consisted of alfalfa grown in a farm in Şanlıurfa. The alfalfa (*Medicago sativa* L) used in the study was harvested at full bloom and chopped in a silage machine in sizes of 1.5-2.0 cm. Almond hulls (AH) were taken as dried and ground from an almond processing plant in Şanlıurfa organized industrial zone. The buffering capacity of the fresh alfalfa used in the study was determined according to the method reported by Playne and McDonald (1966). In the study, while alfalfa plant without additives constituted the control group, the trial groups consisted of 1%, 2%, 4% and 6% almond hulls added groups. Control and each trial group were compressed into 1.5-liter glass jars in 4 repetitions. The silages were fermented in a dark environment for 60 days before opening. During this time, the silages were stored at room temperature. After the silages were opened, a 3-5 cm section was discarded from the top of the jar. After the silages were poured into a container, approximately 25 g of silage sample was mixed homogeneously with 100 ml of distilled water with the help of a blender. The macerated silage samples were filtered through two layers of cheesecloth and the pH values of the filtrate were measured with a laboratory pH meter (Hanna instruments, Romania (WTW-7310)) (Polan et al.,1998). 10 ml samples were taken from the obtained silage liquid in blender and placed in tubes after filtering. In addition to the silage liquid, 0.1 ml of 1 M HCl was added to the tubes prepared for ammonia nitrogen (NH_3-N) analysis. For the analysis of lactic acid and volatile fatty acids, 0.25 ml of 25% metaphosphoric acid was added to the prepared tubes.The tubes prepared for ammonia nitrogen, lactic acid and volatile fatty acids analyzes were stored in the deep freezer until analysis. NH_3-N/TN analyzes of the silage samples were performed according to the method reported by Broderick and Kang (1980). Volatile fatty acids such as propionic acid, acetic acid and butyric acid and lactic acid were determined as reported by Suzuki and Lund (1980). For this reason, high performance liquid chromatography (HPLC) device (Shimadzu LC-20 AD HPLC pump, Icsep-Coregel (87H3 colon), Shimadzu SIL-20 ADHT Autosampler, Shimadzu cto-20ac Colum oven, Shimadzu SPD M20A Detector (DAD), Japan) was used. The silages obtained in the study were subjected to aerobic stability test in order to determine the CO_2 production values. For this purpose, silages were developed by Ashbell et al. (1991) was exposed to oxygen for 5 days according to the method reported.

While the raw nutrient contents of the silages (such as dry matter, crude ash, crude protein) were made according to the method reported by AOAC (2005), the acid detergent fiber (ADF) and neutral detergent fiber (NDF) analyzes of the silages were performed as reported by Van Soest et al. (1991). Before the raw nutrient analysis, the silages were dried at room temperature and ground in a laboratory mill to pass through a 1 mm sieve and made ready for analysis. While determining the *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME) and *in vitro* methane (CH_4) contents of silages, the method reported by Menke and Steingass (1988) was applied.The gas production values of the silages were determined through the method described by Menke and Steingass (1988) using four glass syringes as replicate. The *in vitro* organic matter digestibility (IVOMD) (g/kg OM) and metabolizable energy (ME) (MJ/kg DM) of silages were calculated using equations reported by Menke and Steingass (1988).

$$ME(MJ/kgDM) = 2.20+0.136\times Gp+0.057\times CP+0.0029\times CP^2,$$

$$IVOMD\ (\%) = 14.88+0.889\times Gp+0.45\times CP+0.0651\times XA,$$

where CP is CP in g/100 g DM, crude ash in g/100 g DM and gas production is the net gas production (ml) from 200 mg DM after 24 h of incubation. After recording 24-h gas production values, gas inside the syringe was taken by three-way syringe system and total gas was injected into computer-assisted infrared methane gas meter (Sensor Europe GmbH, Erkrath, Germany) and then methane content was determined as a percentage of 24 h the total amount of gas

formed (Goel et al.2008) Yeast and mold contents of silages were determined using the method reported by Filya et al. (2000).The conformity of the data to the normality distribution was determined by looking at the Histogram graph. One Way Analysis of Variance (One Way ANOVA) was used in the evaluation of the data obtained as a result of the research. The homogeneity of the variances was examined in the Test of Homogeneity of Variance analysis. Duncan's multiple comparison tests were applied to compare group means by using SPSS (1991) package program. A value of 0.05 was used as the level of significance between the groups.

Results

The nutrient analysis results of alfalfa plant used as silage material and almond hull used as additive in the study are presented in Table 1. When Table 1 was analyzed, DM, CA, CP, ADF, NDF, IVOMD, ME and CH_4 values of alfalfa plant used as silage material are 23.45%, 13.05 DM%, 16.70 DM%, 34.99% DM%, 56.44% DM%, 58.52%, 8.79 MJ, respectively. For the same parameters /kg DM and 14.68%, almond hulls values were determined as 92.50%, 15.50 DM%, 3.80 DM%, 35.59% DM%, 50.76% DM%, 34.39%, 4.98 MJ/kg DM and 1.30%, respectively.

The nutrient contents, IVOMD, ME and *in vitro* CH_4 values of the silages prepared by adding almond hull at different rates (1%, 2%, 4% and 6%) to alfalfa plants are given in Table 2. When Table 2 was analyzed, it was found that the differences between the groups were statistically significant in DM, CA, CP, ADF and NDF, IVOMD, ME values of the silages ($P<0.05$).

Within the scope of this study, the fermentation characteristics of the silages prepared by adding different ratios of almond hull to alfalfa plant as an easily soluble carbohydrate source and the correlation results of the analyses are given in Table 3 and Table 4. When Table 3 was analyzed, it was seen that the differences between the groups were significant when the fermentation characteristics (pH, NH_3-N, LA, AA, yeast, mold, CO_2) of the silages were examined ($P<0.05$). When Table 4 is examined, it has been determined that there is a correlation between the fermentation characteristics of alfalfa silages and the yeast and mold values.

Table 1. Crude nutrient contents of alfalfa plant used as silage material and almond hull used as additive in the study

	BC	DM	CA	CP	ADF	NDF	IVOMD	ME	CH_4
Alfalfa	450	23.45	13.05	16.70	34.99	56.44	58.52	8.79	14.68
Almond hull	-	92.50	15.50	3.80	35.59	50.76	34.39	4.98	1.30

***BC**: Buffering capacity meq/kg DM**, DM:** Dry matter, %; **CA:** Crude ash DM%; **CP:** Crude protein, DM%; **ADF:** Acid detergent fiber, %DM; **NDF:** Neutral detergent fiber, %DM; **IVOMD:**In vitro organic matter digestibility %, **ME:**Metabolizable energyMJ/kg DM, **CH_4:** In vitro methane gas (%).*

Table 2. Nutrient contents and IVOMD, ME and *in vitro* CH_4 values of alfalfa silages prepared by adding almond hull at different rates

Groups	DM±SE	CA±SE	CP±SE	ADF±SE	NDF±SE	IVOMD±SE	ME±SE	CH_4±SE
Control	20.49^{c}±0.23	12.93^{a}±0.14	16.27^{a}±0.04	34.85^{b}±0.67	49.61^{b}±0.27	53.29^{b}±0.50	7.84^{b}±0.07	14.60±0.21
%1 almond hull	20.52^{c}±0.73	12.43^{a}±0.29	16.75^{a}±0.26	35.48^{b}±0.28	50.12^{b}±0.34	53.52^{b}±0.62	7.87^{b}±0.11	13.28±1.17
%2 almond hull	21.41^{c}±0.27	11.42^{b}±0.15	16.82^{a}±0.14	35.99^{ab}±0.40	50.16^{b}±0.64	57.43^{a}±1.27	8.69^{a}±0.07	14.64±0.13
%4 almond hull	23.04^{b}±0.63	11.16^{b}±0.09	15.46^{b}±0.23	36.04^{ab}±0.28	52.66^{ab}±0.97	57.86^{a}±1.46	8.54^{a}±0.28	15.24±0.17
%6 almond hull	25.02^{a}±0.63	11.06^{b}±0.06	15.34^{b}±0.17	37.13^{a}±0.43	54.31^{a}±1.81	58.80^{a}±0.26	8.60^{a}±0.92	14.89±0.31
P	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	>0.05

*$^{a-c}$**:** Values with different letters in the same column were found to be different ($P<0.05$); **SE:**Standard error,**DM:**Dry matter, %; **CA:** Crude ash DM%; **CP:** Crude protein, DM%; **ADF:** Acid detergent fiber, %DM; **NDF:** Neutral detergent fiber, %DM; **IVOMD:**In vitro organic matter digestion %, **ME:**Metabolizable energy MJ/kg DM ,**CH_4:**In vitro methane gas (%).*

Table 3. Fermentation characteristics of alfalfa silages prepared by adding almond hulls at different rates

Groups	pH±SE	NH_3N/TN±SE	LA±SE	AA±SE	PA±SE	BA±SE	YEAST±SE	MOLD±SE	CO_2±SE
Control	5.55^{a}±0.01	33.68^{a}±1.45	4.58^{e}±0.03	6.84^{d}±0.02	0.76^{b}±0.01	9.42^{a}±0.01	3.09^{a}±0.01	5.61^{a}±0.01	2.38^{a}±0.01
%1 almond hull	5.48^{a}±0.03	19.81^{b}±0.73	6.07^{d}±0.07	7.37^{c}±0.01	0.43^{c}±0.01	6.64^{b}±0.02	1.01^{b}±0.00	3.46^{b}±0.01	1.78^{ab}±0.01
%2 almond hull	5.56^{a}±0.18	17.11^{bc}±1.09	8.67^{c}±0.03	7.42^{c}±0.02	1.19^{a}±0.01	6.44^{c}±0.01	0.00^{c}±0.00	3.29^{c}±0.01	1.34^{b}±0.01
%4 almond hull	4.60^{b}±0.18	14.42^{c}±0.40	14.44^{b}±0.02	9.03^{b}±0.02	0.00^{d}±0.00	5.44^{d}±0.02	0.00^{c}±0.00	3.00^{d}±0.01	1.26^{b}±0.01
%6 almond hull	4.49^{b}±0.11	11.58^{d}±0.64	27.33^{a}±0.04	14.03^{a}±0.05	0.00^{d}±0.00	0.00^{e}±0.00	0.00^{c}±0.00	2.35^{e}±0.02	1.25^{b}±0.01
P	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**

$^{a-e:}$ Values with different letters in the same column were found to be different (P<0.05); ***SE:****Standarderror,* ***NH_3-N/TN:*** *Ammonia nitrogen,* ***CO_2:*** *Carbon dioxide g/kg DM,* ***LA:*** *Lactic acid g/kg DM,* ***AA:*** *Acetic acid g/kg DM,* ***PA:*** *Probiotic acid;* ***BA:*** *Butyric acid g/kg DM,* ***Yeast:*** *log10/cfu/g,* ***Mold****: log10/cfu/g*

Table 4. Correlation relationship between fermentation characteristics and yeast and mold values of alfalfa silages prepared by adding almond hull at different rates

		NH_3-N/TN	LA	AA	PA	BA	Yeast	Mold	CO_2	IVOMD	ME	CH_4
pH	**PC**	.657**	-.768**	-.790**	.824**	.650**	.527*	.633**	.469*	-.600**	-.447*	-.337
	P	.002	.000	.000	.000	.002	.017	.003	.037	.005	.048	.146
NH_3.N	**PC**	1	-.603**	-.645**	.476*	.756**	.952**	.973**	.733**	-.655**	-.610**	-.090
	P		.005	.002	.034	.000	.000	.000	.000	.002	.004	.706
LA	**PC**		1	.398	-.668**	-.247	-.600**	-.556*	-.508*	.553*	.482*	.378
	P			.082	.001	.294	.005	.011	.022	.011	.031	.100
AA	**PC**			1	-.671**	-.922**	-.506*	-.683**	-.434	.577**	.432	.226
	P				.001	.000	.023	.001	.056	.008	.057	.338
PA	**PC**				1	.580**	.304	.495*	.272	-.280	-.080	-.128
	P					.007	.192	.027	.245	.232	.737	.591
BA	**PC**					1	.631**	.816**	.490*	-.497*	-.380	-.042
	P						.003	.000	.028	.026	.098	.860
Yeast	**PC**						1	.954**	.747**	-.656**	-.671**	-.147
	P							.000	.000	.002	.001	.537
Mold	**PC**							1	.717**	-.619**	-.579**	-.091
	P								.000	.004	.008	.703
CO_2	**PC**								1	-.468*	-.465*	.043
	P									.037	.039	.856
IVOMD	**PC**									1	.908**	.382
	P										.000	.097
ME	**PC**										1	.377
	P											.101
CH_4	**PC**											1

PC: *Pearson correlation.* ****:*** *Correlation is significant at 0.05 level.* *****:*** *Correlation is significant at 0.01 level.* ***NH_3-N/TN:*** *Ammonia nitrogen.* ***CO_2:*** *Carbon dioxide g/kg DM.* ***LA:*** *Lactic acid g/kg DM.* ***AA:*** *Acetic acid g/kg DM.* ***PA:*** *Probiotic acid g/kg DM;* ***BA:*** *Butyric acid g/kg DM.* ***IVOMD:****In vitro organic matter digestion %.* ***ME:****Metabolizable energy.* ***CH_4:*** *In vitro methane gas (%).*

Discussion and Conclusion

When Table 2 was analysed, it was found that the differences between the groups were statistically significant in DM, CA, CP, ADF and NDF, IVOMD, ME values of the silages, while the differences between the *in vitro* CH_4 values were not statistically significant.

When the DM contents of the silages prepared by adding different ratios of almond hull to alfalfa plants were analysed, an increase in the DM levels was observed in parallel with the increase in the addition of almond hull compared to the control group. The increase in DM level can depend on the high DM level of almond hull. When the CA values were analysed, a decrease was observed due to the addition of almond hulls. Kurtoğlu (2011) reported that this was due to the difference in inorganic matter levels of silage materials and additives. When the CP values of the silages were examined, a decreasing trend was observed in the CP level depending on the addition of 4% and 6% almond hulls. This decrease was due to the low CP content of almond hulls. When ADF and NDF values of the silages were analysed, an increase in ADF and NDF values was observed parallel to the increase in almond hull. In this study, the difference in ADF and NDF values between the control group and the trial groups was considered to be due to the high ADF and NDF contents of almond hull.

Kepekci (2020) reported that the addition of anise seed as an easily soluble carbohydrate source to alfalfa silage increased NDF values. When IVOMD and ME values of silages were examined, increases were observed in all trial groups compared to the control group. It was considered that the main fermentation product in silages was LA and LA was fermented in the rumen and evaluated by ruminants and accordingly increased IVOMD and ME values (Okuyucu et al., 2018). When Table 4 was examined, it was seen that there was a positive correlation between LA and IVOMD (R: 0.553) and LA and ME (R: 0.482). Similarly, Şakalar and Kamalak (2016) reported that the addition of sugar beet pulp as an additive to alfalfa silage increased IVOMD and ME values, which supports the results obtained from this study.

When the fermentation characteristics (pH, NH_3-N, LA, AA, yeast, mold, CO_2) of alfalfa silages prepared by adding almond hulls at different rates were examined, the differences between the groups were found to be significant ($P<0.05$). When the pH values of the obtained silages were analysed, the highest pH value (5.55) was obtained from the control group, while the lowest pH value (4.49) was determined in the group with 6% almond hull addition. As the water-soluble carbohydrate content of the silage material increases, the ideal acidic environment required for obtaining qualified silage is formed. Therefore, it is expected that the silage pH will decrease with the addition of almond hull to alfalfa silage. In this study, the lower pH values in the almond hull added groups compared to the control group may be attributed to the fact that the low water-soluble carbohydrate content of alfalfa plant was tolerated with almond hull additive and the lactic acid values in the additive groups were high due to the effect of this additive. When the correlation table of the silages obtained was examined, it was observed that there was a negative correlation between pH and LA. Similarly, Stallings et al. (1981) reported that the addition of 1% arabinose and 1% glucose to alfalfa plants decreased pH values in silages and increased silage quality.

When the NH_3-N/TN values of the silages prepared in this study were compared, it was determined that although a decrease was observed due to almond hull addition, the highest NH_3-N/TN value (33.68) was in the control group, while the lowest NH_3-N/TN value (11.58) was in the group with 6% almond hull addition. It was considered thought that this decrease in silage NH_3-N/TN values was due to the fact that easily soluble carbohydrate sources had a positive effect on silage fermentation and reduced proteolysis (Bingöl et al., 2009). Similarly, Yakışır and Aksu (2019) reported that dried sugar beet pulp with molasses, which they added to alfalfa silage at different levels, considerably prevented the denaturing of protein fractions. It was reported that the use of additives with easily soluble carbohydrate content as an additive source in silage production from alfalfa plants created a good fermentation environment for lactic acid bacteria, and microorganisms can multiply rapidly and minimize protein degradation by lowering the pH of the environment (Kung et al., 1984). When the correlation table was examined, the observation of a negative correlation (R:-0.603) between LA and NH_3-N/TN supports this report.

When the LA and AA values of the silages were examined, increases in LA and AA values were observed in all trial groups compared to the control group, but the highest values were determined in the group with 6% almond hull addition. Orloff and Muller (2008) reported that the water-soluble carbohydrate content of high-quality alfalfa silage during the fermentation stage was converted to LA and AA, and the pH of the environment was rapidly reduced to the level of 4-5. When the correlation table was examined, it was seen that there was a positive correlation (R: 0.398) between LA and AA. Gao et al. (2021) reported an increase in LA values due to the addition of molasses and fructose to alfalfa silage, which agreed with the present study. Tabacco et al. (2006) reported that the addition of chestnut shell increased the LA value in alfalfa silage prepared by adding 2, 4

and 6% chestnut tannin compared to the control group. In the silages prepared with the addition of almond hulls at different ratios in this study, PA was not observed in the groups with 4% and 6% almond hull addition, while the highest PA value (1.19 g/kg DM) was determined in the group with 2% almond hull addition ($P<0.05$). The BA values of alfalfa silages prepared in this study showed a decreasing trend due to the increase in almond hulls, but it was not observed in the group with 6% almond hull ($P<0.05$). The fact that alfalfa plant has high CP and low DM and WSC content causes a deficiency in lactic acid production, which is necessary to inhibit the growth of *clostridial* bacteria (Weinberg et al., 1988). As a result of this situation, *saccharolytic clostridia* convert WSC and organic acids in the plant structure into butyric acid (Ohshima et al., 1997). Although butyric acid is an undesirable organic acid to be present in silo feed, it is usually present in the silo at the level of 0.1-0.7%, and this value receives the highest score at the level of 0-1.5% according to German Agricultural Society (Deutsche Landwirtschafts-Gesellschaft, DLG) (DLG, 1987). Similarly, Özaslan (2016) reported that the addition of corn syrup as an easily soluble carbohydrate source to alfalfa silage decreased the silage butyric acid value, which supports the present study.

In this study, when the yeast and mould values of the silages prepared with the addition of almond hulls at different rates to alfalfa plants were examined, a decrease was observed due to the increase in almond hull compared to the control group. In her study,

When the correlation table was examined, it was seen that the negative correlation between AA and yeast values and AA and mould supported this statement. In the study, on the 5th day of aerobic stability, the CO_2 production amounts of the silage groups with almond hull addition ranged between (1.25-2.38) g/kg DM, but a decrease was observed in all trial groups due to the increase in almond hull compared to the control group ($P<0.05$). The highest acetic acid, the lowest yeast and mould amount and the lowest CO_2 ratio level supports the statement reported by Ali et al. (2020) that the amount of acetic acid produced by heterolactic LAB fermentation in silages in the additive groups has an inhibitory effect against microorganisms that cause silage deterioration, prevents the reproduction and activity of yeasts, reduces CO_2 production, in other words, improves aerobic stability values. When the correlation table was examined, it was seen that the negative correlation observed between LA, AA and CO_2 supported this report. Yayla (2019) reported that the addition of waste jam to alfalfa silage reduced the CO_2 value and improved aerobic stability compared to the control group.

When the results obtained in this study were evaluated in terms of all parameters, it was concluded that alfalfa silages prepared with the addition of 6% almond hull had positive effects on silage quality, fermentation characteristics and *in vitro* organic matter digestion, almond hull can be used as silage additive and the best results were obtained with the addition of 6% almond hull.

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The Effects of Rosemary *(Rosmarinus officinalis L.)* Extract on the Oxidant Stress Indexes and Proliferation Capacities of SW1353 Chondrosarcoma Cells

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Abstract: Arthritis is characterized by symptoms such as joint pain, swelling, and limitation of movement. The main objectives of treating the disease are eliminating pain and inflammation and protecting joint functions. Several herbal remedies have been reported to be applied in specific forms to treat arthritis or prevent some ailments. In this study, the effects of rosemary extract (RE) were examined in terms of proliferation capacity, total antioxidant status (TAS) and total oxidant status (TOS) and oxidative stress index (OSI) on SW1353 cell line to see whether rosemary extract can be used in the treatment of arthritis. The cytotoxic effect of RE, which appears in high doses, draws attention to its pharmacovigilance in its use. Our findings indicate that RE at low concentrations, regardless of its antioxidant properties, may be utilized to treat arthritis associated with cartilage damage.
Keywords: Antioxidant, chondrosarcoma, oxidative stress index, rosemary extract

Biberiye *(Rosmarinus officinalis L.)* Ekstraktının SW1353 Kondrosarkom Hücrelerinin Oksidan Stres İndeksleri ve Çoğalma Kapasiteleri Üzerindeki Etkileri

Öz: Artrit, eklem ağrısı, şişme ve hareket kısıtlılığı gibi semptomlarla karakterizedir. Hastalığın tedavisinde temel amaç ağrı ve iltihabı ortadan kaldırmak ve eklem fonksiyonlarını korumaktır. Artriti tedavi etmek veya bazı rahatsızlıklarını önlemek için belirli formlarda çeşitli bitkisel ilaçlar uygulanır. Bu çalışmada biberiye ekstraktının SW1353 hücre hattı üzerindeki proliferasyon kapasitesi, toplam antioksidan seviyesi (TAS), toplam oksidan seviyesi (TOS) ve oksidatif stres indeksinin (OSİ) etkileri incelenmiştir. Yüksek dozlarda ortaya çıkan biberiye ekstraktının sitotoksik etkisi, farmakovijilansı kullanımında oldukça dikkat çekmektedir. Bulgularımız, antioksidan özelliklerinden bağımsız olarak düşük konsantrasyonlarda biberiye ekstraktının artritten kaynaklı kıkırdak hasarı tedavisinde kullanılabileceğini göstermektedir.
Anahtar kelimeler: Antioksidan, biberiye özü, kondrosarkom, oksidatif stres indeksi

Introduction

Arthritis can be defined briefly as the inflammation or swelling of one or more joints. Along with joints and neighboring tissues, it may also affect other connective tissues leading to the impairment of internal organs, such as the heart, lungs and kidneys, in conditions such as rheumatoid arthritis, lupus and fibromyalgia (Garcia, 2019; Giles et al., 2020). When cartilage is damaged, the ends of the bones become unprotected, resulting in pain, swelling, and stiffness in the joints (Chua et al., 2019; Kolasinski et al., 2020). In recent years, the developments and inventions observed in pharmacognosy and food science have provided opportunities using scientific methods to support the idea that taking certain natural substances, extracts, or chemicals beneficial for our body in specific forms has effects on preventing discomfort (Ng and Azizudin, 2020; Rehman et al., 2019). Today, several herbal-treatment methods, under the name of alternative medicine, are widely used in treating arthritis and relief of symptoms with both local and systemic applications, in addition to existing modern treatment methods (Ko, 2019; Kose et al., 2019).

While osteoarthritis (OA) is the most common form of arthritis defined by joint degeneration and cartilage loss, rheumatoid arthritis (RA) is a chronic, inflammatory, multi-systemic, and autoimmune disease that affects the joints and can progress with disfigurement. Even though these two diseases have very different etiopathogenesis and reveal other clinical manifestations, they are both characterized by the destruction of connective tissue, especially cartilage

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tissue, leading to inflammation and pain shows similarities in both diseases (Chua et al., 2019; Schroeder et al., 2020).

Free radicals and inflammation cause premature apoptosis of cartilage cells in the joint and slow down the damaged cartilage tissue regeneration, and the disease becomes chronic and permanent (Lepetsos et al., 2019; Saxena and Batra, 2020). An increase in proinflammatory cytokines in chronic inflammatory manifestations causes an increase in oxygen free radicals (OFRs). OFRs are highly reactive compounds that affect macromolecules such as lipids, proteins, DNA and carbohydrates, causing deterioration in their structure (Lepetsos et al., 2019; Pradhan et al., 2019; Saxena and Batra, 2020). Free radicals such as reactive oxygen species, superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), nitric oxide ($NO^{\cdot}$), peroxyl radical ($ROO^{\cdot\,\cdot}$), and non-radical hydrogen peroxide (H_2O_2) in biological systems are among the most critical causes of oxidative stress (Hatai and Banerjee, 2019; Miao et al., 2020). While OFRs are generated endogenously in physiological conditions, their formation increases with exogenous factors such as increased immobility, malnutrition, environmental pollution, pesticides, UV, radiation and X-ray (Wu and Pan, 2019; Zahan et al., 2020).

Rosemary (*Rosmarinus officinalis* L.) has been described as an aromatic self-growing plant and perennial herb, usually within the maquis flora. The chemical structure and antioxidant activity of rosemary extract (RE) has been investigated by various researchers (Cheung and Tai, 2007; Genena et al., 2008). Two main rosemary oils have been reported, the first comprises 1,8-cineole at a rate of more than 40%, and the second contains approximately equal amounts of 1,8-cineole, R-pinene, and camphor. There are potent antioxidants in rosmarinic acid and carnosic acid in rosemary leaves, carnosic acid being the most powerful antioxidant for animal fats. Abietatrien-derived diterpenes are responsible for 90% of the antioxidant effect of carnosic acid and carnosol in RE (Vallverdú-Queralt et al., 2014; González-Vallinas et al., 2015; Andrade et al., 2018). Both raw and refined extracts of rosemary leaves are found in trade. RE is commonly used today to preserve food products (Vlavcheski and Tsiani, 2018; Jaglanian and Tsiani, 2020). Studies showed that RE containing 20% carnosic acid added to the diet of rats reduced oxidative stress in elderly rats and RE reduced the antioxidant enzyme activity, lipid peroxidation and OFRs in the heart and brain tissue, and the OSI activity in the brain; hence, revealed its antioxidant protective effects. Also, RE reduces the neuronal damage caused by H_2O_2 and protects against neurodegenerative diseases due to oxidative stress and apoptosis. We aim to investigate the impact of RE, which can be taken orally with meals and/or applied locally, on articular joints, as a supplement to classical medical treatment methods in arthritis treatment and investigate its acting mechanisms.

Materials and Methods

Rosemary extract preparation

The plant materials used in the study were collected as leafy shoots in the Adana-Kozan region by cutting with scissors. The samples were kept in the shade for 8-10 days and dried. The leaves were separated from the dried leafy branches and the dry leaves were bagged and labeled and kept in a dry and cool place in the laboratory for distillation and extraction processes. Existing rosemary stocks were diluted with DMEM containing 20% dimethyl sulfoxide (DMSO). The final concentration of DMSO is 0.1%, and this concentration does not have a toxic effect on cells. Rosemary solutions were sterilized by passing through a 0.2mm filter and added to cell cultures. Dried rosemary leaves were ground and steeped overnight (16 hours) in a 1:1 mixture of dichloromethane and methanol, followed by filtration. After filtering, the solvent was set aside while the leaves were boiled in methanol for 30 min. After boiling, the solvent was combined with the filtered solvent. Rotary evaporation was used to remove the combined solvent from the final extract, and the green powder was collected and stored at -20^{0}C in a light-protected environment. Aliquots of DMSO were prepared to a final concentration of 100 mg/mL. RE powder was dissolved in DMSO to make a 100 mg/mL stock solution. The RE stock (400 µg/mL) was created by treating the cells with cell culture media (n=3) (Figure 1).

Figure 1. Oxidative stress and inflammatory response were examined by applying Lipopolysaccharide (L).

Cell culture and treatment

SW1353 chondrocyte cells (HTB-94, American Type Culture Collection-ATCC) were cultured in DMEM containing 100 units/mL of penicillin, 100 µg/mL of streptomycin and 0.25 µg/mL of Amphotericin B and FBS (10% v/v) with 5% CO_2, at 37^{0}C. A working stock of RE (400 g/mL in DMEM, stored at -20^{0}C in a light-protected environment) was used to treat the cells and the final concentration of DMSO in the RE-treated cells was less than 0.1%. The cells were indicated in DMSO at the same concentration as cells exposed to RE (vehicle control), which did not affect

the measurements (Ketsawatsakul, 2007).

Cell proliferation assay

SW1353 cell proliferation was evaluated by using WST-1 assay. Absorbance at 450nm was measured with a microplate reader (Synergy HTX, Biotek, US). After seeding cells, the colorimetric assay was performed for 48hr.

Preparation of cell lysates for oxidative stress analyses

A solution containing 15mM TRIS-HCl, 150mM NaCl, 0.1mM EDTA, and 0.5% Triton-X was prepared for lysing cultured cells. The pH was adjusted to 7.5 and the lysing solution was prepared fresh and kept at +4^{o}C until use. After the cells were washed with PBS, chilled lysis buffer was added to the wells. Plates were kept on ice for 10min and cell lysates were transferred into micro-centrifuge tubes by pipetting. Lysates were centrifuged at 5000rpm for 5min. The supernatants were stored at +4^{o}C until the TAS and TOS levels were measured. All biochemical analyzes were performed on the same day, within 1-2h.

Total antioxidant status measurement

The working principle of the method is that Fe2+ -*o*-dianicide complex generates an OH radical by generating a Fenton-type reaction with H_2O_2. This solid reactive oxygen type is reduced and reacts with the colorless *o*-dianicide molecule at low pH to generate yellow-brown dianicidil radicals. Dianicidil radicals increase color formation by participating in advanced oxidation responses. However, antioxidants overturn these oxidation reactions and stop color construction. This reaction calculates the optical density measured at 660nm spectrophotometrically (Rel Assay Diagnostics, Germany) (Erel, 2004). The blue-green colored reduced 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonate) (ABTS) generated during the response was calculated in mmolEq/L. Values for standardization were interpreted by proportioning to the total protein amounts of the cells in each application well.

Total oxidant status measurement

The method is based on the principle that oxidants oxidize the ferrous ion-*o*-dianicide complex to the ferric ion. The glycerol accelerates this reaction and triples it approximately. Ferric ions generate a colored complex with xylenol orange in an acidic environment. The color reaction is directly related to the number of oxidants determined by spectrophotometric evaluation. The number of total oxidant molecules generated by measurements at 530nm was expressed in H_2O_2 formed (mmolH_2O_2Eq/L) (Rel Assay Diagnostics, Germany) (Erel, 2005). Values for standardization were represented in proportion to the total protein amounts of the cells. Values for standardization were interpreted by proportioning to the total protein amount of the cells in the application well.

Calculation of oxidative stress index

Considering that it would be a more meaningful parameter in determining the degree of oxidative stress (Bolukbas et al., 2005; Demirbag et al., 2007).

OSI = [TOS (µmolEq/L) / TAS (mmolEq/L) x 100]

Statistical evaluation

The data are the mean±SD (Mean±Standard Deviation) error mean of the indicated number of independent experiments. All the data were subjected to statistical analyses using the ONE-Way ANOVA and Tukey's test for the multiple comparisons of means. The homogeneity of variances was analyzed according to the Brown-Forsythe and Bartlett test. The statistical significance level was assumed at P<0.05. Statistical tests were performed using GraphPad Prism software.

Results

Effects of RE on SW1353 chondrosarcoma cell proliferation

Treatment with RE resulted in a dose-dependent inhibition of cell proliferation. A significant increase in cell proliferation (P<0.001) was seen with 1 and 50 µg/mL doses of RE, while a maximum decrease was caught between 50µg/mL and C (P=<0.001), suggesting a toxic effect of RE on cells (Figure 2). Treatment of the cells with 1-10µg/mL did not result in any significant inhibition of cell proliferation. In contrast, treatment with 50µg/mL showed a considerable increase in cell proliferation compared to D and L (P< 0.05).

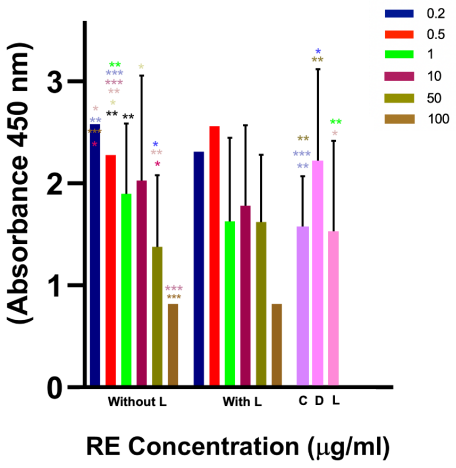

Figure 2. WST-1 cell proliferation assay with different doses with L (1-50µg/mL ***p=0.0005, 50-Cµg/mL ***p=0.0002, 50-Dµg/mL **p=0.0038, 50-Lµg/mL **p=0.0019).

Cells treated with 0.2-50mg/ml concentrations of RE for 48h showed no difference in TOS values compared to the control (C=no treatment), DMSO (D=0.1% DMSO) and L (L=0.1% Lipopolysaccharide) groups (Figure 3A). 100mg/ml dose of RE demonstrated the highest increase in TOS value either with/without L. A significant decrease in TOS activity was observed with RE (0.2 and 0.5mg/ml) at 48h compared to 100mg/ml concentration (P<0.001), both in groups treated with/without L, which correlated with the proliferation capacity of the cells.

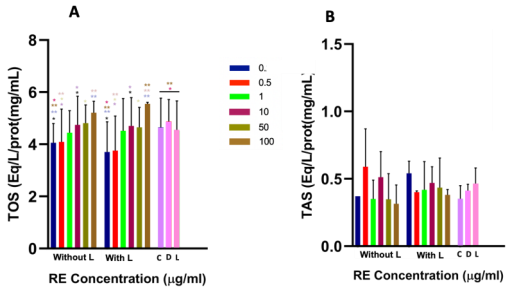

Figure 3. Expression of RE with/without L obtained from (A) TOS, (B) TAS in different doses comparison with C.

TAS assay measured whether RE could inhibit the antioxidant capacity of SW1353 cells. No statistically significant difference was observed in TAS values among the study groups (Figure 3B). In the group where 0.5 mg/ml RE dose was applied, an increase in TAS value was observed compared to C and L-treated groups. The TAS effect of 0.5mg/ml RE on cells was higher than the TAS effect of C and L-treated groups. Again, cells with 0.5mg/ml RE dose were more sensitive than the others.

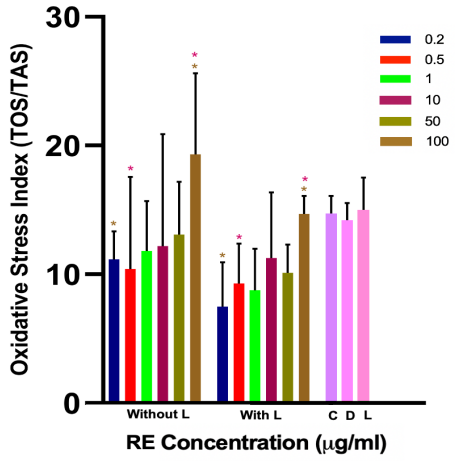

Figure 4. Expression of RE with/without L obtained from OSI in TOS/TAS in different doses comparison with C.

The effects of RE on oxidative stress in cells were evaluated by calculating the oxidative stress index (OSI=TOS/TAS) (Figure 4). When the index values were analyzed, 0.2 mg/ml RE treatment created the lowest ratio, whereas 50mg/ml RE treatment caused the highest TOS/TAS ratio. At dosages of 0.2 and 0.5, it was shown that the oxidative stress was lower compared to the C. OSI index, which seemed to be affected by inflammation. In the presence of inflammation, the L-treated group had decreased OSI, which was not anticipated. Additionally, the dose-dependent impact of RE becomes hazardous in higher doses compared to the C and low dose-treatment groups.

Discussion and Conclusion

Under normal conditions, certain levels of free radical formation exist in all aerobic cells and all cell sections. Free oxygen radicals generated in the organism are eliminated by intracellular free radical converting enzymes (superoxide dismutase, catalase, and glutathione peroxidase, etc.) or substances such as exogenous vitamin C (ascorbic acid) and vitamin E (alpha-tocopherol). While determining the oxidative stress, it is helpful to examine intracellular xanthine oxidase (Schmidt et al., 2019; Manivasagam et al., 2020), adenosine deaminase, SOD, GSH-Px, and CAT or vitamin C and E levels (Sinbad et al., 2019) to learn about antioxidant capacity while looking at the levels of free radicals (Suleman et al., 2019), thiobarbituric acid reactive products, nitric oxide (Poprac et al., 2017; Tejero et al., 2019).

The balance between oxidants and antioxidants is essential for cellular hemostasis. In recent years, the relationship between oxidant and antioxidant capacity and the role of imbalance in cell destruction has been frequently examined (Bagherifard et al., 2020). In particular, the importance of exogenous and endogenous free radicals generated in the cell in terms of toxicity and their roles in the pathogenesis of several diseases such as cancer, emphysema, hyperoxidation, bronchopulmonary dysplasia, arteriosclerosis and pancreatitis have been shown in many researches (Moussa et al., 2019; Neha et al., 2019).

Our work intended to question the mechanism of RE on arthritic chondrocytes by looking at the cells' proliferation capacity and antioxidant/oxidant status. It revealed that RE improves the antioxidant/oxidant status of SW1353 cells in both standard and inflammatory conditions. However, this effect is dose and condition dependent.

A subsequent investigation is whether RE can inhibit SW1353 cell proliferation was evaluated. A significant decrease in cell number was observed at 50mg/ml when the proliferation was individually compared to the C group. The proliferative capacity of the cells

was higher at 50µg/mL RE with L doses compared to the C.

SW1353 cells are commonly used as a model in the literature to evaluate response to inflammation and study chondrocyte matrix destruction. Nevertheless, the proliferation capacities of SW1353 cells increase in the presence of an inflammatory inflammation (Ngo et al., 2019; Zhao et al., 2019). RE has been reported to have an anti-inflammatory effect, especially by inhibiting the production of COX-2 (Mengoni et al., 2011). Therefore, the high dose of RE's antiproliferative impact in our study is consistent with the literature.

RE demonstrated an antiproliferative effect on SW1353 cells *in vitro*; its inhibitory role on proliferation was statistically significant in 0.2 and 0.5mg/ml RE treatment groups. TOS/TAS results indicated a statistically significant decrease for the 0.5mg/ml dose group. At higher concentrations, however, it was observed that RE led to a rise in both TOS and TAS values in SW1353 cells. Although not statistically meaningful, this increase was more pronounced in TOS values than in TAS values.

The antioxidant activity of RE also depends on how the extract is obtained. It is especially recommended to use dried RE as an antioxidant. When we calculated the OSI of cells treated with RE, OSI was highest with 100mg/ml RE concentration; it was observed that 0.2mg/ml significantly decreased cell proliferation compared to the others; this increase may be due to the increased TOS, causing a toxic effect on SW1353 cells *in vitro* with L, in other words, the decreased antioxidant status could not compensate this TOS activity. The antioxidant capacity was higher at lower doses of RE (0.2-0.5 ug/mL) treatment, which also impacted OSI and decreased stress, especially in the L-treated group. The benefit of assessing the oxidative status and antioxidant capacity together and expressing the differences in values with an index is obvious when evaluating the oxidant stress.

When the effects of RE on cell proliferation and OSI are evaluated together, it may be stated that the impact of RE in protecting cartilage cells at low concentrations (≤1mg/ml) and proliferation enhancement occurs independently of the antioxidant effect of the herbal extract. The decrease in cell proliferation at high concentrations is likely due to the increased oxidative effect of RE on cells. Indeed, in the literature, the antiproliferative effects of RE on cancer cells by blocking COX-2 expression have been reported (Moore et al., 2016; Scheckel et al., 2008). Since SW1353 cells originate from chondrosarcoma cells, these results may also indicate that applying a high dose of RE may be an additional/supplemental treatment method for existing cartilage-bone origin mesenchymal tumors. Similarly, studies report reduced proliferation capacity in cancer cells treated with 100mg/ml) RE. This unwanted effect at high doses can be prevented by topical -low-dose use of the RE in people with the arthritic disease.

Although it grows naturally on the western and southern coasts of Turkey, rosemary spreads widely in Çanakkale, Mersin, Adana, Tarsus and Hatay provinces, especially in Mersin and Adana region, in maquis flora, in forest spaces, on the edges of fields and vineyards, and in protected forestation areas (Malayoğlu, 2010). Furthermore, it is reported that RE originated from different regions and may have divergent chemical compound contents. The antioxidant, antiproliferative and anti-inflammatory effects of these substances have been emphasized in different studies (Oliviero et al., 2018). Researchers also emphasized that there might be differences in phenolic and chemotype compounds and therapeutic effects obtained from different rosemary species and herbs gathered at other times of the year (Tsiani, 2019; Shen et al., 2020).

The cytotoxic effect of rosemary appearing in high doses draws attention to the importance of the dose arrangement in its use. Current results indicate that rosemary at low concentrations may be a good candidate as an alternative medicine in the therapy of arthritis characterized by cartilage damage in terms of its antioxidant properties. On the other hand, the unexpected effect of RE observed in SW1353 chondrosarcoma cells brings out the possibility of using rosemary as an addition/supplement to existing treatments in high-grade tumors such as chondrosarcoma, which has limited medical treatment but can be treated only with surgical intervention.

When the OSI was calculated, although a change was observed in the groups where low RE concentrations (0.2-1mg/ml) were applied, this was not statistically significant compared to the C group. In comparison, OSI increased at the higher doses of RE treatment (50-100mg/ml). It is observed to decrease at low concentrations of RE treatment (0.2-0.5mg/ml) in SW1353 cells, compared to the C group. In conclusion, the findings indicate that having antioxidant properties at low concentrations, rosemary may be utilized in treating cartilage damage in arthritis. On the other hand, the cytotoxic effects of rosemary at high doses draw attention to the importance of tightly controlled usage of the herbal extract. This unexpected effect we observed in SW1353 cells suggests using rosemary as a supplement/support to the existing chemotherapeutic regimes in high-grade tumors such as chondrosarcoma, which has limited medical treatment but can be treated with surgical intervention.

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Electron Microscopic Examination of Anatomical Structure of Tongue Papillae in Turkish Grey*,**

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Abstract: This study was carried out to determine the surface of the papillae in the dorsal and lateral parts of the tongue surface and the features in the non-papillary parts of Turkish Grey. In the study, 7 male Turkish Grey tongues were used. Materials obtained from the apex, corpus and radix lingua of the upper (dorsal) side of the tongue were taken for scanning electron microscopic examinations. The routine procedure was applied for scanning electron microscopic images.In the study, the presence of large ruminant tongue papillae, papilla filiformis, papilla fungiformis, papilla conica, papilla lentiformis and papilla vallata, was observed. It was determined that the root part of Papilla filiformes had a depression in the form of a ditch. It was observed that the papilla filiformis at the tip of the tongue had very few secondary papillae. Papilla filiformis in the corpus lingua was found to have 2-4 secondary papillae.On the surface of the papilla fungiformis, cell borders were seen at 2000 magnification, taste pores were detected, and the microridge structure was clearly detected at 10000 magnification. A weak annular pad was seen in the papilla vallata. Cell borders were seen at 2500 magnification on surface images. Taste pores were detected at 5000 magnification. In this study, the tongue papillae of Turkish gray, which is very few inTurkeyand resistant to harsh conditions, were examined, and it was aimed to contribute and own the missing literature about tongue papillae.
Keywords: Papilla, scanning electron microscopy, tongue, Turkish Grey

Boz Irk Sığırlarda Dil Papillalarının Anatomik Yapısının Elektron Mikroskobik İncelenmesi

Öz: Bu çalışma, boz ırk sığırda dil yüzeyinin dorsal ve lateral kısımlarındaki papillaların yüzeyinin ve papillasız kısımlardaki özelliklerin belirlenmesi amacıyla yapılmıştır. Çalışmada, 7 adet erkek Boz Irk sığır dili kullanılmıştır. Dilin üst (dorsal) tarafındaki apeks, korpus ve radiks lingua'dan elde edilen materyaller taramalı elektron mikroskobik incelemeler için alındı. Taramalı elektron mikroskobik görüntüler için rutin prosedür uygulandı. Yapılan çalışmada büyük ruminant dil papillalarından, papilla filiformis, papilla fungiformis, papilla conica, papilla lentiformis ve papilla vallata'nın varlığı gözlendi. Papilla filiformes'lerin kök kısmının hendek şeklinde bir çöküntüye sahip olduğu saptandı. Dilin uç kısmındaki papilla filiformis'lerin çok az sekonder papillaya sahip olduğu görüldü. Corpus lingua'daki papilla filiformis'lerin ise 2-4 arası sekonder papilla'ya sahip olduğu saptandı. Papilla fungiformis yüzeyinde 2000 büyütmede hücre sınırları görüldü, tat porları saptandı, 10000 büyütmede ise mikroridge yapısı çok net saptandı. Papilla vallata'da zayıf bir annular pad görüldü. Yüzey görüntülerinde 2500'lük büyütmede hücre sınırları görüldü 5000'lik büyütmede tat porları saptandı. Çalışmamızda zor şartlara dayanıklı, ülkemizde sayısı çok az olan bu ırkın dil papillaları incelenmiş olup, dil papillaları hakkında eksik olan literatüre katkı sağlanması, bu türün gündeme gelmesi ve sahiplenmesi hedeflenmiştir.
Anahtar kelimeler: Boz Irk, dil, papilla, taramalı elektron mikroskop

Introduction

The tongue plays a vital role in nutrition, along with other organs in the oral cavity. In all mammalian species, structural differences in language reflect differences in food sources and the specific habitat of each species. Therefore, the morphological and histological features of the tongue in mammals are indicative of the differences between mammalian lifestyles (Iwasaki, 2002).

In vertebrates, the mucosa of the tongue consists of various papillary systems that perform taste and mechanical functions, and the tongue is covered with multilayered keratinized epithelium (Tadjalli and Pazhoomand, 2004; Kurtul and Atalgın, 2008). Most of the tongue is covered with various papillae concentrated mainly on the dorsal surface, which arise in connection with local modifications of the mucosa and perform mechanical or taste functions. The scattering, volume, number and shape of the papillae differ in each species (König and Liebich, 2015). It has been reported that functionally, some primary and secondary papillae can assist in the transport of food and liquid from the periphery of the tongue to the center and then towards the pharynx (Ojima,

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2001).

Papilla filiformis, which provides a relatively suitable surface for the movement and crushing of nutrients in the mouth towards the esophagus, were detected on the dorsal surface of the tongue, especially in the apex and corpus lingua. It has been reported that the papilla filiformis has significant variations in shape and size in different animals.

Turkish Grey cattle breed, consisting of *Bos taurus primigenus,* is the native breed of Anatolia and Thrace. Turkish Grey cattle, also called steppe and Pleven cattle, are called Anatolian Grey or Turkish Grey in the international literature. In Turkiye, there is a spreading region that starts from Sivrihisar and covers the Aegean and Marmara sides (Alpan and Aksoy, 2012).

Turkish Grey cattle are breeds that can survive in strong living conditions and are relatively resistant to diseases and microorganisms compared to different species. They have the ability to survive without human assistance. In the winter months, except for the very cold periods of the weather, they continue outside in the form of free communities for most of the year. Forages that are not of good quality can be evaluated much better than different breeds. The cost of breeders in the breeding of this species is almost zero (Kök, 1992).

Material and Methods

In the study, 7 adult male Turkish Grey cattle tongues obtained from the slaughterhouse were selected as material. Tongues taken from animals were initially washed with physiological saline. Fragments obtained from the apex, corpus and radix lingua of the upper (dorsal) side of the tongue were taken for scanning electron microscopic examinations. The tongue was fixed with formaldehyde. The samples were kept in 5% glutaraldehyde (0.13 M Sorensen phosphate buffer, pH 7.2) for 2 hours (+4 C refrigerator) for primary fixation. It was washed twice with 0.13 M sorensen phosphate buffer for 30 min. For secondary fixation, it was incubated in 1% Osmium tetroxide (0.13 M sorensen phosphate buffer, pH 7.2) for 1 hour (+4 C refrigerator). It was then soaked in HMDS (Hexamethyldisilazane) for 1 hour for drying. It was then left to dry at room temperature. It was covered with gold and viewed under a scanning electron microscope (EVO50, ZEISS, Germany). Since the materials are taken from the slaughterhouse, there is no need for an ethics committee document.

Nomina Anatomica Veterinaria (2017) is used for anatomical terms.

Results

The materials were found to have all the features of a ruminant language. It was determined that the tongue consisted of apex lingua, corpus lingua and radix lingua. In the measurements, the average length of the tongue was 31.6 cm, the average anterior width was 7.4 cm, the average middle width was 8.3 cm, and the average posterior width was 9.3 cm. It was observed that there were two types of papillae on the tongue. It was observed that the first of these was mechanical papillae and the other was taste papillae. Mechanical papillae were determined as papilla filiformis, papilla lentiformis, papilla conica. Taste papillae were defined as papilla fungiformis and papilla vallata. Papilla foliata was not found.

Macroscopic findings

Papilla filiformis

It was observed that the papilla filiformis (Figure 1) started from the apex of the tongue and spread towards the trunk. An average of 70.5 papilla filiformis was counted in a 1 cm^2 section. It was determined that the free ends of the papilla filiformis were in the caudodorsal direction in the identified tongues. It was determined that especially the parts at the apex were longer in the first anterior 5 cm and decreased as they went posteriorly. It was determined that their length was minimized in the rostral parts of the fossa lingua (Figure 2) and at the tip of the apex lingua. Papilla filiformis was observed with abundant papilla fungiformis (Figure 1-4) in the ventrolateral part of the apex lingua. When the tongue was viewed from the ventral side, approximately 1 cm from the anterior margins of the tongue down, papilla filiformis and especially abundant papilla fungiformis were found. It was found that this part was separated by a sharp border with the ventral part of the tongue that had no papillae (Figure 5). It was found that the papilla filiformis at the tip of the apex lingua was considerably shortened and even macroscopically not observed (Figure 4). No papillae were observed macroscopically in the ventral part of the tongue (Figure 5).

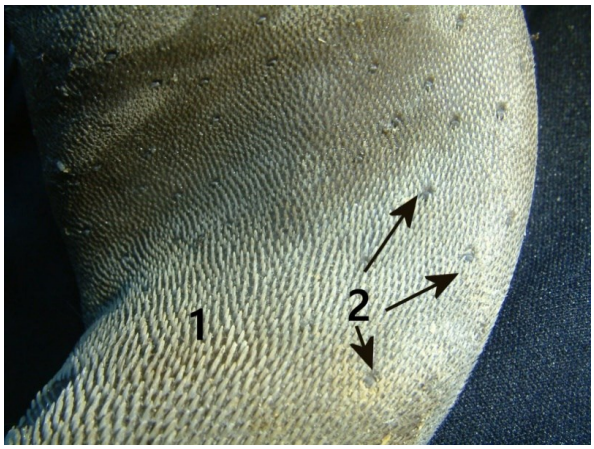

Figure 1. The appearance of the apex lingua in Turkish Grey cattle 1-Papilla filiformis, 2-Papilla fungiformis.

Papilla lentiformis

It was observed that the largest of the mechanical papillae was the papilla lentiformis. Papilla lentiformis (Figure 2-3); observed in the torus lingua in different ways. Papillae, which were not fully evident in the median line, were more prominent in the paramedian part. Papilla lentiformis on average, 223.5 were counted. It was observed that their sizes were different. It was found to be bordered by papilla conica and papilla fungiformis from the sides. They were found to be irregular in shape, pyramidal and round. That's why it was categorized into two different types. The first type was named as pyramidal shaped type 1 because it resembled a pyramid shape, while the other was called round shaped type 2 because it was round shaped.

Papilla conica

Papilla conica; It was observed in the caudal part of the torus lingua of the tongue, especially in the median line, near the radix lingua, and especially in the anterior lateral parts of the torus lingua (Figure 2). On average, 276 papillae were counted. No papilla conica was recorded on the dorsal surface of the corpus lingua. Papilla conicas, which are triangular, conical in shape and relatively thin and small compared to those on the cheek, were found in small amounts in the parts of the torus lingua close to the radix lingua (Figure 2) and in the lateral parts. It was observed that they were of different sizes.

Papilla fungiformis

In the apex lingua, it was observed as mushroom-shaped scattered among abundant papilla filiformis. It was observed less in the median line and more in the lateral line. Papilla fungiformis was also observed in torus lingua. It was observed in the caudal part of the torus lingua, especially in the median line, between the papilla conicas. On the median line was not observed in the parts of the tongue close to the fossa lingua. Small type ones were found in the apex and corpus lingua, while large type ones were found in the torus lingua. It was observed that there was not a complete equality in numbers between the right and left parts of the language. On average, 114.5 papillae on the right and 130.5 on the left were observed. It was determined that there were approximately equal numbers in terms of localization and number only in the part of the fossa close to the tongue. It was detected very rarely in the lateral part of the tongue.

Papilla vallata

Papilla vallata (Figure 2) was detected in the lateral parts behind the torus lingua. It was observed in different sizes. Different numbers of papillae vallata were detected on the right and left sides of the tongue. On average, 20 papillae were counted on the right and 26 on the left. It was observed that the papilla vallata started from the lateral parts after the papilla lentiformis and progressed towards the caudale.

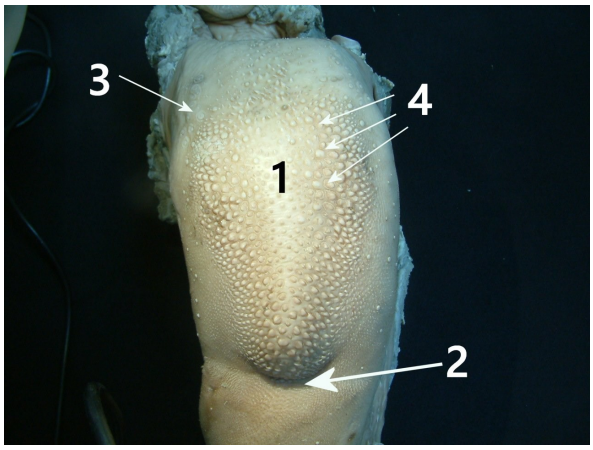

Figure 2. Appearance of the torus lingua of the tongue in Turkish Grey cattle; 1-Torus lingua, 2-Fossa lingua, 3-Papilla vallata, 4-Papilla lentiformis.

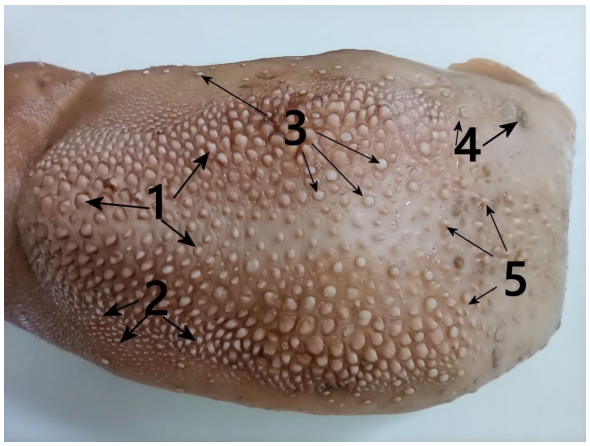

Figure 3. Appearance of papillae on the lingua torus of the tongue in Turkish Grey cattle, 1-Papilla lentiformis, 2-Papilla conica (in the lateral part of the torus lingua), 3-Papilla fungiformis, 4-Papilla vallata, 5-Papilla conica (in the caudal part of the torus lingua).

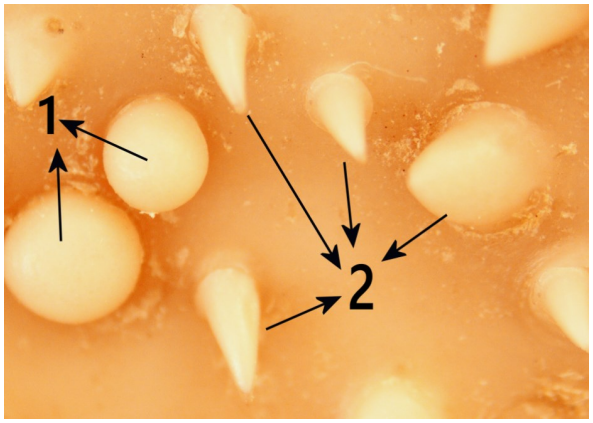

Figure 4. Stereomicroscopy view of some papillae on the torus lingua of the tongue in Turkish Grey cattle, Papilla filiformis was not seen. 1-Papilla fungiformis, 2-Papilla conica.

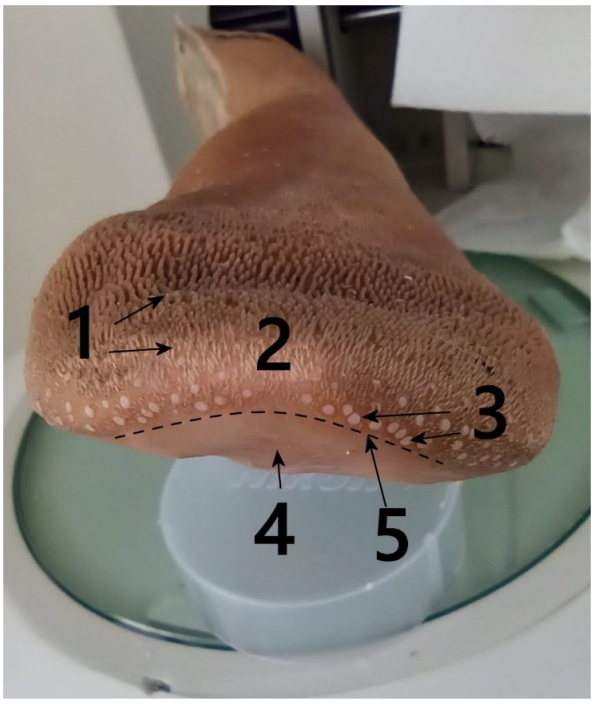

Figure 5. Appearance of papillae on the apex lingua of the tongue in Turkish Grey cattle; 1-Papilla filiformis, 2-Apex lingua, 3-Papilla fungiformis 4-Facies ventralis 5-The part where the tongue has no papillae on its ventral surface and where it is separated by a sharp border with the papillae.

Scanning electron microscopic findings

Papilla filiformis

It was found that the papilla filiformis, which can be seen easily, is quite long and wide in width (Figure 6). Their lengths were measured ranging from 1233 µm to 1420 µm. When measured in the middle, its width was found to vary between 185 µm and 295 µm. It was determined that the apex of the papilla filiformis ended bluntly, not pointedly (Figure 6-7). Some apexes were found to have an unsymmetrical termination as if they were cut unilaterally (Figure 6). A trench-like pit was found in the part where the papilla filiformis attaches to the tongue (Figure 8). It was determined that there were secondary papillae extending from the outer part of this pit towards the body of the papilla filiformis (Figure 8), these papillae were sometimes not seen at all, and some were only one or two. It was found that the width of the secondary papillae ranged from 33 µm to 45 µm when measured from the middle. Its length was determined to be between 86 µm and 115 µm.

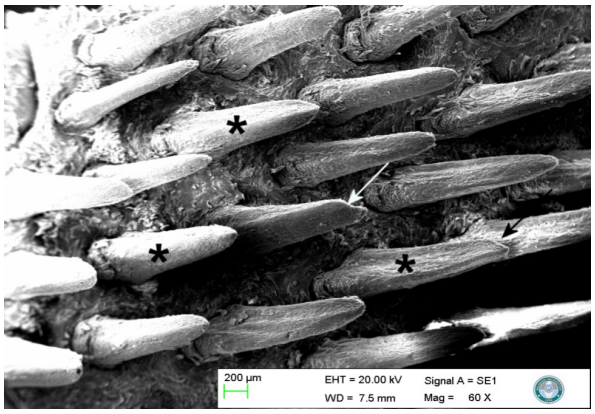

Figure 6. View of the dorsal side of the tongue in Turkish Grey cattle, x60. Asteriks; papilla filiformis, arrow; papilla filiformis with unsymmetrical apex endings.

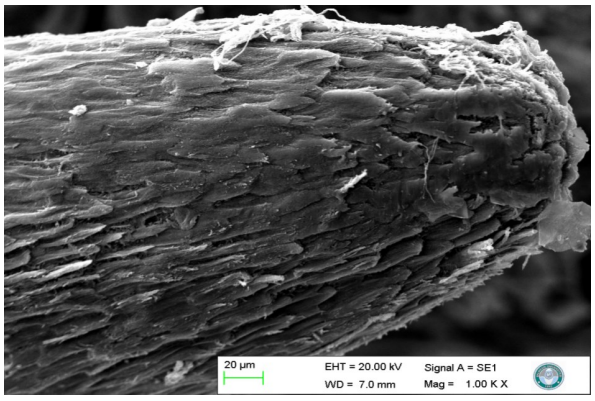

Figure 7. Papilla filiformis tip, x1000.

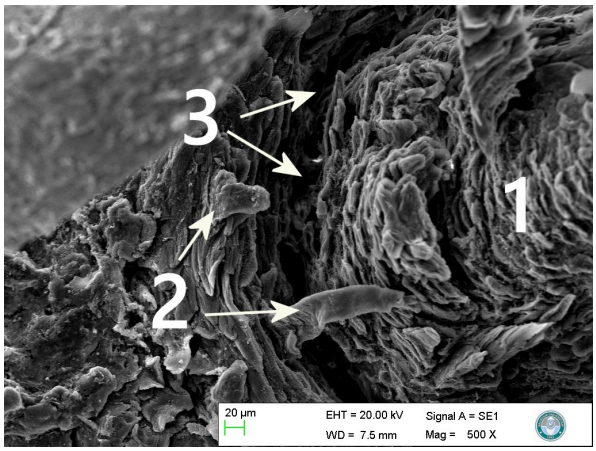

Figure 8. Root part of papilla filiformis x500; 1-Papilla filiformis: 2-Secondary papillae: 3-Trench at the root of papilla filiformis.

It was determined that the papilla filiformis in the middle part of the tongue was smaller than the papilla filiformis in the apex of the tongue. The tips of the papilla filiformis in this section were found to be pointed. It was determined that the length of the trunks of the papilla filiformis ranged from 112 µm to 177 µm. Papilla fungiformis, which provides the sense of taste of the tongue, was seen in this region (Figure 9). These papillae were surrounded by papilla filiformis, heterogeneous and sparsely observed.

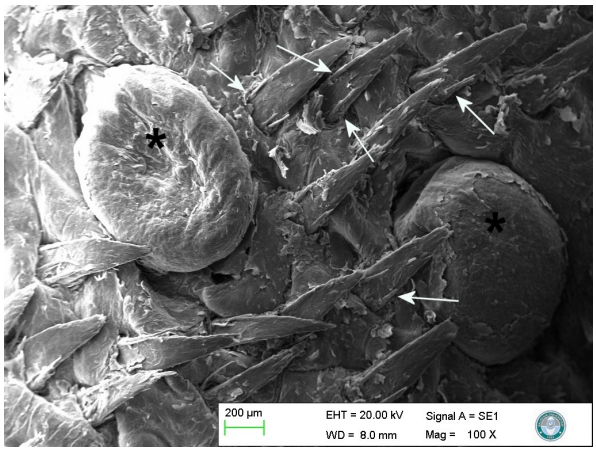

Figure 9. View of the dorsal side of the tongue in gray cattle. Asteriks: Papillafungiformis; Arrows: Secondary papillae of papilla filiformis. x100.

Papilla conica

Conical shaped, triangular shaped papillae conicas, which are relatively thin and small compared to those on the cheek, were found in small amounts in the parts of the torus close to the radix lingua (Figure 3) and in the lateral parts of the torus lingua. Very sparse and small papilla conicas were seen on the lateral aspect of the torus lingua. The lengths of the sparse papilla conicas were measured to vary between 120 µm and 221 µm. It was observed that the width of these papillae in the middle part varied between 43 and 60 µm. It was observed that they were of different sizes. It was determined that these papillae did not have any secondary papillae or protrusion, and also did not have a ditch in the part where they were separated from the tongue. On the other hand, no taste pore was found in the surface enlargement (Figure 10).

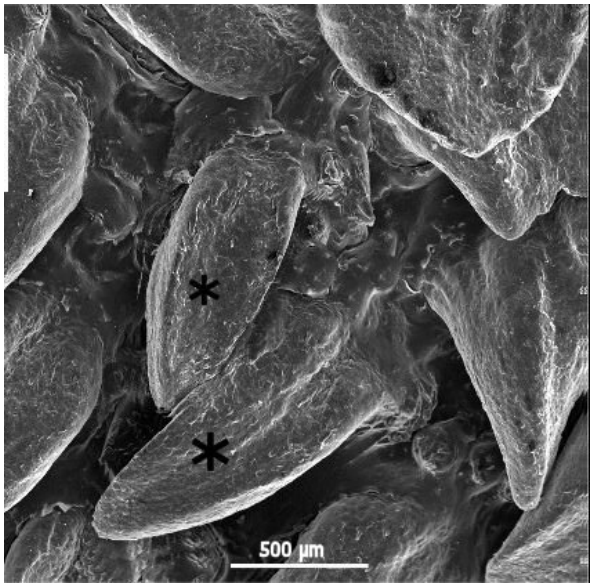

Figure 10. Electron microscopic view of papilla conica, Asteriks: Papillae conicae.

Papilla lentiformis

Papilla lentiformis (Figure 2, 3, 16) was observed in the torus lingua. They were observed to be irregular, round and pyramid-like. No taste pores, ditch or any other formation were found in the surface enlargements.

Papilla fungiformis

The papilla fungiformis was oval and round, and the outer surface was convex. Its diameter was found to be 852 µm on average. It was observed that it was scattered between the papilla filiformis in the anterior and body parts of the tongue. It was seen that there was a groove around it. The surface of the papilla fungiformis was examined. When looking at the surface of the papilla fungiformis at a magnification of 2000, the irregular cell borders were clearly observed (Figure 11). Cell borders were irregularly rectangular, pentagonal and hexagonal (Figure 11). Taste pores were seen at this magnification (Figure 11). The diameter of the taste pores was measured to be 2.3 µm

on average. In the same region, microridges were seen regularly at a magnification of 10000 (Figure 12).

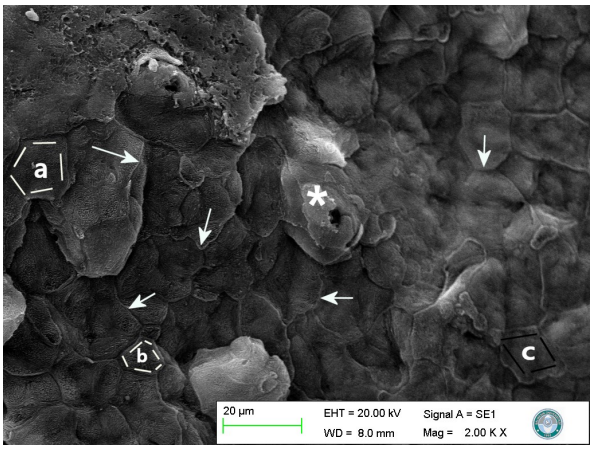

Figure 11. Electron microscopic view of papilla fungiformis, arrows; Cell borders, asterisk: Taste pore. x2000 a. Borders of pentagonal cell: b. Boundaries of the hexagonal cell: c. Borders of a quadrilateral cell.

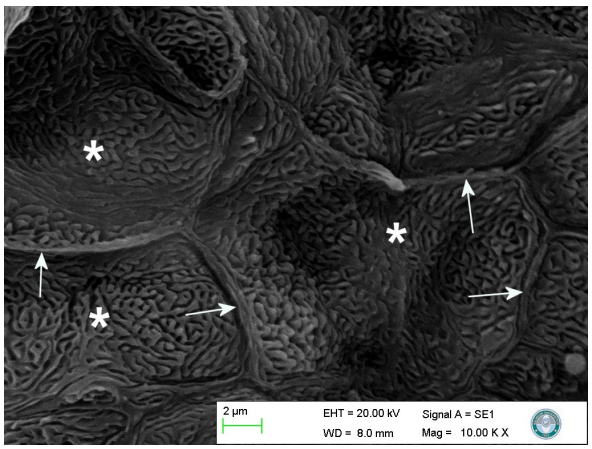

Figure 12. Electron microscopic view of papilla fungiformis; arrows: cell borders; asterisk: microridge. x10000.

Papilla vallata

Papilla vallata were visualized with an electron microscope. This papilla was found to be the largest type of papilla (Figure 13-16). A classic papilla vallata image was observed at 150 times magnification (Figure 13). Its diameter was found to be 879 µm on average. A weak anular pad was visible on the sides of the ditch, surrounding it. Cell borders were detected in 2500 images on the surface of the papilla (Figure 14). Taste pores were found at a magnification of 5000 (Figure 14). It was observed that the diameter of the taste pores was 1.8 µm on average. An average of 10 in the right section and 13 in the left were counted. It was observed that the papilla vallata was slightly convex and had very sparse papillae in the lateral and caudal parts, and only papilla conica was found in the rostral part.

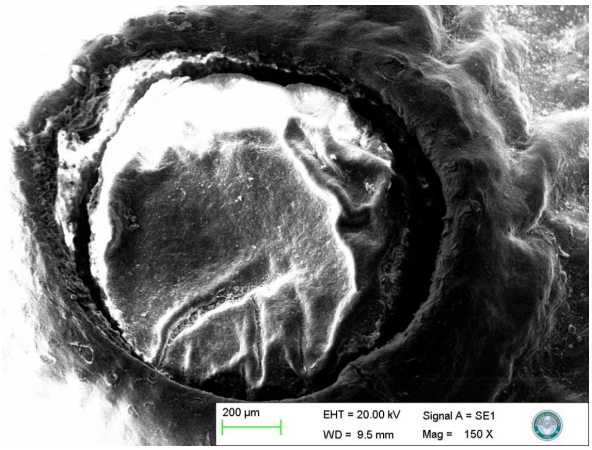

Figure 13. Electron microscopic view of papilla vallata. x150.

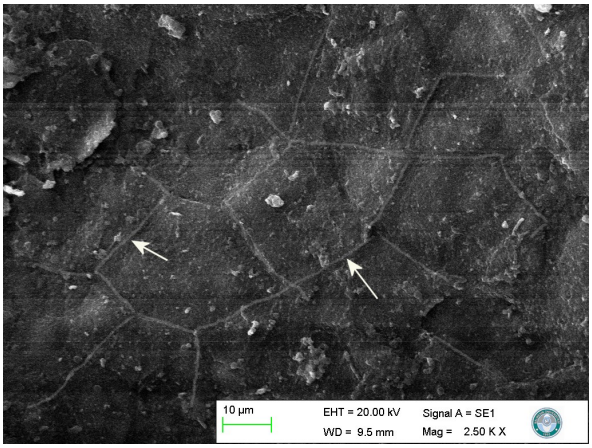

Figure 14. Electron microscopic image of the surface of papilla vallata. Arrows: cell borders, x2500.

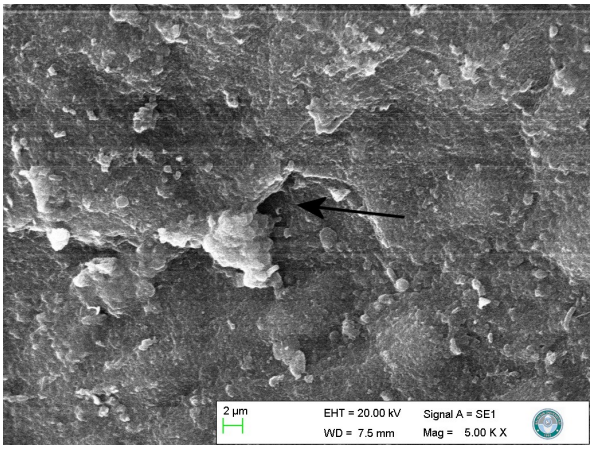

Figure 15. Electron microscopic image of the surface of papilla vallata. Arrow: taste pore, x5000.

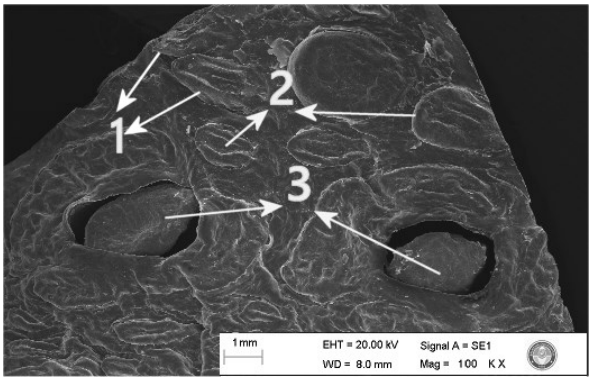

Figure 16. Electron microscopic view of papilla lentiformis and papilla vallata. x10. 1-Papilla lentiformis (pyramidal shaped, type 1): 2- Papilla lentiformis (round shaped, type 2): 3-Papilla vallata.

Discussion and Conclusion

The most important differences of the ruminant tongue from the others are the anatomical features of the caudal part of the tongue. The fact that the formations on the torus lingua are different from the carnivores, sus and equidea is due to the different diet (Adnyane et al., 2010).

Jamunapari goat (Kumar et al., 1998), Saanen goat (Kurtul and Atalgin, 2008), Mehraban lamb (Tadjalli and Pazhoomand, 2004), Barbary sheep (Emura et al., 2000), and Bufallo (Scala et al., 1993) papilla filiformis is reported to be conical in shape, tongue-like in ox (De Paz Cabello et al., 1988), and rod-shaped in Chinese muntjac (Zheng and Kobayashi, 2006). In this study, papilla filiformis was found to be conical in Turkish Grey cattle.

It has been determined that the number of secondary papillae of papilla filiformis in the apex lingua is quite low. In studies, the number of protrusions, also called secondary papillae, at the free end of the papilla filiformis, in connection with the main body, is 3-6 in Jamunapari goat (Kumar et al., 1998), 6-9 in Saanen goat (Kurtul and Atalgın, 2008), Mehraban lamb has 4-8 (Tadjalli and Pazhoomand, 2004), Karacabey merino has two bases (Can et al., 2016), while dromedary camel (Quayyum et al., 1988) has an extension, Chinese muntjak (Zheng and Kobayashi, 2006), bighorn sheep (Takayuki et al., 2002) and goat antelope (Funato et al., 1985) have been reported to have two thin-long visual extensions attached to the main body. In the study conducted in Anatolian buffalo (Can et al., 2015) and dromedary camel (Quayyum et al., 1988), it was stated that secondary papilla was absent. In this study, it was found that the average number of two. In this respect, it was found to be similar to Chinese muntjak (Zheng and Kobayashi 2006), bighorn sheep (Takayuki et al., 2002), goat antelope (Funato et al., 1985) and Karacabey Merino (Can et al., 2016).

The difference in papilla filiformis sizes between Anatolian buffalo (Can et al., 2015) and Turkish Grey breed was remarkable. The papilla filiformis of the Anatolian buffalo varies between 780 and 920 µm in length and between 312 and 422 µm in width. In this study, it was determined that the lengths of the papilla filiformis ranged from 1233 µm to 1420 µm, while their widths ranged from 185 µm to 295 µm. In this respect, it was determined that Turkish Gray papilla filiformis were relatively long and thin compared to the papilla filiformis found in Anatolian buffalo (Can et al., 2015).

In this study, it was determined that the morphology of the papilla filiformis in Turkish Grey cattle was different in the apex and corpus regions of the tongue. In the apex lingua, it was determined that the body of the papillae was longer and easily recognizable, and the apex part of the papilla was conical in shape, some of them were cut on one side and the ends were not symmetrical. No such definition was found in the scanned articles.

It is reported that the papilla lentiformis, which is the largest of the mechanical papillae, provides complementary protection on the linguae surface (Tadjalli and Pazhoomand, 2004).Presence in many animals such as ox (De Paz Cabello et al., 1988), dwarf deer (Agungpriyono et al., 1995), Bactrian camel (Erdunchaolu et al., 2001), Saanen goat (Kurtul and Atalgın, 2008) which was emphasized to be absent, was found in torus lingua in Turkish Grey cattle (Emura et al., 1999), Berber sheep (Emura et al., 2000) and Formosan goat antelope (Takayuki et al., 2002).

In this study, two different forms of papillae, type 1 (pyramidal shaped papillae) and type 2 (round type papillae), belonging to the papilla lentiformis were detected in accordance with the Saanen goat (Kurtul and Atalgin, 2008). Kurtul (Kurtul and Atalgin, 2008) reported in his study that there were papillae with two ends. In this study, however, it was determined that the pyramidal shaped papillae had only one blunt end.

Although in one study (Scala et al., 1993) the presence of a special papilla called “laminari papillae” similar to it, in the torus lingua of buffalo, next to the papilla conicas was mentioned, such a papilla was not seen in this study.

Papilla conica, which was reported to be absent in camels (Quayyum et al., 1988), was found in Barbary sheep (Emura et al., 2000), Japanese goat antelope (Funato et al., 1985), arctic fox (Jackowiak et al., 2009) and land antelope (Emura et al., 1999), it was determined that it is located especially in the posterior part of the torus lingua, less frequently and in a small size on the radix lingua.

Kurtul and Atalgin, (2008) reported that the papilla conica was triangular in shape, without a taste pore on its surface, and had a distinct groove in goats, unlike this study. In this study, it was stated that the papilla conica did not have a secondary papilla, this finding is consistent with some studies (Kumar et al., 1998; Kurtul and Atalgin, 2008; Can et al., 2015).

Papilla conica is found in ruminants like as this study, because it is more resistant to mechanical effects during chewing, but in non-ruminant animals such as the Savanna monkey (Emura et al., 2002), and in ruminant dromedary camels (Qayyum et al., 1988) have been reported to have no papilla conica.Papilla fungiformis were observed in two types in gray cattle, consistent with Formosan goat antelope (Atoji et al., 1998; Takayuki et al., 2002) and Chinese muntjac (Zheng and Kobayashi, 2006). In this study, the small type was found in the apex and corpus lingua, while the large type was reported in the torus lingua. It has been reported in some studies that the surface of the papilla is limited by many different directional grooves (Kumar and Bate, 2004; Nonaka et al., 2008; Adnyane et al., 2010). In this study, however, this type of groove was not observed.

Taste pore was found in both types of papilla fungiformis in gray cattle, different from Akkaraman sheep (Harem et al., 2009) and Jamunapari goat. The findings of this study were found to be consistent with the view that taste buds were present in both types of papillae fungiformis in the Chinese muntjac (Zheng and Kobayashi, 2006).

The number of papillae of papillae vallata located on both sides along the posterior edge of the tongue differs according to the species. Thirty in Barbary sheep (Emura et al., 2000) and black antelope (Emura et al., 1999), 23 in Formosan goat antelope (Atoji et al., 1998; Takayuki et al., 2002), Japanese goat antelope (Funato et al., 1985) and Bighorn sheep (Takayuki et al., 2002), 20 papillae in Saanen goat (Kurtul and Atalgin, 2008) and 10-30 in deer (Adnyane et al., 2010). It was found that there were 23 of them. It has been reported that papilla vallata is absent in prairie rat (Cape Hyrax) (Emura, 2008).

Although it was observed that many papilla conica oriented towards papilla vallata in the savanna monkey (Emura et al., 2002), and the ditch there somewhat closed the gap, such a situation was not observed in this study.

In this study, the papilla surface was smooth, as in the Jamunapari goat (Kumar et al., 1998), ox (Chamorro et al., 1986) and dromedary (Quayyum et al., 1988). It has been reported that the annular pad located on the edge of the papilla vallata regulates the cleaning function of feed residues and saliva accumulated in the ditch with the help of smooth muscles (Chamorro et al., 1986). The annular pad, which is well developed in ruminant species such as the ox (Chamorro et al., 1986), dromedary (Quayyum et al., 1988) and dromedary camel (Eerdunchaolu et al., 2001), has been found in the Formosan goat antelope (Atoji et al., 1998; Takayuki et al., 2002) are reported to be weakly shaped in some species. It was determined that the annular pad was weakly shaped in gray cattle. It has been reported that the annular pad is absent in horses (Chamorro et al., 1986).

In this study, the diameter of the papilla vallata was measured as 897 µm. Accordingly, it was determined to be smaller (1500 µm) than deer (Adnyane et al., 2010), which is one of the measured species.

Papilla foliata can be found in some animals such as pig (Kumar and Bate, 2004), raccoon dog (Emura et al., 2006), rabbit (Nonaka et al., 2008) and horse (Chamorro et al., 1986; Can and Atalgın, 2016) observed and not observed in this study.

In the study, papilla filiformis was found in the Jamunapari goat (Kumar et al., 1998), the ox (Chamorro et al., 1986), the Hellon lamb (Tadjalli and Pazhoomand, 2004) and the buffalo (Scala et al., 1993), papilla fungiformis, papilla conicae, papilla lentiformis, and papilla vallatae have been demonstrated. It shows that the differences and similarities between animal species, the anatomical conditions and characteristics of the papillae in the dorsal and lateral part of the tongue may depend on many factors.

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An Experimental Study on the Use of Exosomes against Acetaminophen-induced Uterine and Fallopian Tubes Damage in Rats

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Abstract: Acetaminophen (APAP) is an antipyretic and analgesic drug that can be bought and used without a prescription worldwide. A dosage of APAP greater than the maximum recommended dosage can increase the risk of organ damage. Mesenchymal stem cells (MSCs) are isolated from various human tissues and used for therapy, in which beneficial effects are attributed mainly to mesenchymal stem cell-derived extracellular vesicles (MSC-EVs). However, no study has focused on the protective effect of exosomes in combination with APAP. Therefore, the present study was carried out to investigate the protective effect of taking exosomes against APAP toxicity on the fallopian tubes and uterus. Forty female Wistar albino rats (12–14 weeks old) were randomly divided into four equal groups: control, APAP (received 1 g/kg APAP), exosome (received 30 µg of exosomes), and APAP+exosome groups that received simultaneously 1 g/kg APAP and were followed three days later by a tail vein injection 30 µg of exosomes. The uterus and fallopian tubes were removed for histological and immunohistochemical analyses after the animals were sacrificed. The results showed that exosomes' administration after APAP decreased APAP's autophagic effects. Moreover, exosome treatment exhibited a protective effect on the immunoreactivity intensities of autuphagy markers (Beclin-1, p62, and LC3). The treatments with exosomes had no adverse effect on the uterus or fallopian tubes. The administration of exosomes after APAP toxicity can decrease cell death through the autophagy effect of APAP. It is suggested that this compound can decrease the toxic effects of APAP. Further studies are needed to evaluate the molecular mechanism of this hyperanalgesic effect.
Keywords: Autophagy, acetaminophen, exosome, pain, rat

Sıçanlarda Asetaminofen Kaynaklı Uterin ve Fallop Tüpleri Hasarına Karşı Eksozomların Kullanımı Üzerine Deneysel Bir Çalışma

Öz: Asetaminofen (APAP), dünya çapında reçetesiz satın alınabilen ve kullanılabilen bir antipiretik ve analjezik ilaçtır. Önerilen maksimum dozdan daha yüksek bir APAP dozu, organ hasarı riskini artırabilir. Mezenkimal kök hücreler (MSC'ler), çeşitli insan dokularından izole edilir ve yararlı etkilerin esas olarak mezenkimal kök hücreden türetilen hücre dışı veziküllere (MSC-EV'ler) atfedildiği terapi için kullanılır. Bununla birlikte, hiçbir çalışma eksozomların APAP ile kombinasyon halinde koruyucu etkisine odaklanmamıştır. Bu nedenle bu çalışma APAP toksisitesine karşı eksozom almanın fallop tüpleri ve uterus üzerindeki koruyucu etkisini araştırmak amacıyla yapılmıştır. Kırk dişi Wistar albino sıçan (12-14 haftalık) rastgele dört eşit gruba ayrıldı: kontrol, APAP (1 g/kg APAP aldı), eksozom (30 µg eksozom aldı) ve APAP+eksozom grupları eş zamanlı olarak 1 g/kg APAP ve ardından üç gün sonra kuyruk damarından 30 ug eksozom enjeksiyonu yapıldı. Hayvanlar sakrifiye edildikten sonra histolojik ve immünohistokimyasal analizler için uterus ve fallop tüpleri çıkarıldı. Sonuçlar, eksozomların APAP'tan sonra uygulanmasının APAP'ın otofajik etkilerini azalttığını gösterdi. Ayrıca, eksozom tedavisi, otofaji belirteçlerinin (Beclin-1, p62 ve LC3) immünoreaktivite yoğunlukları üzerinde koruyucu bir etki sergiledi. Eksozomlarla yapılan tedavilerin uterus veya fallop tüpleri üzerinde herhangi bir olumsuz etkisi olmamıştır. APAP toksisitesinden sonra eksozomların uygulanması, APAP'ın otofaji etkisi yoluyla hücre ölümünü azaltabilir. Bu bileşiğin APAP'ın toksik etkilerini azaltabileceği öne sürülmektedir. Bu hiperanaljezik etkinin moleküler mekanizmasını değerlendirmek için ileri çalışmalara ihtiyaç vardır.
Anahtar kelimeler: Ağrı, asetaminofen, eksozom, otofaji, sıçan

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Introduction

The paraminophenol class of drugs includes acetaminophen (APAP: N-acetyl-p-aminophenol; also known as paracetamol), a non-steroidal anti-inflammatory drug. Its chemical name is N-acetyl-p-aminophenol or 4-hydroxyacetanilide ($C_8H_9NO_2$). The most often recommended antipyretic and analgesic, APAP, generally known as paracetamol, is used mostly for fever, migraine, neuralgia, joint pain, etc. (Zhang et al., 2016). Sexual performance, fertility index, implantation index, and number of implants all decreased after receiving repeated oral treatments for 30 days with APAP (500 and 1000 mg/kg) (Ratnasooriya and Jayakody, 2000). Research on rodents shows that APAP reduces the total number of adult follicles and fetal ovarian germ cells, which affects fertility (Holm et al., 2016; Johansson et al., 2016). Little is known about APAP in girls and its potential relationship to the reproductive system.

The human body consists of mesenchymal stem cells (MSCs) in a variety of tissues. To support numerous physiological processes, MSCs exhibit niche-dependent multiline age differentiation and produce therapeutic exosomes. MSCs thus promise targeted therapy and have the capacity to support both cell-based and cell-free therapies (Li et al., 2019; Mehta, 2021). MSCs are found in a range of tissues throughout the human body. MSCs exhibit niche-dependent multiline age differentiation and produce therapeutic exosomes to support a variety of physiological activities. MSCs can therefore support both cell-based and cell-free therapies and promise targeted therapy. Extracellular vesicles (EVs), which transport a variety of organic molecules, are used by cells to communicate with neighboring cells and distant organs (such as protein, genomic DNA, and RNA). These vesicles are classified depending on their biogenesis and size (Czernek et al., 2020). Exosomes are known as heterogeneous collections of vesicles, and they also carry the phenotypic state of the cell from which they were produced (Sharma et al., 2020). They are released from cells and enter bodily fluids, where they affect the behavior of neighboring cells (Czernek et al., 2020). Exosomes contain a lipid bilayer and, like cells derived from them, transport molecules like DNA, protein, and RNA. Exosomes share a lipid bilayer structure with the cell from which they originated (Kalluri, 2016; Yin et al., 2019). They can freely move throughout the body's blood vessels (Sharma et al., 2020). Through the use of signals including growth factors, proteases, and cytokines, they allow communication between nearby or distant cells (Harris et al., 2015).

It is worrying that research has associated APAP exposure with reduced primordial follicle pools and, hence, reduced fertility. Until now, no studies have investigated the efficacy of concurrent exosome administration on potentially enhancing therapeutic outcomes. So, the purpose of this investigation was to determine whether co-administering exosomes and APAP to female rats had any analgesic activity.

Material and Methods

Animal care

The Institutional Animal Care Committee of Erciyes University approved the study according to a protocol that was followed for this study (approval number 23/030). We used male Wistar albino rats in this work (250–300 g). All of the animals included in the study had normal behaviors, were healthy, and belonged to the same species and gender. Rats that had been previously used in other experiments were not included. The animals were given unlimited access to water and food while being kept in a 12-hour cycle of light and darkness. The animals were kept for at least three days prior to use. The animals were put to death immediately following the experiment using a dose of ketamine.

Obtaining dental pulp MSCs derived exosomes

The secretomes of the dental pulp derived MSCs were collected. Exosomes was isolated using a standard commercial kit (ExoQuick-TC Exosome Precipitation Solution Kit). Briefly, after centrifuging the secretomes obtained for exosome isolation at 3000 x g for 15 minutes, the supernatants were placed into sterile tubes. 10 ml of supernatant were mixed with 2 ml of ExoQuick-TC solution, and the mixture was then incubated for 12 hours or overnight. A 30-minute centrifugation at 1500 x g was then performed on the ExoQuick-TC/supernatant mixture. After the supernatants were removed, pellet-like exosomes could be seen at the tube's bottom.

Experimental design

Different treatments were given to various groups, as follows: Control group: administered as usual with saline. APAP group: On the first day of the trial, rats were gavaged with a single dosage of 1 g/kg of APAP (Baravalia and Chanda, 2011). Exosome group: 30 µg of exosomes were given in 100 µl from the tail vein (Salkın and Basaran, 2023). APAP+exosome group: Each rat received a single dose of 1 g/kg of APAP by gavage, followed three days later by a tail vein injection 30 µg of exosomes. At 72 hours after the last application in the study, the animals were profoundly anesthetized by ketamine and xzylazin (60/10 mg/kg), intraperitoneally, before euthanization. The organs of interest the fallopian tubes and uterus were immediately detached and subsequently processed for histological evaluations.

Histopathological studies

In order to evaluate the uterus and fallopian tubes defects of each experimental group histologically, tissue samples taken at the end of the experiment were fixed in a 10% formaldehyde solution. Tissues kept in formaldehyde for 72 hours were washed in running tap water, passed through a series of increasing grades of alcohol, cleared with xylol, then embedded in paraffin and paraffin blocks were prepared. 5 µm sections taken from paraffin blocks containing rat tissues were taken on polylysine-coated slides. The prepared slides were graded in alcohol (100%, 96%, 80%, 70%, 50%) and then rinsed in water after being deparaffinized with xylol using the standard histological staining technique. The sections were processed through an increasing alcohol series, stained with hematoxylin-eosin (H&E) and Masson's trichrome (MT), passed through xylol, covered with a coverslip by dripping entellan, and examined under a light microscope to identify the general histological structure (Olympus BX51, Tokyo, Japan).

Immunohistochemical procedure

The immunohistochemistry (IHC) studies to evaluate Beclin-1, p62, and LC3 activities use the avidin-biotin peroxidase method. Following deparaffinization, the cross sections were incubated in citrate buffer (pH 6.0) and 3% hydrogen peroxide (Lab Vision, Thermo Scientific, Fremont). To avoid non-specific binding, an Ultra-V block (Lab Vision, Thermo Scientific, Fremont) was used. Following the blocking procedure, tissue sections were incubated overnight with Beclin-1 (Novus Biologicals, Littleton, CO), p62 (Novus Biologicals, Littleton, CO), and LC3 (Cell Signaling Technology, Danvers, MA) primer antibodies. The tissue samples were then exposed for 10 minutes to secondary antibodies (Lab Vision, Thermo Fisher Scientific, Fremont). The streptavidin peroxidase complex (Lab Vision, Thermo Fisher Scientific, Fremont) in combined with DAB enabled made the reaction product detectable. A counterstain was applied using Mayer's hematoxylin. Semi-quantitative Beclin-1, p62, and LC3 immunohistochemical analyses were evaluated in paraffin sections of the uterus and fallopian tubes using a computer imaging system. In the research lab of Erciyes University, two histologists independently and semi-quantitatively assessed immunostaining. The Image J software was used to obtain quantitative measurements. The immunoreactivity intensity for Beclin-1, p62, and LC3 was assessed within 5 fields for each animal at a total magnification of x400.

Statistical analysis

PrismTM software version 8.0 (GraphPad Inc., San Diego, CA) was used to analyze the data (GraphPad Software, San Diego, CA, USA). Standard error of the mean (SEM) is used to provide group means for the raw data. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to examine the distribution of the data. Variables with a normal distribution have, one-way variance analysis (ANOVA) was used before a multiple comparison test by Bonferroni. The following significance levels are listed: *P < 0.05; **P < 0.01; ***P < 0.001.

Result

Histological results

Our histological findings showed that the uterine endometrium, myometrium, and serous layers were distinct in the rats from the control group. Rats from the control group were able to distinguish between myometrium, which is composed of smooth uterine muscles, and endometrium, which is composed of ciliated simple columnar epithelium and an underlying lamina propria where the uterine glands and blood vessels are located. In contrast to the other groups, the endometrium, myometrium, and serosa that constitute the uterine histological structures were more difficult to distinguish in the uterus of APAP-treated rats. Comparatively to the untreated rats, ciliated and secretory epithelial cells were not visible, but foamy-like forms with heterochromatic nuclei were in the APAP-treated rats. In comparison to the control group, this group's lamina propria showed less or no uterine glands at a few sites. The fibroblasts, and connective tissue stroma-forming cells in the lamina propria were seen to have edematous regions and extravasation erythrocytes in addition to degenerative changes. The exosome group's rat uterus had the same normal histology as that of the control group, including the endometrium, myometrium, serous layers, and uterine glands. Layers of the rat uterus from the APAP+exosome group were identified as luminal epithelium, lamina propria, myometrium, and serosa. In the luminal epithelium of the uterus of rats in the APAP+exosome group compared to APAP group animals, foamy-like forms containing heterochromatic nuclei were reduced (Figure 1).

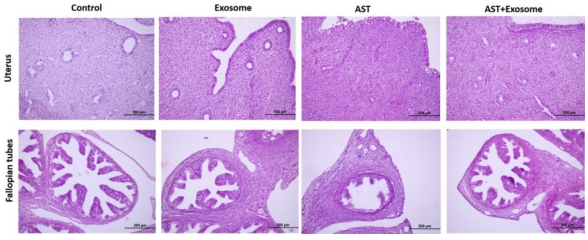

Figure1. Representative micrographs of uterus and fallopian tube stained with H&E are showed at a magnification of ×200. The mucosal folds of the control are covered by simple columnar ciliated epithelium in each tissue. Abnormally loss most of their cilia with the appearance of atypicalpleomorphic nuclei in APAP group. However APAP+exosome group showed the typically normal columnar epithelium covered by numerous cilia (H&E staining).

The control rat fallopian tube was examined using H&E-stained sections, which demonstrated the fimbriae were formed of branched vascular tissue and ciliated columnar epithelium (Figure 1). The stromal and epithelial cells were extensively separated in the damaged tubes. Many cells had pyknotic, darkly pigmented nuclei APAP group. Following exosome therapy, the epithelial cells appeared to return to their usual position, and the pyknotic cells became less noticeable in APAP+exosome group.

In the light microscopic examination of the MT-stained uterus and fallopian tube tissues of the experimental groups, no significant difference was observed in the direction of fibrosis in both tissues (Figure 2).

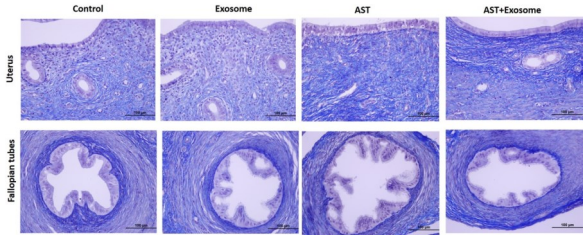

Figure 2. Representative photomicrographs of uterus and fallopian tubes in various groups (MT staining, 390 magnification ×40).

Immunohistochemical results

By using IHC, the semi-quantitative immunoreactivity of the autophagy markers Beclin-1, p62, and LC3 was assessed between the groups. In the endometrium of the rats in the APAP group, we observed that Beclin-1 expression was lower than that of the rats in the control group in surface epithelial cells, glandular epithelial cells, and stromal cells. Rats from the exosome group had similar levels of beclin-1 immunoreactivity in both uterine glands and endometrium compared to the control group (Figure 3).

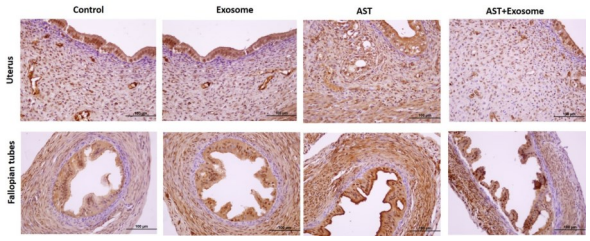

Figure 3. Representative Beclin-1 immunohistochemistry micrographs of uterus and fallopian tubes are showed at a magnification of ×400.

We observed that after APAP administration, Beclin-1, p62, and LC3 immunostaining in surface and glandular epithelial cells and stromal cells in the endometrium increased, while the expression decreased after exosome treatment when we semi-quantitatively compared APAP+exosome with APAP alone (Figure 4).

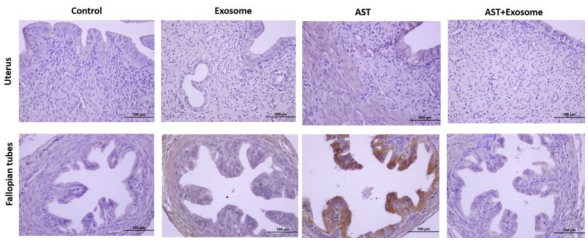

Figure 4. The immunoreactivity of autophagy marker p62 in rat uterus and fallopian tubes from each group. (IHC, magnification ×40).

Rats from the APAP+exosome and APAP groups showed higher levels of LC3 immunoreactivity in glandular epithelial cells, surface epithelial cells, and stromal cells in the endometrium than did rats from the control group. Rats from the exosome group had similar endometrial and uterine gland immunoreactivity to rats from the control group. We found that LC3 immunoreactivity increased after APAP administration in comparison with the control and decreased after exosome administration when we semi-quantitatively compared the APAP+exosome and APAP groups (Figure 5).

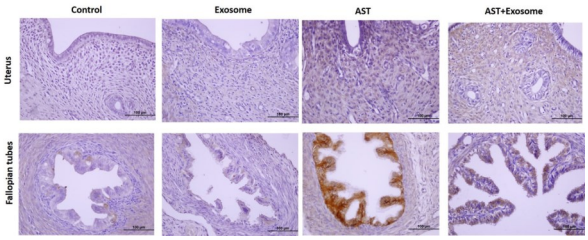

Figure 5. Expression LC3 in uterus and fallopian tubes epithelial cells of the experimental groups (IHC, magnification ×40). The highest intensity expression in APAP group, the lowest in the control group.

The statistical differences between the expression levels of autophagy markers in the uterus and fallopian tube tissues of the experimental groups are shown in Figure 6.

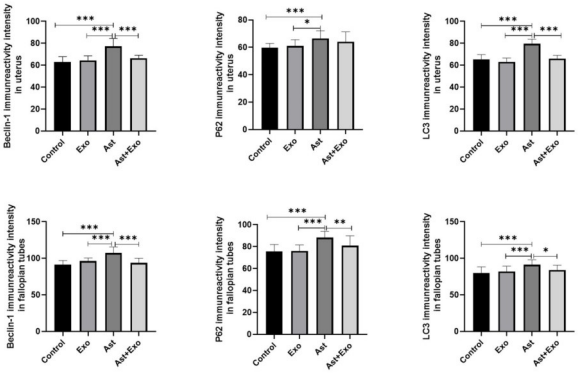

Figure 6. The autophagy markers (Beclin-1, p62, and LC3) in the uterus and fallopian tube were enumerated in immunohistochemical stains. The values in all panels are depicted as mean ± SEM. (*P < 0.05; **P < 0.01; ***P < 0.001).

Discussion and Conclusion

APAP has analgesic, antipyretic, and anti-inflammatory effects on patients with a variety of clinical diseases. Adult fertility and, more broadly, all reproductive functions and processes throughout life are defined by reproductive health. Several research studies have highlighted variations in human reproductive function. The consistent rise in reproductive diseases raises the possibility of environmental and/or lifestyle-related causes (Arendrup et al., 2018). Endocrine disruptors may therefore cause harm throughout generations, particularly in germ cells (Wei et al., 2015), leading to decreased fertility and worse quality gametes, sperm, and oocytes.

APAP is widely used by women. After exposure to APAP during the process of embryogenesis or organogenesis, animal models have demonstrated necrotic and degenerative alterations in the pulmonary, reproductive, and neurological systems (Van den Anker and Allegaert, 2018). The research that is now available also suggests that paracetamol may disrupt several hormonal processes, including steroidogenesis and the depletion of sulfated sex hormones (Cohen et al., 2018), establishing the biological plausibility for any potential change in the embryonic reproductive systems related to paracetamol exposure. When pregnant rats were given indomethacin (0.8 mg/kg/day between 15.5 and 18.5 dpc) or APAP (350 mg/kg/day between 13.5 and 21.5 dpc), the number of germ cells in the fetal ovaries was decreased (by 40–50%). As a result, in utero exposure to APAP and indomethacin impacted the size of the adult ovary and the fertility of F1 females (as determined by the number of pups per generation) (Dean et al., 2016). The sexual development of female offspring was also affected by maternal APAP exposure (350 mg/kg/day from 6 dpc until delivery and from 6 dpc until weaning), which increased plasma estradiol levels, decreased follicle reserve, and impaired sexual behavior in female offspring (Aleixo et al., 2020). In prenatal exposure to APAP in mice (50 or 150 mg/kg/day from 7 dpc to delivery), similar reductions in the number of primordial germ cells were observed at 13.5 dpc, which resulted in a reduced follicular pool in exposed ovaries than in controls. Hence, research suggests that therapeutic APAP dosages have an impact on female fertility (Holm et al., 2016). Growing human epidemiological data over the past 20 years has also raised worry about early life paracetamol exposure and an increased incidence of neurodevelopmental, atopic, and reproductive adverse effects (Bardanzellu et al., 2017). Conversely, the consequences of postpartum ovarian and female reproductive health exposure are unknown. Therefore, the effects of APAP on reproductive health should also be examined. However, we show for the first time that APAP alters the architecture of the uterus and fallopian tubes, which can have toxic effects on female rats.

Exosomes were formerly thought to function as a method for the cell to expel waste. Exosomes were shown to be involved in intercellular communication in the 2000s. Exosomes have been shown to load a wide range of substances and transport them among cells by acting as cargo in the decades that followed (Zhao et al., 2020). Through the application of signals including growth factors, proteases, and cytokines, they enable communication between near or distant cells (Harris et al., 2015). Considering that they include miRNA, exosomes offer a crucial idea in the regulation of changes in cellular activity through genetic material transfer (Valadi et al., 2007). Exosomes behave and function differently depending on where they come from (Börger et al., 2017). Exosomes have an additional benefit over mesenchymal stem cells in that they are a cell-free therapy, preventing immunological reactions and other undesirable effects (Timmers et al., 2011). Because of its better safety profile and reduced immunogenicity compared to using MSCs directly, using exosomes may offer noticeable advantages (Mendt et al., 2019).

In order to promote embryonic implantation during the conceptive cycle and periods of shedding and regeneration during the subsequent non-conception cycle, the endometrium is a dynamic, complex tissue that passes through stages of proliferation and differentiation in succession (Tabibzadeh, 1996). The fallopian tube is essential for several reproductive processes, including sperm transport and capacity, ovary retrieval and transport, fertilization, and the nutrition and transportation of early embryos, in addition to functioning as a passive pathway for gametes and early embryos (Patil, 2009). In order to maintain cellular homeostasis, autophagy mediates the lysosome-mediated degradation of cytoplasmic components such as damaged mitochondria and protein aggre-

gates (Parzych and Klionsky, 2014). In multicellular organisms, autophagy genes play a crucial role in controlling a broad spectrum of vital cellular processes, including cell proliferation, cell death, inflammation, and a wide range of innate and adaptive immune responses (Levine and Kroemer, 2008). As autophagy may be protective or harmful depending on the biological context, the relationship between autophagy and disease pathogenesis is currently a subject of significant research.

Recent studies have demonstrated the importance of autophagy in the physiological and pathological functions of the endometrium, including cyclic menstruation, decidualization, implantation, and disorders such as endometrial hyperplasia, endometrial cancer, and endometriosis (Oestreich et al., 2020). Human uterine epithelial cells from postmenopausal women had higher autophagy levels than those from premenopausal women, suggesting that autophagy began to develop in response to estrogen deficiency (Zhou et al., 2016). This is consistent with the finding that ceasing progesterone or estrogen causes modifications to the menstrual cycle (Choi et al., 2012) by significantly increasing the expression of LC3 in the endometrial Ishikawa cell line. These results suggest that the basal levels of autophagy that are maintained in the cycling endometrium are under the control of ovarian steroid hormone levels. A further interesting observation in this study is that APAP administration, immunostaining of Beclin-1, p62, and LC3 autophagy markers in epithelial and stromal cells in the endometrium increased, while the expression decreased after exosome treatment. Moreover, animals in the exosome group had endometrium and uterine glands that immunoreactive similarly to control rats for Beclin -1, p62, and LC3.

The rat uterus and fallopian tubes exhibited histological and morphometric alterations as a result of exposure to APAP. Exosome treatment can also prevent the degenerative changes caused by APAP in the uterus and fallopian tubes. It appears that APAP has a detrimental impact on the uterine and fallopian tube ultrastructure in rats until it is demonstrated differently. Exosomes can also be used to improve this histology process. Studies utilizing combinations of analgesics should be generalized based on our findings because people frequently take analgesics or are exposed to different cocktails of anti-androgenic or anti-estrogenic chemicals in the environment. Due to the widespread usage of analgesics, more rodent research is needed to evaluate the harmful effects of these drugs.

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Investigating an Outbreak of *Aspergillus fumigatus* Infection in a Racing Pigeon (*Columba livia domestica*) Flock

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Abstract: In this study, the clinical course, pathological findings, and potential risk factors of a systemic aspergillosis outbreak caused by *Aspergillus fumigatus* in racing pigeons (*Columba livia domestica*) were investigated. The disease was reported during the period of intense exercise in a 120 head racing pigeon flock. The exercise period coincided with the high environmental temperatures. Affected birds displayed symptoms, such as weakness, fatigue, depression, inability to stand, rapidly developing weakness, incoordination, convulsions, and death. Necropsy revealed varying sized, prominent nodular or plaque-like lesions on the lungs, air sacs, and visceral peritoneum. Single or multiple caseonecrotic fungal granulomas invading the existing and/or adjacent tissues were seen histopathologically. Malacic lesions associated with fungal elements were observed in the central nervous system. Histopathologic and cytological findings revealed the presence of characteristic *A. fumigatus* elements and lesions. Considering the fact that the environmental temperatures were above the seasonal norms during the emergence of the disease as well as subjugation of pigeons to intense exercise for race preparations, *A. fumigatus* colonization and its spread in racing pigeons was seen likely due to the combined effects of these risk factors.
Keywords: Aspergillosis, exercise, pathology, predisposing factors, racing pigeon

Bir Yarış Güvercini (*Columba livia domestica*) Sürüsünde *Aspergillus fumigatus* Salgınının Araştırılması

Öz: Bu çalışmada yarış güvercinlerinde (*Columba livia domestica*) *Aspergillus fumigatus*'un neden olduğu sistemik aspergillozis salgınının klinik seyri, patolojik bulguları ve muhtemel risk faktörleri araştırıldı. Yüzyirmi adet yarış güvercini bulunan sürüde yoğun egzersiz periyodu ile ilişkili hastalık salgını rapor edildi. Hastalığın ortaya çıkışı yoğun egzersiz programı ve hava sıcaklıklarının yüksek seviyelerde seyretmesi ile ilişkiliydi. Etkilenen kanatlılarda halsizlik, çabuk yorulma, depresif durum, ayağa kalkamama, hızlı gelişen zayıflama, inkoordinasyon, çırpınma gibi belirtiler ve ölüm görüldü. Nekropside akciğer, hava keseleri ve viseral peritoneumun yüzeyinde değişen büyüklüklerde, nodüller veya plak benzeri lezyonlar dikkati çekti. Histopatolojik olarak parenkimatöz organları etkileyen ve bu organların çevresindeki dokulara invazyon gösteren fungal heterofilik granülomlar belirlendi. Santral sinir sisteminde fungal yapılar ile ilişkili malasik lezyonlar görüldü. Histopatolojik ve sitolojik bulgular *A. fumigatus*'a ilişkin karakteristik bulguları ortaya koydu. Hastalığın çıkış döneminde hava sıcaklıklarının mevsim normallerinin üstünde seyretmesi ve bu tarihlerde yarışlara hazırlık için güvercinlere yoğun egzersiz yaptırıldığı göz önüne alındığında *A. fumigatus*'un güvercinlerde kolonize olarak yayılmasında bu faktörlerin olası rolü olduğu düşünüldü.
Anahtar kelimeler: Aspergillozis, egzersiz, patoloji, predispozan faktörler, yarış güvercini

Introduction

Despite being uncommon in mammals (Hazıroğlu et al., 2006; Arné et al., 2011), aspergillosis, primarily caused by *Aspergillus fumigatus*(Arné and Lee, 2020), is a major deadly mycotic disease of free-living, captive, and domestic birds (Akan et al., 2002; Atasever et al., 2004; Beytut et al., 2004; Beyaz et al., 2008; Özmen et al., 2013; Aslan et al., 2015; Gulcubuk et al., 2018). Aspergillus fungi are common in nature (Arné and Lee, 2020), and aspergillosis is commonly associated with contaminated feed or litter (Martin et al., 2007). Fungi sporulate in favorable climatic conditions, increasing the number of conidia in the air and thus the risk of air-borne infection. Despite the presence of aerial mycoflora birds, immunosuppression plays a major role in the development of aspergillosis (Fulleringer et al., 2006; Cafarchia et al., 2014).

Pulmonary infection, which includes diffuse miliary plaques or nodular fungal lesions of lungs and air sacs, is the most common type of aspergillosis. Depending on the severity of lesion, infected birds exhibit varying degrees of dyspnea, gasping, and accelerated breathing (Arné and Lee, 2020) The infection may spread to neighboring organs through local inva-

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sion (Martin et al., 2007) or, in rare cases, through circulation to other organs and systems, most notably the eyes (Beckman et al., 1994) and brain (Hubben, 1958; Akan et al., 2002; Ozmen and Dorrestein, 2004). Encephalitic lesions can be granulomatous or malacic in nature and are clinically manifested by ataxia, tremor, and other symptoms (Hubben, 1958; Akan et al., 2002; Arné and Lee, 2020).Other avian granulomatous infections with similar clinical signs may be confused with this disease. The presense of a fungal element in a tissue specimen, a touch-impression smear of the cut surface, or in culture and cytology aids in the final diagnosis (Arné et al., 2011; Arné and Lee, 2020)

The present study describes the clinical and pathological findings and potential risk factors for aspergillosis caused by *A. fumigatus* in racing pigeons, affecting the lungs, air sacs, visceral organs, and brain.

Materials and Methods

History

In October 2022, a disease outbreak resulting in deaths was reported in a flock of 120 racing pigeons (*Columba livia domestica*) aged 3 to 5 months. The breeder stated an average of 1-2 deaths daily at the onset of the disease that increased to an average of 8-10 deaths daily following the vaccination against Newcastle disease and infectious bronchitis while there were no reports of disease or death in the area. The occurrence of disease coincided with the exposure of pigeons to temperatures above seasonal norms (36 °C - the highest) and excessive physical exercise while preparing for competitions. Housing and rearing conditions like ventilation, relative humidity, and lighting were compliant with the standards. Fatigue, depression, inability to stand, rapidly developing weakness, incoordination, convulsions, and death were among the clinical symptoms seen in the affected birds. In the course of this outbreak, 75 pigeons died, 15 of which were presented to the Department of Pathology for necropsy. The study did not require ethical approval from the institutional committee since the samples used in the study were collected within the scope of diagnostic purposes.

Pathological examination

Gross pathological findings were documented during necropsy. Lactophenol cotton blue was used to stain touch-impression smears of the lesions' cut surfaces. Tissue samples were then fixed in 10% neutral buffered formalin for 48 h before being dehydrated in ascending concentrations of ethanol, cleared in xylene, and embedded in paraffin. The paraffin blocks were cut into serial sections (4-5 µm) and stained with hematoxyline and eosin. To visualize the microbial agents, selected tissue sections were stained with periodic acid Schiff (PAS), Gridley fungus (GFS), Brown-Breen (BB), and Ziehl-Neelsen (ZN) stains. Slides were examined using a light microscope (#BX51, Olympus) and a digital camera (#SC180, Olympus), and photomicrographs were taken.

Immunohistochemistry

To investigate the possibility of Newcastle virus infection, immunohistochemical staining was performed on sections of lesioned organs (brain, kidney, liver, heart, air sacs, and lung). After deparaffinization and rehydration, the tissues' peroxidase activity was inhibited, antigenic epitopes were exposed, and non-specific binding sites were blocked. The sections were then incubated for 1 h each with polyclonal rabbit anti-Newcastle virus and HRP-labeled anti-rabbit (#MRT621, Biocare, USA), before being stopped with 3,3′-diaminobenzidine tetrahydrochloride. As a positive control, tissues with confirmed Newcastle disease virus were used.

Result

Gross pathology

All of the birds were in poor physical condition, with moderate muscle mass loss. On the lungs, there were randomly distributed multifocal to coalesing consolidated areas with round, oval-shaped, raised dome-like, flat, or umbilical nodules in the center, accompanied by caseous necrosis (Figure 1a-b). Fibrinous adhesions from the pleura to the thorax were occasionally found in the lesion areas. In the tracheal lumen, mucoid exudate or, less commonly, caseous exudate adhering to the mucosa was observed. In sporadic cases, the mucosa at the level of pharyngeal papillae was quite swollen and hyperemic, with numerous gray-to-white nodular formations of varying sizes on the surface (Figure 1c). Hearts were generally hypertrophic, with thickened pericardial sacs and gray-to-white areas on the epicardium on occasion (Figure 1d). The air sacs had thickened and become opaque, with cream-colored plaque-like lesions protruding from the surface. The thoracic air sacs had the most prominent lesions. Plaque-like lesions of varying sizes were also observed at random on the surface of the kidneys and the intestine serosa. Kidney lesions were more common and larger near the air sacs (Figure 1e-f). Except for hyperemia in leptomeningeal vessels, no lesions were found in the central nervous system (CNS).

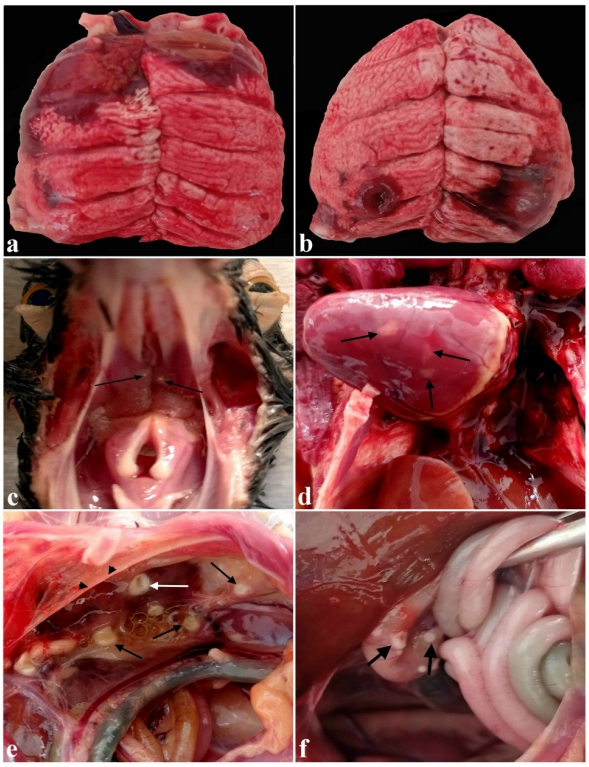

Figure 1. Macroscopic lesions of aspergillosis in racing pigeons. a) extensive consolidated areas with necrosis on the cranial regions of the lung; b) varying sized, multifocal consolidated areas with round to oval, raised dome-like, or umbilical nodules on the lung; c) The mucosa at the level of pharyngeal papillae is prominent with gray to white nodular lesions (arrows) on its surface; d) gray to white amorphic areas on the surface of epicardium (arrows); e) the air sacs are thickened and opaque (arrowheads) and plaque-like lesions on air sacs (black arrows) and surface of the kidneys (white arrow); f) flat plaque-like lesions on the serosa of the intestines (arrows).

Histopathological findings

On gross examination, the nodular or plaque-like lesions seen in various organs and tissues were heterophilic granulomas with intralesional fungal hyphae. Granulomas were observed replacing, distorting, or compressing existing tissue. The granulomas had caseous necrotic exudate in the center, which was surrounded by a palisade of radially organized multinucleated giant cells and an intense infiltration of macrophages mixed with heterophils without an outermost fibrous capsule. Giant cells were occasionally missing from the granulomas. At the periphery of granulomas, clusters of lymphoid cells were seen sporadically. In the lesion areas, there was total or segmental fibrinoid necrotic vasculitis with thrombosis, hemorrhages, and fungal mycelium invading the vessel wall, as well as perivascular inflammatory cell infiltration. With mixed inflammatory cells and edema, the visceral peritoneum was slightly thickened.

Multifocal heterophilic granulomas were found in the parabronchi of the lungs. Heterophilic fibrinous exudate or less commonly diffuse granulomatous infiltrates filled the secondary bronchi, infundibulum, and atria surrounding the granulomas (Figure 2a). In severe cases, coalescence of these lesions affected a larger area opening to the adjacent parabrochi, where numerous dichotomously branched septated hyphae, conidia, and a few conidiophores (characteristics of *Aspergillus* spp.) were seen (Figure 2b). Widespread thrombotic necrosis areas affecting the adjacent granulomas along with intense fungal elements invasion were seen in some cases. The pleura and air sacs had thickened with fibrin-rich or caseonecrotic exudate with intralesional fungal elements (Figure 2c-d). The tracheal submucosa thickened due to edema and inflammatory cell infiltration. Caseous exudate occasionally covered epithelial damage in the mucosa. Small granulomas were seen scattered throughout the oropharynx's submucosa. Mild-to-moderate macrophage infiltrates mixed with heterophils and edema thickened the pericardium, occasionally invading the myocardium (Figure 2e). The pericardial lesion was more visible at the heart's base. Similar lesions were found in the adventitia of the vessels that enter and exit the heart. In one case, small granulomas were also found in the subendocardium. Typical granulomas was also observed on the surface of kidney (Figure 2f). The leptomeninges and choroid plexuses in the CNS (n=2) were thickened with edema and inflammatory cell infiltrations. Malacic lesions with inflammatory cells infiltrates were found adjacent to these lesions (Figure 3a-b). In the liver (n=3), there was inflammatory cell infiltration consisting of macrophages and fewer heterophils scattered randomly in the parenchyme or perivenular areas, with occasional lymphoid cell hyperplasia and necrotic foci. In the liver, parenchymal degeneration observed, which was more prominent in subcapsular and periacinar regions and was accompanied by fatty degeneration in some areas.

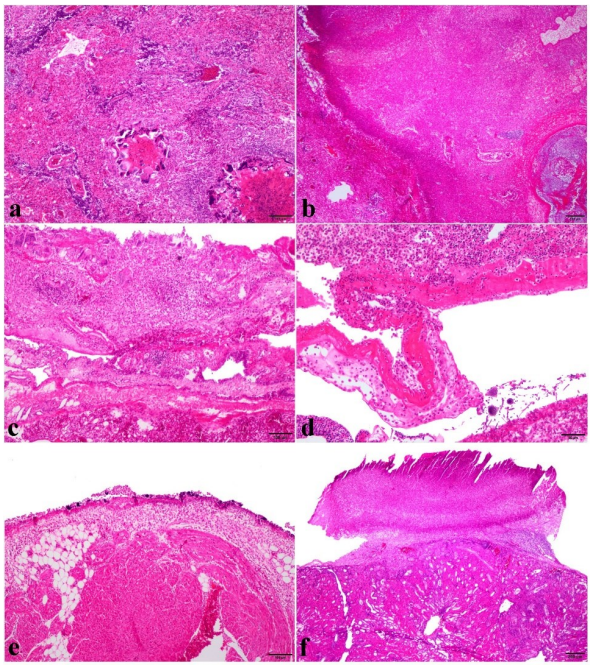

Figure 2. Histopathological lesions of aspergillosis in racing pigeons, H&E stainings. a) varying sized granulomas on the lung, characterized by central caseonecrotic exudate surrounded by a palisade of radially organized multinucleated giant cells and epitheloid macrophages, Bar: 100 µm; b) a large granuloma with intralesional intense fungal elements and thrombosed vessel on the lung parenchyma, Bar: 200 µm; c) thickened pleura with fibrin-rich exudate comprising of macrophages, heterophiles, and multinucleated giant cells, Bar: 100 µm; d) caseified necrotic exudate that includes hyphae, conidia, and conidiophores covered the air sacs, Bar: 50 µm; Inset: close view of fungal elements in caseified necrotic exudate; e) thickened pericardium by moderate infiltrates of macrophages admixed with heterophils and edema invading the myocardium, Bar: 100 µm; f) laminar appearance caseous exudate typically protruding from the surface of kidney, Bar: 200 µm.

In the center of granulomatous foci and encephalomalacic areas, GFS and PAS staining revealed branched, septated fungal hyphae and spores invading the surrounding tissue (Figure 3c-d). The BB and ZN stains were negative. H&E stained tissue sections and lactophenol cotton blue stained touch-impression smears revealed the characteristic of *A. fumigatus* as septate hyphae and typical unbranched conidiophores, each with a single conidial head or vesicle. There were typical columnar and unseriate conidial heads, as well as short conidiophore stipes with conical-shaped terminal vesicles. On the upper two thirds of the vesicle, conidial vesicles were seen supporting a single row of phialides (Figure 4).

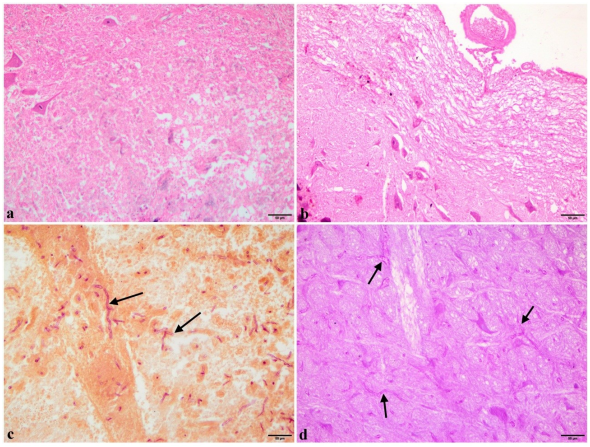

Figure 3. Mycotic encephalomalacia in racing pigeons. a-b) cerebrum showing extensive malacia, H&E, Bars: 50 µm. GFS (c) and PAS (d) stainings showing numerous dichotomously branched, septate fungal hyphae, typical of *Aspergillus* spp. in the areas of malacia, Bars: 50 µm.

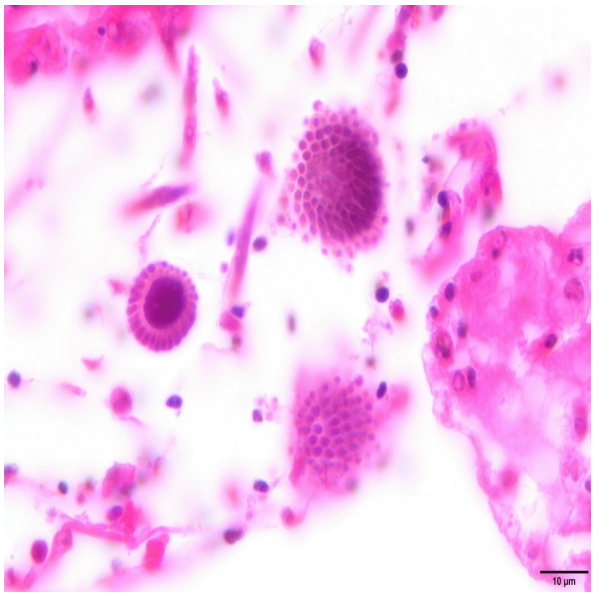

Figure 4. Tissue section showing fungal elements (hyphae, conidia, and conidiophores) specific to *A. fumigatus*. H&E staining, Bar: 10 µm.

Immunohistochemical findings

There was no anti-Newcastle disease virus antibody immunoreactivity.

Discussion and Conclusion

Despite being uncommon in mammals (Tunca and Hazıroğlu, 2004; Hazıroğlu et al., 2006; Tunca et al., 2006), fungal infections especially aspergillosis continues to result in considerable morbidity and mortali-

ty especially in birds (Akan et al., 2002; Atasever et al., 2004; Beytut et al., 2004; Beyaz et al., 2008; Özmen et al., 2013; Aslan et al., 2015; Gulcubuk et al., 2018). In this epidemic, clinical, macroscopic, microscopic, and cytological examinations revealed *A. fumigatus* systemic infection. The clinical signs of the disease differ depending on the organs and tissues involved, as well as the disease's progression (localized or systemic) (Arné and Lee, 2020). In this outbreak, clinical respiratory signs associated with disease were subtle that might be linked with the lesser extent of the pulmonary lesions. Previous research has shown that mild pulmonary symptoms can occur in spontaneous outbreaks (Jensen et al., 1997) and experimentally infections of *A. fumigatus* (Thierry et al., 2013). Signs of the involvement of nervous system like inability to stand up, incoordination and convulsions were seen in this study as described previous reports (Richard et al., 1982; Arné and Lee, 2020). Earlier studies have shown that these findings are caused by granulomatous (Ozmen and Dorrestein, 2004) or malacic meningoencephalitis (Akan et al., 2002). In our study, nervous signs in some pigeons might have resulted from malacic lesions caused by fungal elements. Despite the neurological symptoms, some studies have found that histopathological lesions in the CNS may be absent in aspergillosis outbreaks. These findings suggest that neurological symptoms may occur as a result of fungi -produced toxins (Richard et al., 1981; Özmen et al., 2013)

Mold-contaminated litter and feed is the main source of Aspergillosis (Martin et al., 2007). Under favorable climatic conditions, fungi sporulate in these materials, increasing the mycofloral load in the air. Birds exposed to large amounts of conidia are more likely to become infected. Nonetheless, the factors that cause immunosuppression in birds are equally important in the progression of infection. These factors include inadequate ventilation, high temperature and humidity, mucosal irritants, physical activity, and exogenous administration of corticosteroids (Fulleringer et al., 2006; Cafarchia et al., 2014). Physical stress, such as intense exercise, activates the sympathomedullary pathway and the hypothalamus-pituitary-adrenal axis, allowing stress hormones to be released(Caplin et al., 2021). Excess catecholamine secretion during increased physical activity may contribute to immunosuppression (Kruk et al., 2020). During the summer and autumn, racing pigeons in this outbreak were subjected to intense exercise and a special nutritional regimen for long-distance flight. This rigorous exercise regimen may be one of the factors that predisposes pigeons to aspergillosis by causing immunosuppression. Furthermore, environmental temperatures were above seasonal norms during the current outbreak, indicating that heat stress might have played a role in the disease's emergence. Moreover, the increased mortality following vaccination in the current outbreak was consistent with the findings of a previous study reported that vaccine administration is a risk factor for aspergillosis (Barton et al., 1992).

Because the infectious agent usually enters through the air, the pulmonary system is the primary infection site. Conidia inhaled are typically eliminated by an activated immune system. The conidia of *A. fumigatus* are very small organisms that can cross the physical barrier and infiltrate deep into the respiratory system to initiate the infection before the host immune response is effective (Martin et al., 2007; Arné et al., 2011; Arné and Lee, 2020). Consolidation in the lungs with caseous necrotic noduls and fibrinous adhesions, as well as plaque-like lesions in the air sacs and visceral peritoneum, was also observed in our study, as previously reported (Akan et al. 2002; Arné and Lee, 2020). The present study's widespread and severe pulmonary lesions suggest that hyphae adhesion and colonization most likely began in the lung and thoracic air sacs and then spread to other tissues. Because of the presence of smaller lesions in organs adjacent to the air sacs, as well as hyphal lesions in brain, endocardium, and parenchyma of other organs, we believe that systemic infection occurred via both hematogenous route and by direct invasion. The presence of multiple parenchymal lesions in the same organ suggests that hematogenous spread is a continuous process.

Ocular lesions have been reported in previous aspergillosis outbreaks, with two different localizations involving either the corneal and conjuctiva or the vitreous humor (Richard et al., 1984; Beckman et al., 1994; Dalton and Ainsworth, 2011). While corneal and conjuctival involvement develops as a result of direct contact with superficial fungal elements (Beckman et al., 1994; Dalton and Ainsworth, 2011), choroid and ciliary retinal involvement develops as a result of fungi being spread hematogenously from pulmonary lesions (Richard et al., 1984). Histopathological evidence of corneal and intraocular lesions was not found in this study. Other factors may be associated with the absence of external eye lesions. In fact, a previous study suggested that mycotic keratoconjuctivitis develops as a result of corneal damage caused by toxic gases, such as ammonia (Beckman et al., 1994).

Multifocal to coalescing distributed fungal granulomas surrounded by giant cells in lungs, air sacs, and visceral peritoneum in addition to diffuse granulomatous infiltration in lungs in severe cases was consistent with previous studies (Richard and Thurston, 1983; Akan et al., 2002; Cafarchia et al., 2014). We easily detected the fungal elements using PAS and GFS stainings, as previous reported (Ozmen and Dorrestein, 2004). According to one experimental study (Richard and Thurston, 1983), granulomas

begin to be surrounded by a fibrous capsule during the subacute stage, and the fibrous capsule becomes more prominent later on. In our study, granulomas that were not surrounded by a prominent fibrous capsule suggests that infection occurred early in the disease's progression. Infected birds with non-viable *A. fumigatus* conidia do not exhibit the typical pneumonia lesions surrounded by giant cells (Kunkle and Rimler, 1996)

The present study demonstrates that *A. fumigatus* caused this outbreak with a mortality rate of more than 60%. In racing pigeons, high environmental temperature and intense physical activity played a role in the systemic course of the disease involving the CNS by probable immunosuppression.

Declaration of Competing Interest

The authors declare that no commercial funding was obtained that may be construed as potential conflict of interest.

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Memeli Testisinde Kan-Testis Bariyeri'nin Bileşenleri ve Üreme ile İlişkileri

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Öz: Memelilerde vücudun bazı özel bölümlerindeki molleküllerin kan ve dokular arasındaki hareketi "kan-doku bariyeri" adı verilen yapılar tarafından kontrol edilir. Bu bariyerlerin başlıcaları kan-beyin, -plasenta, -retina, -timus, -testis ve -epididimis bariyerleridir. Kan-testis bariyeri (BTB) ve kan-epididimis bariyeri (BEB) erkek üreme sistemindeki iki önemli hücresel bariyerdir. Seminifer epitelde yerleşen ve komşu Sertoli hücreleri arasında bulunan BTB, tight junction, gap junction (geçit bağlantıları), desmozom (macula adherens) ve adherens junction (bazal ektoplazmik özelleşme-testise özgü bir yapışma bağlantısı) tipi bağlantılar tarafından oluşturulur. Bu bariyer gelişmekte olan germ hücrelerini, özellikle postmayotik spermatidleri, kan ve lenf yoluyla buraya taşınan zararlı ajanlardan (ilaçlar, toksik kimyasallar ve mutajenler gibi) koruyan ve farklılaşmış germ hücrelerine karşı oluşabilecek otoimmun tepkileri önleyen biyokimyasal ve immünolojik bir mikro çevre oluşturur. BTB seminifer tübül epitelini bazal ve adluminal bölmelere ayırarak hücre polaritesi sağlar ve tübül lümenindeki sıvının kimyasal bileşiminin korunmasına yardımcı olur. BTB spermatogenez sırasında yeniden yapılanmaya uğrar, ancak bütünlüğü bozulmaz. Böylece germ hücreleri bu benzersiz yapı sayesinde seminifer epitel boyunca taşınır. Bariyeri oluşturan bileşenlerden herhangi birinde bozulma olması durumunda germ hücreleri gelişimlerini tamamlayamaz ve erkeklerde infertilite şekillenir. Ayrıca, gelişmemiş germ hücreleri sekonder oositi dölleyemediğinden dişi fertilitesi de dolaylı olarak bu durumdan etkilenebilir. Özetle bu bariyer germ hücrelerinin hayatta kalması ve normal spermatogenezin devamlılığı için kritik bir öneme sahiptir. Bu derlemenin amacı, memelilerde erkek infertilitesinde önemli rol oynayan kan-testis bariyerini oluşturan bağlantı komplekslerinin moleküler bileşenleri hakkında bilgi vermektir.
Anahtar kelimeler: Kan-testis bariyeri, sıkı bağlantılar, testis, tutundurucu bağlantılar

Components of the Blood-Testis Barrier in the Mammalian Testis and Their Relationship with Fertility

Abstract: In some special parts of the mammalian body, the movement of molecules between blood and tissues is controlled by structures called "blood-tissue barriers." These barriers are mainly the blood-brain, -placenta, -retina, -thymus, -testis, and -epididymis barriers. The blood-testis barrier (BTB) and the blood-epididymis barrier (BEB) are the two important cellular barriers in the male reproductive system. BTB is localized between adjacent Sertoli cells in the seminiferous epithelium of the testis and is formed by tight junctions, GAP junctions, desmosomes (macula adherens), and adherens junctions (ectoplasmic specialization-a testis-specific adhesion junction). The BTB creates a biochemical and immunological microenvironment that protects developing germ cells, especially post-meiotic spermatids, from harmful agents (such as drugs, toxic chemicals, and mutagens) carried there by blood and lymph and that prevents autoimmune responses against differentiated germ cells. The BTB divides the seminiferous tubule epithelium into basal and adluminal compartments, ensuring cell polarity and helping to maintain the chemical composition of the fluid in the tubule lumen. BTB undergoes remodeling during spermatogenesis, but its integrity remains intact. Thus, thanks to this unique structure, the germ cells are transported across the seminiferous epithelium. Any disruption in the components that make up the barrier can adversely affect Sertoli-germ cell interactions, preventing germ cells from completing their development and leading to male infertility. In addition, female fertility may be indirectly affected as immature germ cells cannot fertilize the secondary oocyte. In summary, this barrier is critical for germ cell survival and maintenance of normal spermatogenesis. This review aims to provide information about the molecular components of the junction complexes that form the blood-testis barrier, which plays an important role in male infertility in mammals.
Keywords: Adherens junction, blood-testis barrier, testis, tight junction

Giriş

Memeli testisleri, erkek üreme hücresi olan spermiyumları ve erkeklik hormonları olan androjenleri üreten bir çift organdır. Testis dokusu başlıca seminifer tübüller ve interstisyum olmak üzere iki bölümden oluşur. Spermatogenezisin gerçekleştiği kanalcıklar olan seminifer tübüller somatik Sertoli hücreleri ile germ hücrelerini (spermatogonyum, spermatosit ve spermatid) içeren çok katlı epitelle örtülüdürler. Sertoli hücreleri, seminifer tübülün bazal membranından

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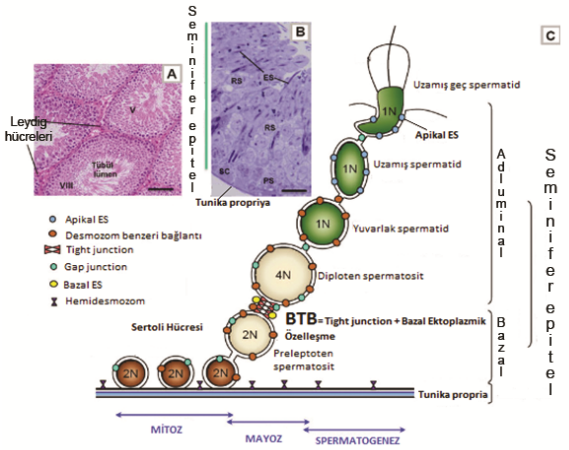
lümenine kadar uzanan polarize epitel hücreleridir. Bu hücreler seminifer epiteli bazal ve adluminal olmak üzere iki bölmeye (kompartıman) ayıran kan-testis bariyerini oluştururlar. Spermatogonyum ve preleptoten spermatositler bazal bölmede bulunurken, primer ve sekonder spermatositler, yuvarlak ve uzayan/uzamış spermatidler adluminal bölmede bulunur (Şekil 1). Seminifer tübüller etrafında motilite yeteneği olmayan, spermatozoanın tübül lümeninden epididimise atılmasında görevli kontraktil peritübüler miyoid hücreler bulunur. İnterstisyum ise androjenlerin üretiminden sorumlu olan Leydig hücrelerinin yanısıra fibroblastları, makrofajları, lenfositleri, dendritik hücreleri, mast hücrelerini, kan ve lenf damarlarını içeren bağ dokudan oluşur (Mruk ve Cheng, 2015).



Şekil 1. Spermatogenez sürecinde Sertoli hücresi-Sertoli hücresi ve Sertoli hücresi- germ hücresi sıkı bağlantılarının lokalizasyonu (Cheng and Mruk, 2009).

Spermatogenez

Spermatogenez, erkeklerde diploid spermatogonyal kök hücrelerden haploid germ hücrelerinin (spermatozoa) oluştuğu hücresel dönüşüm sürecidir. Bu döngü, memeli testisinin fonksiyonel birimi olan seminifer tübüller içinde, Sertoli hücrelerinin yapısal ve besinsel desteği ile çeşitli endokrin faktörlerin kontrolü altında gerçekleşir (Hess ve Renato de Franca, 2008).

Spermatogenez, birbiriyle ilişkili ancak birbirinden farklı dört hücresel olaydan oluşur:

Mitoz bölünme ve farklılaşma (diferensiyasyon): Seminifer tubüllerde bazal lamina üzerinde yerleşen A tipi spermatogonyanın çoğalması ve bazılarının B tipi spermatogonyaya farklılaşması aşamasıdır. Bu faz seminifer tübüllerin bazal kompartmanında gerçekleşir. B tipi spermatogonyumlar da preleptoten ve leptoten spermatositlere farklılaşırlar.

Mayoz bölünme: Primer spermatositlerin birinci mayoz bölünme ile sekonder spermatositleri, sekonder spermatositlerin de ikinci mayoz bölünme ile haploid spermatidleri oluşturduğu aşamadır. Bu aşama büyük ölçüde tübülün adluminal kompartmanında gerçekleşir.

Spermiyogenez: Spermatidlerin spermatozoaya morfogenezidir. Spermiyogenez süreci, dört aşamaya ayrılır: Golgi aşaması, akrozom aşaması, kuyruk oluşumu, olgunlaşma aşaması.

Spermiasyon: Bu aşamada gelişimini tamamlayan Sertoli hücrelerinden ayrılarak seminifer epiteli terk eder ve rete testise iletilir. Bu olay adluminal bölmede gerçekleşir (Fiorini ve ark., 2004).

Seminifer tübülde birbirini takip eden bu hücresel gelişim evreleri spermatojenik döngüyü (spermatojenik siklus) oluşturur. Spematojenik döngü germ hücrelerinin morfolojik değişikliklerine, varlığına, konumuna veya spermatidlerdeki akrozomun gelişimine göre aşamalara ayrılır. Bu aşamaların sayısı türlere göre değişmekte olup, insanda 6, sıçan ve farede 14, boğa, koç, kedi, köpek ve atta 8 aşama bulunmaktadır (Johnson, 1995; Liman ve ark., 2013). Bu aşamalar seminifer tübül boyunca seminifer epitel dalgasına (spermatogenic wave) yol açmaktadır (Cheng ve Mruk, 2002).

Kan-Testis Bariyeri (BTB)

Memeli vücudunda kan-beyin bariyeri, kan-hava bariyeri, kan-retina bariyeri, kan-timus bariyeri, kan plasenta bariyeri gibi birçok bariyer mevcuttur. Kan-testis ve kan-epididimis bariyerleri de erkek genital sistemde bulunan önemli hücresel bariyerlerdendir. Kan testis bariyerinin üç ana bileşenden oluştuğu kabul edilir:

a) Sertoli hücre bağlantılarından oluşan fiziksel bariyer,

b) Maddelerin lümenden lümene hareketini kontrol eden, Sertoli hücre taşıyıcılarından oluşan fizyolojik bariyer,

c) Bağışıklık düzenleyici faktörler ve tolerans mekanizmalarının sağladığı immünolojik bariyer.

Bu üç bileşen, BTB'de koordineli hareket ederek fonksiyonel olarak adluminal bölge içinde mayotik ve post-mayotik germ hücre olgunlaşması için hem immunolojik hem de biyokimyasal olarak uygun bir ortam sağlar. Fiziksel bariyerler, moleküllerin lümene geçişini kısıtlarken, Sertoli hücrelerinin bazolateral ve apikal membranları boyunca yer alan spesifik taşıyıcılar, moleküllerin lümen içine veya dışına hareketini düzenler (Stanton, 2016).

Testiste, damar endotelinin geçirgenliği oldukça faz-

ladır ve bu sayede dolaşımdaki maddeler interstisyuma serbestçe geçebilir (Kaur ve ark., 2013). Ayrıca seminifer tübüllerin lümenindeki sıvı, plazmaya göre daha az protein ve glikoz, daha fazla androjen, östrojen ve potasyum içerir. Hem seminifer tübül duvarında gelişmekte olan germ hücrelerinin ve hem de tübül lumenindeki sıvının içeriğinin korunması için kan-testis bariyerinin varlığı büyük önem taşımaktadır. Kan-testis bariyerinin birinci işlevi Sertoli hücrelerinin arasından seminifer tübül lümenine doğru su, elektrolitler, iyonlar, hormonlar, parakrin faktörler ve çeşitli biyolojik moleküllerin geçişini kısıtlamaktır. İkinci işlevi ise preleptoten evresinden itibaren bazal kompartmandan adluminal kompartmana geçmiş olan spermatositlerin izole bir mikro çevrede spermatogenezi tamamlamasını sağlamaktadır (Mruk ve Cheng, 2004). BTB, hem lökositlerin ve spermiyogenezis sırasında oluşabilecek anti-sperm antikorlarının (ASA) seminifer tübüllere girmesini engelleyerek, hem de mayoz bölünme için gerekli olan benzersiz bir immünolojik ve biyokimyasal mikro ortam oluşturarak infertiliteyi önler (Li ve ark., 2018).

Bu derlemede hücresel bir bariyer olan BTB'nin yapısına katılan bağlantı kompleksleri hakkında bilgi verilecektir.

Kan-Testis Bariyeri Bağlantı Kompleksleri ve Proteinleri

Kan-beyin bariyeri ve kan-retina bariyeri gibi diğer bariyer yapıları kapilar damarların endotel hücreleri arasındaki sıkı bağlantılar (tight junction) ile damar dışında destekleyici olarak bulunan perisit ve perivasküler makrofajlardan meydana gelir. Memeli testislerinde seminifer tübül epitelinde bulunan BTB ise komşu Sertoli hücreleri arasında yerleşen özelleşmiş bağlantılar [tight junction'lar, sıkı bağlantılar, TJs), gap junction'lar (geçit bağlantıları), desmozomlar (macula adherens) ve adherens junction'lar (ektoplazmik özelleşmeler-testise özgü bir yapışma bağlantısı)] tarafından oluşturulur (Şekil 1). Bu bağlantıların spermatogenez ve fertilite için gerekli olan BTB bütünlüğünün korunmasında birlikte işlev gördüğü bilinmektedir (Cheng ve ark., 2011). BTB'de farklı bağlantı türlerinin bir arada bulunması veya birbirine karışması, bu bariyeri kan-beyin bariyeri ve kan-retina bariyeri gibi diğer tüm kan-doku bariyerlerinden farklı kılar (Wong ve Cheng, 2005).

Memelilerde BTB'nin gelişimi, pubertede seminifer tübüllerde ilk spermatogenez dalgasının şekillenmesi ile başlar. BTB, kemirgenlerde doğum sonrası, insanlarda ise ergenlik döneminde gelişir (Gerber ve ark., 2016). BTB'de, spermatositlerin seminifer epitelin adluminal kompartmanına girebilmeleri için döngüsel olarak yeniden yapılandırıldığına inanılmaktadır ve bu kısmen bağlantı proteinleri, sitokinler, proteazlar/ proteaz inhibitörleri, hormonlar ve endositik/trafficking proteinleri gibi bir dizi molekül tarafından gerçekleştirilir (Mruk ve Cheng, 2004; Li ve ark., 2009).

Tıkayıcı Bağlantılar

1. *Sıkı bağlantılar [Tight junction (TJ), Zonula occludens]*

Sıkı bağlantılar, bitişik hücreler arasında hem geçirimsiz bir bariyer görevi görür hem de epitel hücrelerinde karakteristik olarak bulunarak hücresel bir polarite oluşturur. Bu durum özellikle seminifer tübüllerde, Sertoli hücreleri ve BTB'ler arasında oldukça belirgindir (Gerber ve ark., 2016). BTB'nin sıkı bağlantıları bazı yazarlar tarafından "Sertoli hücresi-Sertoli hücresi sıkı bağlantıları" olarak da adlandırılır. Bu bağlantılar, tübüllerin bazalindeki Sertoli hücreleri arasında ve Sertoli hücrelerine bağlı spermatidler arasındaki temas bölgelerinde bulunur (Mital ve ark., 2011).

Bu bağlantılar okludinler, klaudinler ve bağlantı adezyon moleküllerinden oluşmaları bakımından diğer epitel hücrelerinde bulunan sıkı bağlantılara benzer, ancak apikalden ziyade bazalde yer almalarından ötürü diğer epiteliyal sıkı bağlantılarına göre benzersiz olarak nitelendirilirler. Son veriler, sıkı bağlantı proteinleri ve sinyal molekülleri arasındaki fiziksel etkileşimlerin, sıkı bağlantının sinyal iletimi için bir platform olduğunu ve bu platformun bozulmasının Sertoli hücre fonksiyonunu etkileyebileceğini göstermektedir (Fiorini ve ark., 2004). Sertoli hücresi-Sertoli hücresi sıkı bağlantılarının kurucu proteinleri arasında integral transmembran proteinleri [okludin ve klaudinler], adaptör proteinler [zona okludens proteinleri 1, 2 ve 3 (TJP 1, 2 ve 3)] ve kavşak yapışma molekülleri [junctional adhesion molecules, JAMs] bulunmaktadır (Mitic ve ark., 2000).

1.1. *İntegral transmembran proteinleri [Okludin ve klaudinler]*

Okludin, dört transmembran alanı, bir hücre içi ve iki hücre dışı döngüsü olan 60 ila 65 kDa'lık, Ca^{+2}'den bağımsız hücreler arası adezyon (yapışma) molekülüdür. Sıçanlarda ve farelerde Sertoli hücreleri arasındaki sıkı bağlantılarda bulunur, ancak kobay veya insanda bulunmaz (Cheng ve ark., 2001). Okludin, Sertoli hücrelerinde TJ'lara yerleşir ve paraselüler sızdırmazlığın oluşturulmasında da önemli bir rol oynar (Chung ve Cheng, 2001).

Klaudinler, dört transmembran alanı, kısa bir N-terminali ve daha uzun C-terminalinden oluşan küçük moleküler ağırlıklı (21-28 kDa) integral membran proteinleridir (Tsukita ve ark., 2001). Klaudinler TJ'ların sitoplazmik yüzeyinde, adaptör veya iskelet molekülleri, özellikle üç zonula okludens proteini (ZOP'ler: ZO-1, ZO-2 ve ZO-3) aracılığıyla aktin hücre iskeletine bağlanırlar (Xu ve ark., 2009). Memelilerde klaudinler en az 27 üyeden oluşur ve işlevlerine göre kategorize edilirler (Mineta ve ark., 2011). Geçirgenliği azaltan klaudinler "sıkı" veya sızdırmaz (veya bariyer oluşturan) klaudinler (1, 3, 5, 11, 14, 19) olarak adlandırılırken, geçirgenliği artıranlar "sızdıran"

veya kanal oluşturan" veya “por oluşturan klaudinler” olarak adlandırılır. Por oluşturan klaudinler de katyon seçici (2, 10b, 15) ve anyon seçici (10a, 17) klaudinler olarak ikiye ayrılır. Diğer iki grup ise tutarsız (4, 7, 8 ve 16) veya bilinmeyen (6, 9, 12, 13, 18, 20-27) işlevlere sahip klaudinler içerir (Amasheh ve ark., 2009). TJ'lar tipik olarak birden fazla klaudin izoformu içerir. Klaudinler tüm epitellerin sıkı bağlantılarında bulunur, ancak doku dağılımları spesifiktir ve türler arasında değişiklik gösterir (Günzel ve Yu, 2013; Liman ve Ateş, 2020). Bugüne kadar bazı memelilerin testislerinde yedi farklı klaudin (klaudin-1, -3, -4, -5, -7, -8 ve -11) bulunduğu gösterilmiştir (Mazaud-Guittot ve ark., 2010; McMillan ve ark., 2014; Stammler ve ark., 2016; Pörtner ve ark., 2020; Liman, 2023). Klaudin-11 testisteki Sertoli hücreleri arasındaki sıkı bağlantılarda bulunur, ancak germ hücrelerinde bulunmaz (Siu ve Cheng, 2004). Klaudin - 3 ve -4 hem TJ'lerin hem de bazal ektoplazmik özelleşmelerin (ES'ler) bir bileşenidir ve klaudin-3, yeni BTB oluşumunun bir göstergesi ve önemli bir aracısıdır (Li ve ark., 2018). Çeşitli çalışmalar, klaudin-11 geni bulunmayan (claudin-11-null) farelerde Sertoli hücre polaritesinin ve TJ fonksiyonunun bozulduğunu ve bunun da infertilite ile sonuçlandığını, ayrıca klaudin-11'in testiste sıkı bağlantı oluşumu ve bariyer bütünlüğü için zorunlu bir protein olduğunu göstermişlerdir (Mazaud-Guittot ve ark., 2010; Stammler ve ark., 2016; Pörtner ve ark., 2020). Ayrıca, McMillan ve ark. (2014) klaudin-8'in sığır testisindeki spermatogonial kök hücre nişinde rol oynayabileceğini ileri sürmüştür.

1.2. *Adaptör proteinler (Zonula okludens proteinleri, ZO)*

Zonula okkludens proteinleri membranla ilişkili guanilat kinaz (MAGUK) benzeri protein süper ailesine aittir. Bunlar, hücreler arası bağlantıların sitoplazmik yüzeyinde çok proteinli komplekslerin bir araya gelmesi için yapısal temel sağlayan iskelet proteinlerdir. Ayrıca aktin hücre iskeletine bağlanmak için okludin ve klaudin gibi integral membran TJ proteinleri tarafından kullanılan adaptör proteinlerdir. Hücre-hücre temaslarındaki yapısal işlevlerinin yanı sıra, ZO proteinlerinin hücre büyümesi ve çoğalmasının düzenlenmesine katıldığı görülmektedir. ZO proteinleri ZO-1 (TJP1), ZO-2 (TJP2) ve ZO-3 (TJP3) sıkı bağlantı proteini olmak üzere üç üyeden oluşur (González-Mariscal ve ark., 2000). Testiste bugüne kadar sadece ZO-1 ve ZO-2 pozitif olarak tanımlanmıştır ve ilk TJ periferik proteini ZO-1'dir (moleküler ağırlığı: 220 kDa) (Lui ve ark., 2003). Testiste, ZO-1 kan-testis bariyerine ve aynı zamanda uzamış spermatidleri çevreleyen apikal ektoplazmik özelleşmeye lokalize olur. ZO-1 ve ZO-2 ayrıca TJ montajı için gerekli olan klaudin polimerizasyonunu da destekler (Mruk ve Cheng, 2015). ZO-2, testisteki gap junction proteini olan Cx43 ile birleşir. Sertoli hücreleri arasındaki TJ'lerin yapısında ve ek olarak, ZO-2 ayrıca germ hücreleri ve Sertoli hücreleri arasındaki yapışmada ve germ hücre göçü ve farklılaşması sırasında bağlantıların yeniden şekillenmesinde de rol oynar (Xu ve ark., 2009).

1.3. *Bağlantı yapışma molekülü (Junction adhesion molecule, JAM)*

Bağlantı yapışma molekülü (JAM), immünoglobulin süper ailesinin bir üyesi olan bir proteindir ve lökositler, trombositler, epitel ve endotel hücreleri gibi çeşitli dokularda ifade edilir (Ebnet, 2017). JAM, TJ'lerde tanımlanan üçüncü tip TJ-integral membran proteinidir. Hem epitel hem de endotel hücreleri arasındaki sınıra lokalize olur. Ailenin birçok üyesi hücre polaritesine, endotel geçirgenliğine ve lökositlerin göçüne aracılık eder (Garrido-Urbani ve ark., 2014). JAM ailesinin birkaç üyesi, ZO-1, klaudin ve afadin gibi proteinlerle etkileşime girerek hücre-hücre temasının olgunlaşmasını ve TJ'ler ile yapışma bağlantıları (Adherens Junction, AJ) gibi bağlantı komplekslerinin oluşumunu düzenler (Hartmann ve ark., 2020).

Bugüne kadar JAM-A (-1), -B (-2), -C (-3) ve -L (-4) olarak adlandırılan dört JAM molekülü tanımlanmıştır (Wang ve Liu, 2022). Genel olarak JAM, spermatogonia-spermatogonia, spermatogonia-Sertoli hücreleri ve Sertoli hücreleri-Sertoli hücreleri arasındaki homofilik hücre yapışmasına katılır. JAM-A prostat, seminal vezikül ve epididimisin her üç bölgesinde de bulunur. JAM-A hem Sertoli hem de bazı bazal germ hücrelerinde (GC), özellikle Sertoli hücreleri arası bağlantılarda ve kemirgenlerin epididimisi içindeki uzun spermatidlerin kuyruklarında bulunur; luminal sıvıdaki epididimozomlarda salgılanır ve in vitro olarak spermlere iletilir (Wu ve ark., 2017). Premayotik germ hücrelerinde eksprese edilen JAM-A, BTB boyunca germ hücresi göçünü kolaylaştırır ve daha sonra adluminal kompartmanda yerleşik çoğu GC'de kaybolur (Wang ve Liu, 2022). Sertoli hücresi sıkı bağlantı noktası in vitro olarak bozulduğunda, BTB ile ilişkili proteinler JAM-A hücre-hücre arayüzünden kaybolur (Yan ve ark., 2007). Memeli testisinde JAM-B, Sertoli hücreleri ile Sertoli ve germ hücreleri arasındaki apikal ektoplazmik özelleşmeler arasındaki BTB'de bulunur ve gelişmekte olan germ hücrelerinin kan-testis bariyerinden geçişini ve olgun spermatidlerin zamanında salınmasını teşvik eder. Özetle JAM-A ve JAM-B BTB'nin polaritesine katkıda bulunur (Wang ve Liu, 2022).

JAM-C germ hücre aktivitelerini modüle eden önemli bir proteindir. JAM-C, germ/Sertoli hücre temaslarına lokalize olur; akrozom oluşumuna ve germ hücre polaritesine, özellikle de yuvarlak spermatidlere katılır. İlginç bir şekilde, JAM-C fare testislerinde yerleşimi BTB yerine apikal ektoplazmik özelleşme (ES) ile sınırlıdır. JAM-C eksikliği olan erkekler yaklaşık %50 daha küçük testislere sahiptirler ve kısırdırlar. Farklılaşmış uzun spermatidlerden yoksundurlar ve olgun

sperm hücreleri üretemezler. Dolayısıyla JAM-C spermatidlerin gelişiminin birincil düzenleyicisidir (Pellegrini ve ark., 2011). Sertoli hücreleri içinde JAM -C, hem yuvarlak hem de uzun spermatidlerin polaritesini etkilemek için JAM-B ile etkileşime girer (Rehder ve ark., 2006). Çalışmalar ayrıca JAM-C'nin inaktivasyonunun erkek germ hücre gelişimini ve proliferasyonunu bloke ederek fertiliteyi önemli ölçüde engellediğini göstermiştir (Ebnet, 2017).

Diğer bir üye olan JAM-L (JAM-4) proteini gonositler, spermatogonyumlar ve Sertoli hücrelerinde lokalizedir ve BTB oluşumu sırasında testiste bir sıkı bağlantı proteini yerine bir hücre yapışma molekülü olarak işlev görür (Nagamatsu ve ark., 2006).

Tutundurucu Bağlantılar

Tutundurucu veya bağlayıcı bağlantılar Sertoli hücreleri, Sertoli ve germ hücreleri ve germ hücreleri arasında bulunan bağlantılardır. Seminifer epitelde testise özgü iki tür tutundurucu veya bağlayıcı bağlantı bulunur: Bunlar; Desmozom-benzeri bağlantılar ve Ektoplazmik özelleşmeler (ES)'dir (Kopera ve ark., 2010).

1. Desmozom-benzeri bağlantılar

Desmozomlar, hücre-hücre temasına ve yapışmasına aracılık eden özelleşmiş ve oldukça düzenli membran alanlarıdır. Desmozomlar hem hücre-hücre adezyonuna hem de hücre iskeleti bağlantılarına aracılık ederek, hücreleri dokulara mekanik olarak entegre eder ve böylece mekanik strese direnme işlevi görür. Son çalışmalarda, hücre yapışması dışında hücre proliferasyonu, farklılaşma, göç ve morfogenez ile ilgili sinyal yolaklarını organize etmede ve düzenlemede önemli işlev gördüğü ifade edilmektedir (Kopera ve ark., 2010).

Desmozomal proteinler üç ana gen ailesi tarafından kodlanır: Desmozomal kaderinler, Armadillo (Arm) ailesi proteinleri ve plakin ailesi (Kowalczyk ve Green, 2013).

i. Desmozomal kaderinler: Desmogleinler ve desmokollinler olarak adlandırılan iki alt tipten oluşan desmozomal kaderinler, kalsiyuma bağlı hücre-hücre adezyonuna aracılık eden kaderin süper ailesinin bir alt ailesidirler. İnsanlarda dört gen desmogleinleri (Dsg1-4) ve üç gen desmokollinleri (Dsc1-3) kodlar. Desmozomal kaderin genleri dokuya ve farklılaşmaya özgü bir şekilde ifade edilirler (Saito ve ark., 2012).

ii. Armadillo (Arm) ailesi proteinleri: Armadillo (Arm) ailesi proteinleri, doku bütünlüğü ve hücre sinyalizasyonunda önemli roller oynayan ve kaderinleri çeşitli hücre iskeleti filamentlerine bağlamada ve bağlantı düzeneğini düzenlemede görevli olan proteinlerdir. Armadillo ailesi, armadillo alanı olarak adlandırılan 42 amino asitlik tekrarlanan bir motifin varlığıyla tanımlanır. Desmozomlarda iki tip armadillo proteini bulunur. Plakoglobin ve β-katenin bu gen ailesinin orijinal kurucu üyeleri arasındadır ve her iki protein de doğrudan kaderinlerin sitoplazmik alanına (domain) bağlanır (Kowalczyk ve ark., 1999; Hatzfeld, 2007). Desmozomlar ayrıca Armadillo alt ailesinin üyeleri olan p120 catenin (p120ctn), p0071 (plakofilin-4 olarak da bilinir), Velo-kardiyo-fasiyal sendromda silinmiş Armadillo tekrar proteini (Armadillo repeat protein deleted in velo-cardio-facial syndrome, ARVCF), delta-katenin (δ-catenin) ve plakofilinler 1-3'ü (PKP1-3) içerir (Hatzfeld, 2007).

iii. Plakin ailesi: Desmozomal proteinleri kodlayan üçüncü büyük gen ailesi plakin ailesidir (Sonnenberg ve Liem, 2007). Üyeleri arasında bulunan desmoplakin, intermediyer (ara) filamentleri desmozomal plağa bağlayan zorunlu bir desmozomal proteindir. Desmoplakin'in amino-terminusu, doğrudan plakoglobin ve plakofilinlere bağlanır (Kowalczyk ve ark., 1999).

Testiste desmozomlar ilk olarak Lonnie Russell (1977) tarafından incelenmiş ve bu ara filament bazlı yapılar 'desmozom benzeri' (alternatif olarak 'desmozom-gap' olarak da ifade edilmiştir) olarak tanımlanmıştır. Çünkü ultrastrüktürel olarak deride veya kalpte bulunan sıkı ve Ca^{2+}-bağımsız (yani aşırı yapışkan-hiper-adeziv) desmozomlara benzemedikleri görülmüştür. Bugün, BTB'deki Sertoli hücreleri arasında ve Sertoli hücreleri ile gelişimin 8. basamağındaki uzayan spermatidlere kadar (ancak bunlar dahil değil) tüm germ hücreleri arasında bulunan desmozom benzeri bağlantıların, diğer organlardaki desmozomların yapısına katılan proteinlerin çoğunu (örn. desmogleinler, desmokollinler, plakoglobin, plakofilinler ve desmoplakinler) içerdiği bilinmektedir (Lie ve ark., 2010). Testiste, BTB'nin yanısıra, Sertoli hücresi -spermatid veya Sertoli hücresi-spermatosit/ spermatogonyum arayüzünde desmozomlar bulunabilir (Cheng ve ark., 2011). Bununla birlikte Domke ve ark. (2014) insan, sığır, domuz, sıçan ve farenin seminifer epitelinin desmozom veya "desmozom benzeri" bağlantılar ya da desmozoma özgü kaderinlerden, yani desmoglein ve desmokollinlerden herhangi birine veya desmozoma özgü sitoplazmik plak proteinlerinden, yani desmoplakin veya plakofilin (Pkp) 1-3'ten birine de sahip olmadıklarını göstermişlerdir. Dolayısıyla BTB'de desmozom benzeri proteinlerin varlığı hala tartışmalı bir konudur.

2. Adherens junction (Ektoplazmik özelleşmeler)

Ektoplazmik özelleşmeler (ES), testiste iki farklı bölgede bulunan aktin bazlı bağlantı noktalarıdır. İlk yerleşim yeri, seminifer epitelde postmayotik spermatidler ile Sertoli hücreleri arasıdır. Uzayan ve uzamış spermatidlerin başının tamamını çevreleyen bu ES tipi apikal ES olarak da bilinir (Mruk ve ark., 2008). Apikal ES, Sertoli hücreleri ile kemiricilerde spermatidler arasında bulunan, hücre-hücre aktin bazlı testi-

se özgü atipik adherens bağlantı noktası olarak tanımlanır. Apikal ES, ES'nin seminifer epiteldeki tek ve en güçlü bağlayıcı bağlantı noktasıdır. Çünkü daha erken evredeki spermatidler ile Sertoli hücreleri arasında bulunan desmozom-gap bağlantılarının tümü kaybolurken (Wolski ve ark., 2005), apikal ES seminifer epitel döngüsü boyunca mevcuttur ve ilk olarak Sertoli hücreleri ve yuvarlak spermatidler arasında sıçan testisinde VII. evrenin sonlarında ve VIII. evrenin başlarında görülür. Seminifer epitelde varlığını sürdürür ve tübülobulbar kompleksin (TBC) ortaya çıkmasından önce farede 19. aşamaya kadar olan spermatidlerle ilişki kurar (Vogl ve ark., 2000). TBC'ler sperm salınımından (spermiyasyon) hemen ortaya çıkan ve Sertoli hücreleri ile uzamış geç spermatidler arasında bulunan özel yapılardır. TBC'ler uzamış geç spermatidlerin sitoplazmik evaginasyonlarıdır. Bunlar Sertoli hücre sitoplazmasına nüfuz ederler ve hem tübüler hem de ampul benzeri kısımlardan oluşurlar. Tübüler kısmler aktin filamanları ile, ampul kısımları ise granülsüz endoplazmik retikulum kesecikleri ile kuşatılmıştır. Bu TBC'lerin çevresinde çok sayıda çift membranlı vezikül mevcuttur. Sıçanda seminifer epitel döngüsünün VII. evresinin başında, spermatidler Sertoli hücresinin derin girintilerinden seminifer epitelin lümenine doğru hareket ederken çok sayıda oluşurlar. TBC'ler sıçan, keseli sıçan, tarla faresi, kobay, fare, hamster, tavşan, köpek, koç, maymun ve insan olmak üzere on memeli türünün testislerinde incelenmiş olup ve spermatid başına 24 kadar TBC'nin oluştuğu tespit edilmiştir (Upadhyay ve ark., 2012).

ES'nin ikinci tipinin yerleşim yeri BTB'deki komşu Sertoli hücreleri arasında olup bu bağlantı bazal ES olarak bilinir (Cheng ve Mruk, 2010). Tek başına var olan apikal ES'nin aksine, bazal ES sıkı bağlantılar, desmozomlar ve gap junction'lar ile bir aradadır ve BTB'nin immünolojik bariyer işlevine katkıda bulunur (Mruk ve ark., 2008).

Ultrastrüktürel olarak ES'ler, Sertoli hücrelerinin endoplazmik retikulum sisternaları ile plazma membranı arasına yerleşmiş aktin filamanı demetlerinin varlığıyla karakterizedir (Vogl ve ark., 2000). Bazal ES'de endoplazmik retikulum sisternaları ile plazma membranı arasına sıkışmış aktin filament demetleri komşu her iki Sertoli hücresinde de bulunur, yani bazal ES iki dizi aktin filament demetinden oluşur. Apikal ES, Sertoli hücresi plazma membranı ile endoplazmik retikulum sisternaları arasına sıkışmış, uzayan/uzamış spermatidlerin neredeyse tüm baş bölgesini çevreleyen, altıgen olarak paketlenmiş aktin filament demetlerinden oluşur (Wong ve ark., 2008). Bitişik uzayan/uzamış spermatidlerdeki integral membran bağlantı molekülleri hakkında çok az şey bilinmektedir. Bununla birlikte, son çalışmalar nektin-2, nektin-3, N-cadherin, laminin γ3, zyxin ve diğerlerinin apikal ES bölgesinde bulunduğunu ve uzayan/uzamış spermatidlerle ilişkili olduğunu gösterilmiştir (Lee ve ark., 2003; Siu ve Cheng, 2004). Bu ultrastrüktürel özellikler ES'ye ve testise özgüdür; yani, memeli vücudundaki herhangi bir organdaki başka bir hücre tipinde tespit edilemezler. Her iki ES'nin birincil işlevi germ hücresi hareketini kolaylaştırmaktır; buna ek olarak germ hücrelerini, özellikle de spermatidleri spermiyasyon gerçekleşene kadar epitelde tutma işlevi de bulunmaktadır (Vogl ve ark., 2000; Toyama ve ark., 2003).

İlginç bir şekilde, ES aynı zamanda TJ'lar, gap junction'lar, desmozomlar, hemidesmozomlar ve diğer epitellerdeki fokal bağlantılarda bulunan proteinlerden oluşur, bu da ES'yi hibrit bir kavşak haline getirir (Yan ve ark., 2007; Wong ve ark., 2008). Ayrıca, apikal ve bazal ES ultrastrüktürel olarak aynı olsa da apikal ES'de bulunan proteinlerden β1-integrin ve laminin α3, β3, γ3 bazal ES'de tespit edilmezken, bazı proteinler (örneğin N-cadherin) her iki bölgede de bulunur (Johnson ve Boekelheide, 2002; Lee ve ark., 2003; Siu ve Cheng, 2004; Yan ve Cheng, 2006).

Gelişen germ hücreleri, memelilerin testislerindeki seminifer epitel döngüsü boyunca, dezmozom benzeri bağlantılar ve apikal ektoplazmik özelleşmeler (ES) yoluyla Sertoli hücrelerine yapışır ve bu hücreler arasındaki yapışma herhangi bir şekilde tehlikeye girerse, germ hücreleri seminifer epitelden erken ayrılır ve kısırlık meydana gelebilir. Apikal ES'nin, Sertoli hücreleri ile uzamış spermatidler arasında bulunan ve spermatid hareketinde (yani spermiasyon) önemli rol oynadığı öne sürülen benzersiz bir bağlantı noktası olduğu ifade edilmiştir. Bazal ES'nin de BTB bütünlüğüne katkıda bulunduğuna inanılsa da BTB'nin aynı zamanda TJ'lerden ve dezmozom benzeri bağlantılardan oluşmasından dolayı bunu nasıl başardığı henüz bilinmemektedir (Kopera ve ark., 2010).

Ektoplazma dinamikleri arasında yer alan protein komplekslerinin hücre yapışmasını kolaylaştırmaya ek olarak, hücrelerin endotel boyunca göçünde de işlev gördüğü ifade edilmektedir. Bu protein kompleksleri şunlardır:

a) Multiprotein kompleksleri: Bugüne kadar ES'de kaderin/katenin, nektin/afadin/ponsin, integrin/laminin ve JAM-PAR/CAR multi-protein kompleksleri olmak üzere dört çoklu protein kompleksi bulunmuştur Kaderin/katenin ve nektin/afadin komplekslerinin her ikisi de apikal ve bazal ES bölgelerinde tespit edilirken, integrin/laminin kompleksi çoğunlukla apikal ES bölgesiyle sınırlıdır (Cheng ve Mruk, 2002; Takai ve Nakanishi, 2003).

(i) Kaderin-katenin multi-protein kompleksi: Bu kompleks, epitel ve endotel hücrelerinde en çok incelenen aktin bazlı yapışma birimidir. Testis'te bir dizi farklı kaderin tanımlanmıştır ve bu durum geçmişte tartışmalı bir konu olmasına rağmen, N-Cadherin'in apikal ES'de bulunduğu artık iyi bilinmektedir (Kopera ve

ark., 2010).

(ii) İntegrin-laminin multi-protein kompleksi: İntegrinler, lamininler gibi ligandlara bağlanarak hücre-hücre ve hücre-matriks yapışmasına aracılık eden ve transmembran heterodimerlerinden oluşan hücre yüzeyi reseptörleridir. Yapışma rolüne ek olarak integrinler hücrelerle dış ortam arasında önemli sinyal dönüştürücüleridir. Memeli testisinin seminifer epitelindeki integrin/laminin kompleksi, Sertoli hücresi-uzamış spermatid arayüzündeki apikal ES'de ve Sertoli hücre -hücre membranındaki hemidesmozom arayüzünde olmak üzere iki yapışma bölgesine yerleşir. Son çalışmalar, integrinlerin ve lamininlerin apikal ES, hemidesmozom ve BTB arasındaki bağlantıya aracılık ettiğini ve bariyerin yeniden yapılandırmasını kolaylaştırmak için lokal bir otokrin eksen oluşturduğunu göstermektedir (Lie ve ark., 2013).

(iii) Nektin-afadin multi-protein kompleksi: Sertoli hücresi-spermatid bağlantılarının nektin-2 ve afadinden oluşan nektin-afadin sistemine bağlı olduğu bildirilmektedir (Takai ve Nakanishi, 2003).

(iv) JAM-PAR/CAR multi-protein kompleksi: TJ'ların varlığı seminifer epiteldeki BTB ile sınırlı olmasına karşılık, bağlantı adezyon molekülü (JAM), Coxsaccie virus ve adenovirüs reseptörü (CAR) ve PAR proteinlerini içeren TJ proteinlerinin apikal ES'de lokalize olduğu ifade edilmiştir. JAM-A ve -B, BTB'deki Sertoli hücrelerinde ve yuvarlak ve uzun spermatidlerde bulunurken, JAM-C sadece apikal ES'de Sertoli hücresi-spermatid yapışması ve germ hücre konumlandırma ve polarizasyonda işlev gördüğü belirtilmiştir. CAR ise hücre yapışmasında rol oynar (Kopera ve ark., 2010).

b) Hemidesmozom: Hemidesmozom, Sertoli hücresi ile bazal membran arasındaki arayüzde bulunan intermediyer filaman bazlı bir hücre-matriks bağlanma kavşağıdır. 2008 yılında laminin α2 ve β1-integrini hemidesmozomun iki bileşen proteini olarak tanımlayan bir çalışma dışında (Yan ve ark., 2008), testisteki moleküler bileşimi şimdiye kadar araştırılmamıştır. Diğer epitel dokularındaki TJ'lar hemidesmozomdan en uzakta bulunurken, seminifer epitelde ise BTB TJ'ları hemidesmozomun yakınında yer alır; bu da TJ'ların, bazal ES'nin, desmozomların ve GJ'ların BTB bütünlüğünü korumak için hemidesmozomlarla etkileşim halinde olabileceğini göstermektedir. Ayrıca son çalışmalar hemidesmozomdaki β1-integrin işlevinin bozulmasının TJ bariyer işlevini etkileyeceğini göstermekte ve hemidesmozom ile BTB'nin bağlantı birimleri arasında fizyolojik bir bağlantı olduğunu ortaya koymaktadır (Cheng ve ark., 2011).

3. Geçit bağlantıları (Gap junction, GJ)

Geçit bağlantıları (Gap junction, GJ) iki hücrenin sitoplazmasını doğrudan birbirine bağlayarak çeşitli metabolitlerin, ikinci habercilerin, iyonların, 1 kDa'dan küçük diğer moleküllerin ve elektriksel uyarıların difüzyonuna izin veren hücre-hücre kanallarıdır (Mruk ve Cheng, 2015). GJ, her biri "konneksin" adı verilen 6 adet proteinin bir por etrafında dizilmesiyle oluşan ve "konnekson" olarak adlandırılan iki yarım kanaldan oluşur (Kumar ve Gilula, 1996). İnsanlarda ve kemirgenlerde konneksin 43 (Cx43), konneksin 33 (Cx33) ve konneksin 26 (Cx26) gibi en az 20 konneksin proteini bulunur (Cheng ve ark., 2011). BTB'de GJ'lar komşu iki Sertoli hücresi arasındaki ve Sertoli hücreleri ile germ hücreleri arasındaki hücreler arası iletişimi düzenler ve spermatogenezde hayati işlevler görür (Gerber ve ark., 2016). CX43 testiste belirlenen birkaç konneksin türü arasında en bol eksprese edilen konneksin proteinidir (Mital ve ark., 2011). Cx43 ile desmozom proteini plakofilin-2'nin, Sertoli-Sertoli hücre arayüzünde TJ ile ilişkili proteinlerin dağılımını düzenleyerek kan testis bariyeri bütünlüğünü düzenlemek için sinerjistik olarak çalıştığı ifade edilmiştir (Li ve ark., 2009).

Erkek İnfertilitesinde Oksidatif Stresin Kan-Testis Bariyeri Üzerine Etkisi

Vücutta antioksidanlar ve reaktif oksijen türleri (ROS) arasındaki dengesizliğin oksidatif strese neden olduğu ve ROS'lerinin erkek infertilitesinde %30-80 oranında rol oynadığı bildirilmiştir (Takeshima ve ark., 2020). Testislerin daha fazla oksijene ihtiyaç duyan hassas bir organ olduğu için oksidatif strese daha yatkın olduğu, bununla birlikte testisin sahip olduğu antioksidan enzim sistemi sayesinde normal spermatogenez ve steroidogenezi sürdürülebildiği tespit edilmiştir (Mruk ve ark., 2002; Gram ve ark., 2022). Son yıllarda yapılan çalışmalar çevredeki toksik maddeler, kemoterapötik ilaçlar, ısı ve çeşitli hastalıkların aşırı ROS oluşumuna neden olduğunu (Agarwal ve ark., 2003) ve ROS' lerinin de Sertoli hücre yapısını ve işlevini bozarak BTB'nin bütünlüğünün bozulmasına neden olduğunu ortaya koymuştur (Yi ve ark., 2018; Wu ve ark., 2019). ROS'un Sertoli hücrelerinde yaygın apoptoza, ZO-1, β-katenin ve Cx43 ekspresyonunda belirgin azalmaya (Yi ve ark., 2018; Zhang ve ark., 2020) ve Sertoli hücrelerinin işlevini bozarak spermatogoniyal kök hücrelerin korunmasında başarısızlığa neden olduğu bildirilmiştir (Zhang ve ark., 2020). Bu sonuçlara dayanarak oksidatif stresi inhibe ettiği bilinen çeşitli antioksidan ilaçların (örneğin askorbik asit, metformin, fluvastatin gibi) kullanılmasının BTB hasarının hem önlenmesi hem de hafifletilmesi için potansiyel bir tedavi yöntemi olabileceği öne sürülmüştür (Gurel ve ark., 2019; Ye ve ark., 2019).

Sonuç

Bu derlemede, kan-testis bariyerindeki bağlantı komplekslerinin bileşenleri ile bunların testis fonksiyonu ve spermatogenez için fizyolojik önemi konusundaki son gelişmelerden bazıları özetlenmiştir. Son yirmi yılda yapılan Sertoli-germ bağlantılarını araştı-

ran çalışmalar büyük ölçüde Sertoli veya germ hücrelerinin salgılama işlevi ve aktivitesine odaklanmıştır. Bu çalışmalarla seminifer tübüldeki bu hücrelerin salgılama aktivitelerindeki müteakip değişikliklerin başlangıçta hücre bağlantıları düzeyinde gerçekleştiği ortaya konmuştur. Yine de testisin seminifer tübüllerindeki TJ'ların ve ES gibi AJ'ların biyokimyasal ve moleküler yapısı ve mimarisi henüz tam anlamıyla açıklanamamıştır. Son güncel çalışmalarda BTB'nin spermatogenez sırasında, gelişen preleptoten ve leptoten spermatositlerin adluminal bölgeye geçisi ve gelişmesini tamamlamış spermatidlerin spermiyasyon sırasında tübüler lümene salınabilmesi için açılıp kapandığı ve bu açılıp kapanmasının bir dizi sinyal yolağı ve molekül tarafından düzenlendiği ileri sürülmüştür (Zhou ve Wang, 2022). Bu yönüyle kan testis bariyerinin fonksiyonunu düzenleyen mekanizmaları açıklayacak ileri moleküler çalışmalara gerek vardır.

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Histopathological Findings and Treatment of Intermuscular Lipoma in a Dog: A Rare Case

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Abstract: In this study, the histopathological diagnosis and treatment of an intermuscular lipoma were reported in a dog. In the clinical inspection, there was a large mass in the caudal of the left femur and that the animal had difficulty in moving. On palpation, the mass was solid and painful. A decision was made to totally extirpate the mass, which was suspected to be a tumor. A wide skin incision was made from the caudal part of the left femur; the mass was separated from the surrounding tissues by blunt dissection and extirpated. Histopathological examination revealed that the mass was an intermuscular lipoma. In the postoperative period, the patient recovered completely and returned to its normal life.
Keywords: Dog, histopathology, intermuscular lipoma, mass

Bir Köpekte İntermüsküler Lipomun Histopatolojik Bulguları ve Tedavisi: Nadir Bir Vaka

Öz: Bu çalışmada bir köpekte intermüsküler lipomun histopatolojik tanısı ve uzaklaştırılması rapor edilmiştir. Hastanın inspeksiyon ile yapılan muayenesinde, sol femurun kaudalinde büyük bir kitle olduğu ve hayvanın hareket etmekte güçlük yaşadığı belirlendi. Palpasyonla yapılan muayenede, kitlenin solid yapıda olduğu ve hastanın ağrı duyduğu tespit edildi. Tümör olabileceğinden şüphelenilen kitlenin total olarak ekstirpe edilmesine karar verildi. Hastanın sol femurunun kaudal kısmından geniş bir deri ensizyon yapıldı. Kitleye ulaşıldıktan sonra, kitle çevre dokulardan künt diseksiyon ile ayrılarak ekstirpe edildi. Histopatolojik incelemeler sonucunda kitlenin intermüsküler lipom olduğu belirlendi. Postoperatif dönemde yapılan muayenelerde ise, hastanın tamamen iyileştiği ve normal yaşamına döndüğü belirlendi.
Anahtar kelimeler: Histopatoloji, intermüsküler lipom, kitle, köpek

Introduction

Lipomas are benign tumors of soft tissues of mesenchymal origin, usually caused by the differentiation of mature adipocytes in older and obese dogs (Huppes et al., 2016; Kim et al., 2015; Leriquier et al., 2017; O'Neill et al., 2018; Sasikala et al., 2020; Veena et al., 2013). Lipomas affect approximately 16% of dogs (Huppes et al., 2016; Sasikala et al., 2020) and female dogs are more prone to lipoma development (Aydogan and Metin, 2013; Huppes et al., 2016; Julie et al., 2013; O'Neill et al., 2018; Veena et al., 2013; Vigneshwaran et al., 2020). Doberman Pinscher and Labrador Retriever are the most common dog breeds with lipoma cases (Gough et al., 2018). Lipomas are classified according to their histological features as lipoma, fibrolipoma, angiolipoma, myolipoma, pleomorphic lipoma and spindle cell lipoma (Kim et al., 2015; Sasikala et al., 2020). Size of lipomas can vary between 1-30 cm (Veena et al., 2013; Vigneshwaran et al., 2020).

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Infiltrative and intermuscular lipomas are types of lipomas that are rarely encountered in dogs (Azizi et al., 2011; Leriquier et al., 2017; Olle et al., 2002). In infiltrative lipoma cases, the adipose tissue invades deeply into the muscle and connective tissue, separating the muscle fibers and causing atrophy (Aydogan and Metin, 2013; Azizi et al, 2011; Leriquier et al., 2017; McChesney et al., 1980). Intermuscular lipomas, on the other hand, are subcutaneous lipoma variants that are located between the muscles that do not invade surrounding tissues. Intermuscular lipomas occur mostly in the caudal aspect of the pelvic extremity (especially in the area close to the semitendinosus and semimembranosus muscles) in both humans and dogs (Huppes et al., 2016; Leriquier et al., 2017; Pakanati et al., 2019; Trebacz and Galanty, 2016). The treatment of both intermuscular lipomas and infiltrative lipomas is the surgical extirpation of the masses (Huppes et al., 2016; McChesney et al., 1980; Sasikala et al., 2020). Complications such as seroma, wound infection and nerve damage may occur postoperatively (Huppes et al., 2016).

Erciyes Üniv Vet Fak Derg 2023; 20(2): 152-155

In this case report, we aimed to contribute to the literature by discussing the histopathological diagnosis and treatment of intermuscular lipoma encountered in the left hind leg (between the semimembranosus and semitendinosus muscles) of a 7-year-old English Cocker Spaniel dog. The fact that it is the first case report in Turkey about intermuscular lipomas, which is rarely encountered in dogs, shows the importance of the study.

Case Description

In this case report, a 7-year-old female English Cocker Spaniel dog brought to Firat University Animal Hospital with the complaint of a large mass in her left hind leg is discussed.

The animal owner stated that the dog had a large mass on the left hind leg for about two months and had been limping while walking. In clinical examination, a large mass was detected caudal to the left femur of the dog (Figure 1). On palpation the mass was painful and was solid in structure with clear borders. Since the mass was thought to be a tumor, a decision was made for total extirpation.

The wide area from the proximal of the left femur to the middle of the tibia was prepared for the operation.

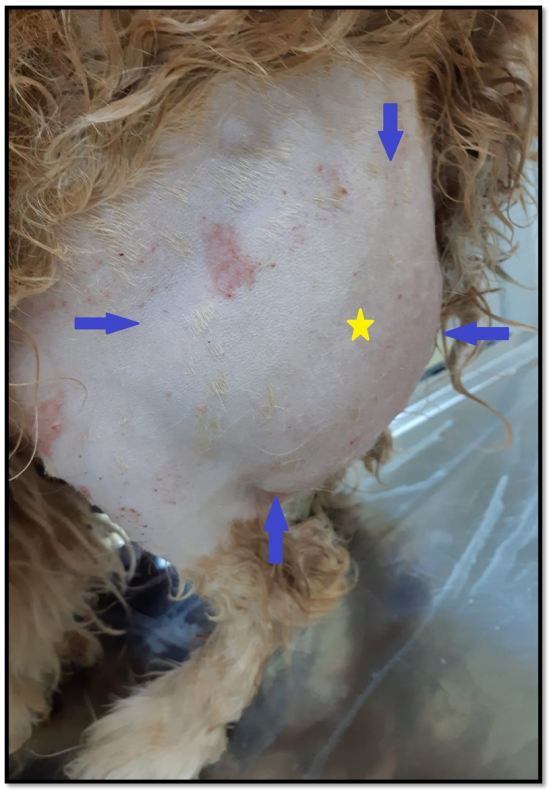

Figure 1. View of the solid mass (yellow star) and its borders (blue arrows) encountered in the caudal part of the femur.

In order to provide analgesia before the operation, 0.5 mg/kg meloxicam (Bavet Meloksikam, 5 mg/ml, Bavet, Turkey) was administered subcutaneously to the dog. Ten minutes after the patient was given 1 mg/kg xylazine hydrochloride (Xylazinbio 2%, 23.32 mg/ml, Bioveta, Czech Republic) intramuscularly, inhalation anesthesia was administered using isoflurane with the help of a mask. After the patient was anesthetized, a wide skin incision was made from the caudal part of the femur. After the incision was made, it was observed that there was a mass resembling adipose tissue with clear borders on the semitendinosus and semimembranosus muscles. With blunt dissections, the mass was carefully separated from the surrounding tissues and totally extirpated (Figure 2a). The subcutaneous connective tissue and skin were then sutured according to routine surgical procedures. The extirpated mass (Figure 2b) was sent to Firat University Veterinary Faculty Pathology Department Laboratory for examination. Postoperatively, penicillin at a dose of 20,000 IU/kg (Iecilline, 400,000 IU, İE Ulagay, İstanbul) was administered intramuscularly for one week. In addition, 0.2 mg/kg meloxicam (Bavet Meloxicam, 5 mg/ml, Bavet İlaç San. Ltd., İstanbul) was administered subcutaneously once every three days for one week. The dog returned to its normal life in a short time postoperatively (Figure 3a, 3b).

Biopsy specimens were fixed in a 10% solution of

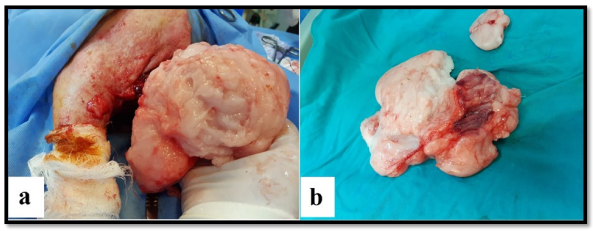

Figure 2. Total extirpation of the mass (a) and the extirpated mass (b).

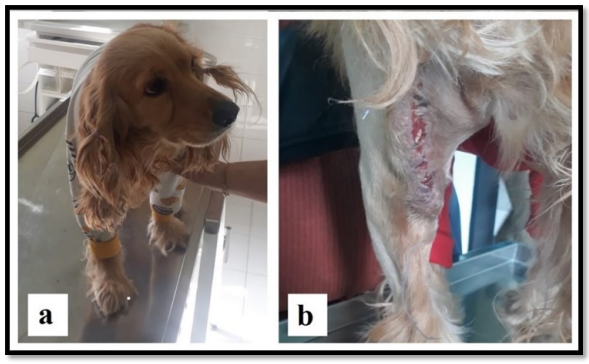

Figure 3. The appearance of the dog (a) and the operation wound (b) on the 15th postoperative day.

neutral formalin solution before being routinely processed and stained with hematoxylin and eosin (H&E) for light microscopic examination (Luna, 1968). The mass was sharply circumscribed, but not encapsulated. The neoplasm consisted of well-differentiated lipocytes that had infiltrated and replaced muscle and collagen fibers. Most of the adipocytes showed necrotic changes. Tumor cells destroyed and replaced the muscle cells and connective tissues. Atrophic muscle tissue remnants were found only in the surgical margins (Figure 4a, 4b). As a result, the mass was diagnosed as infiltrative intermuscular lipoma.

Discussion and Conclusion

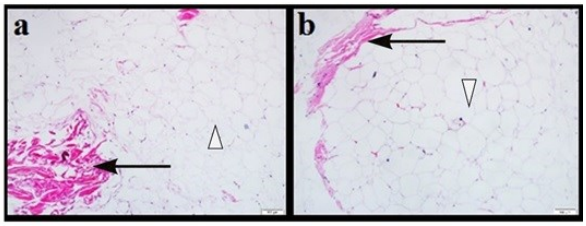

Figure 4. The lipocytes (arrowheads) invading the muscle cells (arrows). The muscle cells were atrophic and lipocytes were mostly necrotic (a,b). Hematoxylin -eosin staining.

Intermuscular lipomas are lipoma variants that are less common than simple lipomas and whose biological significance is unknown (Azizi et al., 2011; Olle et al., 2002). Lipoma cases are mostly seen in old and obese dogs (Huppes et al., 2016; Leriquier et al., 2017; O'Neill et al., 2018; Sasikala et al., 2020; Vigneshwaran et al., 2020). Huppes et al. (2016) reported that all five dogs in which they detected intermuscular lipoma were older than six years of age. In the same study, the majority of dogs were heavy and obese according to their breed characteristics. Kazemi and Neshat-Gharamaleki, (2021) reported that a dog with an intermuscular lipoma on the right hind leg was 10.5 years old. In our study, the dog with the intermuscular lipoma on the left hind leg was a 7-year-old geriatric dog.

Lipoma cases are more common in female dogs compared to male dogs (Huppes et al., 2016; Julie et al., 2013; O'Neill et al., 2018; Veena et al., 2013; Vigneshwaran et al., 2020). Although studies on breed predisposition are insufficient, some studies have reported that lipomas are more common in Doberman Pinscher and Labrador Retriever dogs (Gough et al., 2018; O'Neill et al., 2018). In the study of Huppes et al. (2016) three of the five dogs with intermuscular lipoma were female. Two of the dogs in the same study were reported to be Labrador Retrievers. Kazemi and Neshat-Gharamaleki, (2021) reported that the dog they detected an intermuscular lipoma on the right hind leg was a female Shih Tzu Terrier breed. In our study, the dog with an intermuscular lipoma on the left hind leg was a female English Cocker Spaniel dog.

Intermuscular lipomas are mostly detected in the chest, neck, gluteal muscles and proximal hind legs (especially between the semitendinosus and semimembranosus muscles) (Azizi et al., 2011; Huppes et al., 2016; Kazemi and Neshat-Gharamaleki, 2021; Leriquier et al., 2017; Trebacz and Galanty, 2016). Huppes et al. (2016) reported that the masses were seen in the hind legs in four of the five dogs in which they detected intermuscular lipoma. In many studies the masses were detected between the semitendinosus and semimembranosus muscles (Huppes et al., 2016; Kazemi and Neshat-Gharamaleki, 2021; Trebacz and Galanty, 2016) similar to our study.

The most important and effective treatment method for all lipomas, especially intermuscular lipomas, is the surgical removal of the masses (Huppes et al., 2016; McChesney et al., 1980; Sasikala et al., 2020). In many studies it has been reported that lipomas do not recur after surgical removal and patients return to their normal lives in a short time (Huppes et al., 2016; Vigneshwaran et al., 2020). In this study, the intermuscular lipoma mass extirpated from the left hind leg of a 7-year-old dog did not recur. The most common complications after surgical removal of intermuscular lipomas are seroma, wound infections and nerve damage (Huppes et al., 2016). In our case, no complications occurred in the postoperative period and the patient returned to its normal life shortly.

The gold standard for the definitive diagnosis of intermuscular lipomas is histopathology (Azizi et al., 2011; Kazemi and Neshat-Gharamaleki, 2021). Sasikala et al. (2020), in their study in which they extracted a giant lipoma mass from the prescapular region of a 9-year-old dog, reported that the adipocytes had histopathologically clear borders and the nuclei were located eccentrically. Veena et al. (2013) also reported that in the histopathological examination of the lipoma they detected in the neck of a dog, the cell nuclei were eccentrically located and there were multiple vacuoles in the cytoplasm. In our study, the histopathological examination of the extirpated mass revealed, well-differentiated necrotic adipocytes. Tumor cells infiltrated the muscle and connective tissue only within the tumor borders, and atrophic muscle tissue residues were found in these regions.

As a result, as reported in many studies, the case of intermuscular lipoma, which was the subject of this study, was encountered in an obese, geriatric and female dog. Intermuscular lipoma, which was reported to be found in different dog breeds in many studies, was encountered in this study in a dog breed that was rarely reported before. Intermuscular lipoma was encountered caudal to the pelvic extremity in this study, as in most cases reported in human and veterinary medicine. Apart from these, this study is also

very important in terms of bringing a case of intermuscular lipoma, which is very rare in dogs, to the literature for the first time in Turkey.

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Yazım Kuralları

1. Erciyes Üniversitesi Veteriner Fakültesi Dergisi'nde veteriner bilimlerini ilgilendiren alanlarda orijinal araştırmalar, olgu sunumları, araştırma notları, kısa bildiri, derleme ve editöre mektup yayımlanır.
2. Dergide yayımlanacak yayınlar için resmi dil Türkçe'dir. İngilizce yazılmış eserler de yayımlanabilir. **İngilizce hazırlanmış makalelerin yayımlanmasına öncelik verilir**.
3. Yayınlar A4 tipi formatta, çift aralık, Arial, 10 punto ve iki yana yaslı olarak yazılmalıdır. Her kenardan 2.5 cm boşluk bırakılarak, sayfaların sağ altına numara verilmelidir. Resimler, şekiller ve kaynaklar dâhil orijinal makaleler ve derlemeler 14, olgu sunumları, araştırma notu ve kısa bildiriler 7 sayfayı geçmemelidir.
4. Yazılar, ercvet@gmail.com adresine gönderilmelidir. Yazışmalar için, makale kapak sayfasında, sorumlu yazarın yazar adı, unvanı, ORCID numarası ve E-posta adresi yazılmalıdır.
5. Daha önce kongrelerde tebliğ edilmiş ve özeti yayımlanmış çalışmalar, bu durum kapak sayfasında belirtilmek üzere kabul edilir.
6. Araştırma herhangi bir kuruluş tarafından desteklenmiş ise kapak sayfasında dipnot olarak belirtilir.
7. Kapak sayfasında Türkçe makale başlığı (koyu ve ilk harfleri büyük), İngilizce başlık (ilk harfler büyük), kısa başlık (40 karakteri geçmemeli ve ilk kelimenin ilk harfi büyük, diğerleri küçük olarak yazılmalıdır), yazar adları (unvansız), çalıştıkları kuruma ait bilgiler (soyadı üstüne numara konulup dipnot olarak) verilmelidir.
8. Türkçe ve İngilizce özetlerin bir sonraki sayfaya yazılması gerekir. Bu sayfa, paragrafsız olarak Türkçe ve İngilizce özetleri (en fazla 250 kelime) içermelidir. Anahtar kelimeler özetlerin altına alfabetik olarak (virgülle ayrılmış şekilde) yazılmalıdır. Yalnızca ilk anahtar kelime büyük harfle başlamalıdır. **Türkçe Bilmeyen yazarlar için Türkçe özet ve anahtar kelimeler yazma zorunluluğu bulunmamaktadır.**
9. Araştırma makalesi; Kapak Sayfası - Özet (Türkçe ve İngilizce) - Anahtar kelimeler (Türkçe ve İngilizce), Giriş, Gereç ve Yöntem, Bulgular, Tartışma ve Sonuç, Teşekkür, Kaynaklar, Tablo ve Şekiller, Sorumlu yazar (Correspondence Author) bölümlerini içerecek şekilde düzenlenmelidir. Metin içindeki tüm başlıklar koyu yazılmalıdır. Metin içinde paragraf girintisi yapılmamalı, devamlı satır numarası verilmelidir.
10. Derlemeler, orijinal olması, en son yenilikleri içermesi, yazarların konu ile doğrudan ilişkili **en az 3 adet** çalışmalarının olması ve bunların derleme içinde kullanılması durumunda yayınlanmak üzere kabul edilebilecektir. Derlemeler kapak sayfası, Özet (Türkçe ve İngilizce), Anahtar kelimeler (Türkçe ve İngilizce), Giriş, konunun kendine ait alt başlıkları, Sonuç, Kaynaklar, Tablo ve Şekiller ve Sorumlu yazar (Correspondence) bölümlerini içerecek şekilde düzenlenmelidir.
11. Olgu Sunumları, Özet (Türkçe ve İngilizce), Anahtar kelimeler (Türkçe ve İngilizce), Giriş, Olgu(lar), Tartışma ve Sonuç, Kaynaklar, Tablo ve Şekiller ve Sorumlu yazar bölümlerini içermelidir.
12. Etik kurul onayı gerektiren çalışmalarda Etik Kurul onayı alınan kurumun adı ve onay numarası, çalışmanın Gereç ve Yöntem kısmında belirtilmelidir.
13. Tablo ve şekillerin metinde geçeceği yer, altı ve üstü çizgili olarak belirtilmelidir.
14. Ondalık ifadelerde nokta kullanılmalıdır.
15. Tür isimleri ve anatomik terimler gibi Latince ifadeler *italik* karakterle yazılmalıdır. Tüm ölçü birimleri SI (*Systeme Internationale*)'e göre verilmelidir.
16. Tablolar kaynaklar kısmından sonra, her bir tablo ayrı sayfada olacak şekilde verilmelidir. Tablo başlıklarının yalnızca ilk harfleri büyük olmalıdır. Tablo başlıkları tablonun üzerinde bulunmalı ve **Tablo 1.** şeklinde numaralandırılmalıdır. Tablolarda iç ve yan kılavuz çizgiler kullanılmamalıdır. Tanımlayıcı bilgi ve açıklamalar tabloların altına yerleştirilmelidir.
17. Her resim, grafik ve çizim; şekil olarak kabul edilip **Şekil 1.** gibi yazılmalı, her biri ayrı sayfada olacak şekilde verilmelidir. Tanımlayıcı bilgi ve açıklamalar şekil ismi ile birlikte şeklin altına yerleştirilmelidir. Resimler 300dpi çözünürlükte olmalıdır.
18. Kaynaklar metin içinde cümle sonunda belirtilmelidir. Yazar soy isimleri ve tarihi yazı içinde her kaynağa ait yayın yılı yazar isminden hemen sonra parantez içinde belirtilmelidir. Kaynak iki isimli ise isimler belirtilmeli (örn; Kaldhone ve Nayak, 2008). Kaynakta yazar sayısı ikiden fazla ise sorumlu yazar "ve ark." şeklinde belirtilmelidir (örn, Kaldhone ve ark., 2008). Eğer kaynak cümlenin başında kullanılıyorsa yazar isimlerinden sonra parantez içinde yayın yılı belirtilmelidir.
19. Kaynaklar yazılırken alfabetik sıraya konulmalı, kaynaklar bölümünde 0.5 cm içeri doğru asılı halde yazılmalıdır. Noktalama işaretlerine örneklerde gösterildiği şekilde dikkat edilmelidir. Dergi kısaltmaları *Index Medicus* ile uyum içerisinde olmalıdır. **Orijinal araştırma makaleleri, derlemeler ve olgu sunumları sırasıyla 30, 45 ve 15'ten fazla kaynak içermemelidir.**
    Kaynaklar;

19.1. Kaynak süreli yayın ise;
Örnek: Kaldhone P, Nayak R, Lynne AM, Dvaid DE, McDermott PF. Characterisation of *Salmonella enterica* serovar Heidelberg from Turkey-associated sources. Appl Environ Microbiol 2008; 74(16): 5038-46.

19.2. Kaynak editörlü kitaptan bir bölüm ise;
Örnek: Hornbeck P. Assay for antibody production. Colign JE. Kruisbeek AM. Marguiles DH. eds. In: Current Protocols in Immunology. New York: Greene Publishing Associates, 1991; pp. 105-32.

19.3. Kaynak kitap ise;
Örnek: Fleiss JL. Statistical Methods for Rates and Proportions. Second Edition. New York: John Wiley and Sons,1981; p.103.

19.4. Kaynak editörlü kitap ise;
Örnek: Balows A, Mousier WJ, Herramafl KL, eds. Manual of Clinical Microbiology. Fifth Edition. Washington DC: IRL Press, 1990; p. 37.

19.5. Kaynak kongre bildirisi ise;
Örnek: Entrala E, Mascarp C. New structural findings in *Cryptosporidium parvum* oocysts. Eighth International Congress of Parasitology (ICOPA VIII). October, 10-14, 1994; İzmir-Türkiye.

19.6. Kaynak tez ise;
Örnek: Erdem V. Köpek göz hastalıklarında klinik oftalmoskopik ve ultrasonografik bulguların değerlendirilmesi, Doktora tezi, Ankara Üniv Sağ Bil Ens, Ankara 2003; s. 1-2.

19.7. Kaynak internette bulunan bir web sitesi ise;
Örnek: TUIK. Hayvancılık İstatistikleri. http://www.tuik.gov.tr/hayvancilik.app/hayvancilik.zul; Accessed Date: 14.03.2010.

20. Eserler dergide yayımlandıktan sonra, bütün sorumluluk sahiplerine aittir.
21. Yazılar gönderilirken son kontrol listesi izlenecek ve "Telif Hakkı Devir Formu" tüm yazarlarca isim sırasına göre imzalanacaktır. **Yazım kurallarına uygun olarak hazırlanmayan yayınlar işleme alınmayacaktır.**

Instructions to Authors

1. The Journal of Faculty of Veterinary Medicine, Erciyes University publishes original research articles, short communications, case reports, letter to editor and original review articles related to the field of Veterinary Medicine.
2. Formal language of manuscripts is Turkish. Manuscripts in English are also accepted. **The publication of English-language manuscripts is given priority.**
3. Publications should be in A4 format, double spacing and Arial 10 font size. With a margin of 2.5 cm from each edge, the page number should be placed at the bottom right of the pages. Original articles and reviews should not exceed 14 pages and case reports, research notes and short papers should not exceed 7 pages including illustrations, figures and references.,
4. Manuscripts should be sent to ercvet@gmail.com. For correspondence, author's name, title, ORCID number, and E-mail address should be written on cover page of the manuscripts.
5. Studies were presented in a meeting and published as an abstract can be published with indication of this status at the bottom of the cover page.
6. Information should be included on any institutions financially contributed to the study as a footnote on the cover page.
7. The cover page should be supplied as a separate page and include: Turkish running title (bold and first letters capital), English title (first letters capital), short title (max 40 characters and first letters of first word is capital, others should be written as small), author(s) names (without titles), author(s) affiliations (Superscript numbers should be given to the surnames of authors as affiliation information).
8. The summaries in Turkish and English should be written on the next page. The title page must contain the Turkish and English summaries (up to 250 words) with no paragraph and not more than five Key words in Turkish and English. Key words must be placed below summary with an alphabetical order (comma delimited). Only the first Key word must start with a capital letter. **For non-Turkish authors, there is no obligation to write summary and keywords in Turkish.**
9. Original research paper must be organized as follows: Cover page, Summary (Turkish and English), Key words (Turkish and English), Introduction, Material and Methods, Results, Discussion and Conclusion, Acknowledgements, References, Tables and Figures and Correspondence. All titles in the text should be written in bold. There should be no paragraph indent in the text and continuous line number should be given.
10. Review articles are considered for publications if they are original and contain recent developments and accepted for publication if the authors have **at least 3 papers** directly related to the subject. Reviews must be organized as follows: Summary (Turkish and English), Key Words (Turkish and English), Introduction, Sub-headings of the subject, Conclusion, Acknowledgements, References, Tables and Figures and Correspondence.
11. Case reports must be organized as follows: Summary (Turkish and English), Key Words (Turkish and English), Introduction, Case(s), Discussion and Conclusion, Acknowledgements, References, Tables and Figures and Correspondence.
12. In the studies requiring the ethics approval, the name and approval number of the institution of the Ethics Committee must be specified in the Materials and Methods section of manuscript.
13. The place where the tables and figures belong in the text should be indicated as underlined and upperlined.
14. Decimal expressions should be used in the dot.
15. Species names and anatomical terms in Latin should be italicized. All measurement specifications must follow the SI (Systeme Internationale) units.
16. Tables must be given in a separate page after the text. First letters of first word should be capital, others should be written as small in the headings of the tables. Title of tables and figures should be numbered in order as **Table 1**. Internal and lateral lines should not be used in the tables. Descriptive information and explanations should be placed below the tables.
17. Each picture, graphic and drawing; should be given as figure and should be written as **Figure 1**. Each one should be on a separate page. Descriptive information and explanations should be placed below the figures. Pictures should be the least 300dpi resolution.
18. References should be specified in the text at the end of the sentence. Author surnames and the date of publication should be specified in parentheses. If the reference has two names, the names should be given after the publication year (eg, Kaldhone and Nayak, 2008). If the reference has more than two names should be given as “et al.,” (eg, Kaldhone et al., 2008). If the source is used at the beginning of the sentence, the year of publication should be specified in parentheses after the names of the authors.
19. References should be placed in alphabetical order and hanging 0.5 cm inwards in the references section. Punctuation should be taken into consideration as shown in the examples, Journal abbreviations must be in line with *Index Medicus*. **The reference list must not contain more than 30, 45, and 15 references for original research articles, reviews and case reports, respectively.**
    References;

19.1. If the reference is a periodical, citation must be done as shown below;
    Example: Kaldhone P, Nayak R, Lynne AM, Dvaid DE, McDermott PF, Logue CM, Foley SL. Characterisation of *Salmonella enterica* serovar Heidelberg from turkey-associated sources. Appl Environ Microbiol 2008; 74(16): 5038-46.

19.2. If the reference is from chapter of a book with an editor, citation must be done as shown below;
    Example: Hornbeck P. Assay for antibody production. Colign JE. Kruisbeek AM. Marguiles DH. eds. In: Current Protocols in Immunology. New York: Greene Publishing Associates, 1991; pp. 105-32.

19.3. If the reference is a book, citation must be done as shown below;
    Example: Fleiss JL. Statistical Methods for Rates and Proportions. Second Edition. New York: John Wiley and Sons,1981; p.103.

19.4. If the reference is whole book with an editor, citation must be as below;
    Example: Balows A, Mousier WJ, Herramafl KL, eds. Manual of Clinical Microbiology. Fifth Edition. Washington DC: IRL Press, 1990; p. 37.

19.5. If the reference is from meeting, citation must be done as shown below;
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