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RESEARCH ARTICLE

The Effect of Different Transport Distances and Season on Meat Quality Characteristics of Broiler Chicken in Commercial Slaughter Conditions

Yavuz ÇOBANBAŞI[1], Bülent TEKE[2*]

[1]Amasya Meat Products / Ekur Meat Production, TDİ Fattening OSB Saluca Village Suluova, 05500, Amasya, TURKEY
[2]Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Animal Breeding and Husbandry, 55270, Samsun, TURKEY*

ABSTRACT

The aim of the study was to determine the effect of season and transport distance on meat quality characteristics of broiler chicken in commercial transport and slaughter conditions. The study was carried out on Ross 308 broiler chickens reared under similar commercial conditions from three different seasons (summer, autumn and winter) and three different transport distances at 40 km, 70 km and 130 km. Meat samples were taken on July, October, and December for summer, autumn and winter seasons, respectively during 2018. Broilers in trucks were waited in holding barn for 1 h. A total of 135 broilers, 15 samples per transport distance, were randomly selected to determine meat quality characteristics, (3 seasons × 3 transport distances × 15 samples). Meat colour parameters, pH_{4h}, drip loss, cooking loss and Warner Bratzler shear force (WBSF) was determined. In winter, pH_{4h}, a^*_{24h} and b^*_{24h} were higher than other seasons, while L^*_{24h}, drip loss and WBSF were lower than other seasons. The incidence of pale, soft and exudative (PSE) meat was the highest in summer (26.67%), while the incidence of dark, firm and dry (DFD) meat was the highest in winter (53.33%). The lowest incidence of normal breast meat was in winter season. In conclusion, incidence of normal breast meat decreased when broiler chickens were transported in winter. However, incidence of PSE meat was the highest in summer season. Transport distance affected adversely some meat quality characteristics and this effect was most pronounced in summer season. In order to improve the meat quality, as much as possible, transportation of broiler chickens should be carried out within thermal comfort zone ranges and avoided from long-distance transports especially in summer.

Keywords: Animal welfare, DFD meat, meat quality, PSE meat, transportation

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Ticari Kesim Koşullarında Etlik Piliçlerde Farklı Nakil Mesafelerinin Ve Mevsimin Et Kalitesine Etkisi

ÖZ

Bu araştırma ticari nakil ve kesim koşullarında etlik piliçlerin et kalite özellikleri üzerine mevsimin ve nakil mesafesinin etkisini belirlemek amacıyla yapılmıştır. Bu araştırma benzer ticari koşullar altında yetiştirilen 3 farklı mevsim (yaz, sonbahar ve kış) ve 3 farklı nakil mesafesinde (40 km, 70 km ve 130 km) kesimhaneye nakledilen Ross 308 hattı etlik piliçler üzerinde yürütülmüştür. Et örnekleri 2018 yılında yaz, sonbahar ve kış mevsimleri için sırasıyla Temmuz, Ekim ve Aralık aylarında alınmıştır. Etlik piliçlere nakil aracı içinde 1 saat dinlenme süresi uygulanmıştır. Her mevsimde her bir nakil mesafesi için 15'er örnek olmak üzere toplam 135 etlik piliç (3 mevsim × 3 nakil mesafesi × 15 örnek) et kalite özelliklerinin belirlenmesi için rastgele seçilmiştir. Et rengi, pH_{4h}, damlama kaybı, pişirme kaybı ve Warner Bratzler kesme kuvveti (WBSF) belirlenmiştir. Kış mevsiminde pH_{4h}, a^*_{24h} ve b^*_{24h} değeri diğer mevsimlerden daha yüksek iken L^*_{24h}, damlama kaybı ve WBSF değeri diğer mevsimlerden daha düşük bulunmuştur. PSE (soluk, yumuşak, sulu) etin insidensi yaz mevsiminde en yüksek iken (%26.67), DFD (koyu, sert, kuru) etin insidensi ise kış mevsiminde en yüksek olarak tespit edilmiştir (%53.33). En düşük normal et insidensi kış mevsiminde bulunmuştur. Sonuç olarak, kış mevsiminde etlik piliçlerin nakli normal et insidensinin düşmesi ile sonuçlanmıştır. Diğer taraftan, PSE etin insidensi ise en yüksek yaz mevsiminde bulunmuştur. Nakil mesafesinin artışı bazı et kalite özelliklerini olumsuz olarak etkilemiş ve en çok bu etki yaz mevsiminde gözlenmiştir. Etlik piliçlerin et kalite özelliklerinin olumsuz olarak etkilenmemesi için termal konfor zonu aralıklarında naklin yapılması ve özellikle yaz mevsiminde uzun mesafe nakillerden kaçınılması önerilmektedir.

Anahtar Kelimeler: DFD et, et kalitesi, hayvan refahı, nakil, PSE et.

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ORCID ID; *YÇ: 0000-0003-2511-6125, BT: 1000-0002-1091-643X*
*Corresponding author e-mail: bulentteke@gmail.com

INTRODUCTION

The rapid increase in the human population in the world has closely associated with an increase in protein demand leading to nutritional problems (Makkar et al. 2014). Animal products due to important essential amino acids contents constantly increasing thus, the breeding of animals where animal proteins can be obtained at the lowest price and in the a short time have gained popularity (Altınel 1999). Chicken meat has a very important role in human nutrition because of its low cholesterol and fat levels, high protein and calcium content, sufficient amount of essential amino acids and being cheaper than red meat. In addition, it is economical to breed because it can be produced in the short term, the feed conversion rate is good, and more products can be obtained in a unit area than other animals (Çınar 2007, Öztürk 2016).

Turkey provides 60% of its total meat production from poultry meat. Chicken meat production was 163 thousand tons in 1990 in Turkey, has reached 2 million tons with an increase of 12 times in 2019 and has a share of 2% in the world (Anonymous 2019).

Farm animals can show different reactions to the stress depending on their specific genetic structures during the transport process. Chickens are one of the most sensitive species to stress among farm animals (Isabel Guerrero 2010). They may face with risk and the extent of economic damage become more important considering that chickens are the most transported farm animals in the world (EFSA 2004).

Transport of poultry from farm to slaughterhouse is a multifactorial process. This process includes pre-slaughter stages with varying degrees of stress, such as the start of loading, leaving the social environment, movement, sudden acceleration and stopping, exposure to a new unfamiliar environment, noise, high or low ambient temperature (Appleby 2008). Catching, crating and transporting broilers are the most traumatic events among these stages (Elrom 2000). Pre-slaughter stress factors can cause varying degrees of immune system damage, bone fractures, injuries in different regions, deterioration in meat quality, and even death in broilers. Pre-slaughter stress factors such as transport time and distance, vehicle riding quality, unloading of broilers from the transport vehicle and dispatching to slaughter, pre-slaughter rest period have been studied by various researchers (Warriss et al. 1992, Mitchell and Kettlewell 1998, Knierim and Gocke 2003; Nijdam et al. 2004). Among these factors, especially transport distance and heat stress at pre-slaughter is a significant threat to bird welfare. Heat or cold stress has a negative effect on meat quality and increases the mortality rate. Thus, it can cause great economic loss (Mitchell and Kettewell 1998, Lara and Rostagno 2013).

The most important factor increasing the fear level of the transported broilers is the transportation time (Cashman et al. 1989). Long-distance transport negatively affects both animal welfare and meat quality. Long transports cause fatigue in broilers, especially when they are hungry for a long time, and decrease the glycogen stores in the body. In addition, broilers exposed to heat stress expose to dehydration in long-distance transportation. The stress caused by the negative effects of long transports brings about significant economic losses by damaging the welfare and meat quality of broilers. Therefore, it has been reported that the transport time should be reduced (Burgess and Pickett 2006).

Seasonal conditions of transport may affect meat quality characteristics. The water holding capacity, WBSF and meat color are negatively affected when broilers are exposed to cold weather conditions (Barbut et al. 2005, Dadgar et al. 2010, 2011). Broiler meats exposed to hot weather conditions have more drip loss, cooking loss and pale meat colour with WBSF value (McKee and Sams 1997, Petracci et al. 2004, Bianchi et al. 2005, Langer et al. 2009). On the other hand, it has been reported that the season does not have a significant effect on meat quality characteristics in some studies (Holm and Fletcher 1997, Sandercock et al. 2001, Debut et al. 2003).

Temperature stress can cause inappropriate changes in meat quality parameters such as pale, soft and exudative (PSE) or dark, firm and dry (DFD) meat (Langer et al. 2009). Most of the consumers do not prefer this type of meat because of their colour and low meat quality (Fletcher 1999). Recently, it has been reported by many researchers that there is a close relationship between uncooked breast meat color and defective meat problems (Qiao et al. 2001, Bianchi et al. 2005). It has been reported by some researchers that the brightness value (L*) can be used as an indicator of PSE or DFD meat, as well as to predict the incidence of meat defects (Barbut 1997, Wilkins et al. 2000, Soares et al. 2002, Galobart and Moran 2004, Petracci et al. 2004).

This research was carried out to determine the effect of season and transport distance on meat quality characteristics of broiler chickens under commercial transport and slaughter conditions.

MATERIAL and METHODS

Animals, study design and slaughter process

The study was carried out three different seasons (summer, autumn and winter) and three different transportation distances (short, medium and long distance) reared under the similar commercial conditions in Samsun. The animal material of this study consisted of Ross 308 line broilers. The short distance transportation was determined as 40 km, medium distance transportation was 70 kilometers

and long distance transportation was 130 kilometers. In the research, data were collected for the summer, autumn and winter seasons in July, October and December in 2018. A total of 135 broiler chickens (3 seasons × 3 transport distances × 15 samples) were randomly selected to determine meat quality characteristics.

In the hens, 23 hours of light and 1 hour of darkness were applied, and feed and water were given ad libitum by the producers. Broilers in all poultry houses were starved 8 hours before transport throughout the study. The dimensions of the loading crates used for transport are 80 cm long × 45 cm wide × 30 cm high. The type of trailers was similar and there were 320 crates in each vehicle. Stocking densities in the crates for all transfers were within the range recommended by Anonymous (2011). Average vehicle speed was approximately 40 km/h. The transport vehicles completed the transport without stopping, without sudden acceleration or deceleration during the transport. A temperature and humidity recording device (Testo 174H, Testo Instrument Co. Ltd., Germany) was installed outside the slaughterhouse to record the ambient temperature and humidity. The recorded values throughout the research are given in Table 1.

Table 1. Certain transportation and slaughter characteristics by season ($\overline{X} \pm S\overline{x}$)

Items	Summer	Autumn	Winter
Stocking density (m^2/bird)	0.043±0.001	0.042±0.001	0.040±0.001
Slaughter weight (kg)	2.13±0.05	2.10±0.05	2.14±0.05
Slaughter age (d)	40.89±0.62	40.02±0.36	39.08±0.74
Temperature (°C)	16.61±0.75	4.16±0.45	0.34 ± 0.33
Humidity (%)	90.11±2.54	88.33±0.85	96.89±0.63

The slaughtering process took place between 23:30 and 08:00, depending on the workload in the slaughterhouse. The rest period before slaughter was applied as 1 hour for all transport vehicles. Broilers were unloaded from the crates and hung upside down on the slaughter line at the end of this period. Broilers were stunned electrically at 50 Volt for 10 sec before slaughtering, afterward they were cut by hand, after the blood flow was provided, they were passed through a hot water tank at 60°C and their feathers were automatically plucked. The carcasses were taken to the relevant sections for cooling after the internal organs were removed automatically. Laboratory analyzes of meat quality were carried out at Istanbul University-Cerrahpasa, Faculty of Veterinary Medicine, Department of Animal Breeding and Husbandry, Meat quality laboratory.

Meat quality analysis

M. *pectoralis major* was removed from the 15 carcasses for each application. The pH of this muscle was measured 4 hours after slaughter using a Testo 205 pH meter (Testo Instrument Co. Ltd., Germany) and the result was recorded as pH_{4h}.

Passive Water Loss Measurement

Samples were taken from M. *pectoralis major* approximately 20 g for passive water loss measurement. After the moisture on the outer surfaces of the samples taken for this purpose was carefully dried with a paper towel, it was weighed on a precision balance sensitive to 0.01 g (HT-1000NH+ model, Dikomsan, Istanbul) and recorded as the initial weight ($W_{initial}$). The sample taken was placed in a transparent bag in such a way that it would not touch the bag and was weighed again after it was kept at 4°C for 24 hours. Passive water loss (PWL) is calculated with the following formula, which expresses the ratio of passive water loss resulting from hanging for 24 hours to the initial sample weight (Honikel 1998):

$PWL\ (\%) = [(W_{initial} - W_{last}) / W_{initial}] \times 100$

Meat Colour Measurement

A color measuring device (Minolta CR 400, Konica Minolta Sensing, Inc., Osaka, Japan) measuring with L*, a*, b* coordinate system was used for meat color measurement. The standards reported by CIE (1976) were applied in the measurements, and D65 was used as the light source. The device was calibrated according to the standard white plate (Y=93.8; x=0.316; y=0.3323). Measurements were made from three different places (the bone-facing surface of M. *pectoralis major*, from the lean and undamaged parts of the median line) by means of a colorimeter for color analysis. Samples taken for color measurement were placed on a plastic plate and the first measurement was made as soon as the sample was taken. Afterwards, the samples were kept in a refrigerator at 4°C for 24 hours and then a second color measurement was performed. The colorimeter is set to take three measurements per command and calculate their average. The average value was accepted as the color value of that sample.

Cooking loss analysis

The remaining part of M. *pectoralis major* was used for cooking loss and texture analysis. The samples were weighed, packed with vacuum, and baked in a water bath at 80°C for 20 minutes before cooking At the end of this period, the samples were removed from the water bath and cooled under running water until their internal temperature reached room temperature. The samples were then kept in a refrigerator at 4°C for 24 hours. Afterwards, the samples were taken out of their bags, dried with paper towels and weighed to determine their weights after cooking. Cooking loss (%) was calculated as the ratio of the difference between pre- and post-cooking weights to the initial weight (Honikel 1998).

Texture analysis

Warner-Bratzler blade connected to Instron 3343 device (Instron, Norwood, USA) was used for WB shear force analysis. The samples used in the cooking loss measurement were used in the WBSF analysis. Three sub-samples from each cooked meat samples were cut parallel to the muscle fibres with a cross section of 1×1 cm and 2.5-3 cm length. The average value was accepted as the value of that sample (Pekel et al. 2012).

Classification of Samples

Breast meat samples were classified as PSE, DFD or normal meat according to L^*_{24h} value. Accordingly, if $L^*_{24h} \geq 49.0$ is considered PSE, if $L^*_{24h} \leq 44.0$ is DFD and $44.0 < L^*_{24h} < 49.0$ is considered normal (Barbut 1997, Soares et al. 2002).

Statistical Analysis

Chi-square test was used to compare DFD, normal and PSE meat incidences by season. GLM test was performed to determine the effect of season and transport distance on meat quality characteristics. Season and transport distance were used as the main factors in the model, and the interaction effect of season and transport distance was calculated. Tukey's multiple range tests was used to determine the significance of the difference when the effect of season, transport distance or interaction of both were significant.

RESULTS

The Effect of Season on Meat Quality Characteristics

Average values for meat quality characteristics according to season and transportation distance are shown in Table 2. In this study, the effect of the season on meat quality characteristics was significant ($P<0.01$ and $P<0.001$) except b^*_0, while the effect of transport distance on pH_{4h}, L^*_0 and L^*_{24} values was insignificant ($P>0.05$). The value of pH_{4h}, a^*_0 and a^*_{24} were the lowest in summer, and the highest L^*_{24h} and b^*_{24h} among meat quality characteristics. In addition, it was determined that drip loss, cooking loss and WBSF value were higher in summer than broilers transported in other seasons. On the other hand, it was determined that the effect of the season × transport distance interaction on meat quality characteristics became significant except L^*_0, b^*_0, drip loss and WBSF value ($P>0.05$).

Effect of Transport Distance on Meat Quality Characteristics

Mean meat quality characteristics (±SEM) of different transportation distances are given in Table 3 according to the seasons. Accordingly, it has been determined that meat quality characteristics from different transportation distances are greatly affected in the summer season. It was determined that meat quality characteristics were partially affected or the difference was insignificant at different transportation distances in other seasons.

Incidence of DFD, normal and PSE meat

DFD, normal and PSE meat incidences according to the seasons are given in Table 4. According to these findings, it was determined that DFD meat incidence was highest in winter (53.33%) and lowest in summer (6.66%). The highest incidence of PSE meat was observed in the summer (26.67%) and PSE meat was not found in the winter season. In addition, normal meat incidence was the lowest (46.67%) in winter.

Table 2. Means and importance levels of meat quality characteristics of seasonal and transport distance groups

Meat Quality Characteristics	Seasons (S)			Transport Distance (TD)				Significance		
	Summer	**Autumn**	**Winter**	**Short**	**Medium**	**Long**	**SEM**	**S**	**TD**	**SxTD**
pH_{4h}	6.06^{c}	6.35^{b}	6.50^{a}	6.35	6.31	6.26	0.03	***	ns	***
L^*_0	46.48^{b}	43.37^{a}	44.51^{b}	44.52	45.23	44.61	0.57	***	ns	ns
L^*_{24}	46.67^{a}	44.57^{b}	43.92^{b}	45.37	45.06	44.72	0.23	***	ns	*
a^*_0	2.59^{b}	3.13^{a}	3.03^{a}	2.67^{b}	2.84^{ab}	3.25^{a}	0.12	**	**	**
a^*_{24}	2.58^{b}	2.79^{ab}	3.03^{a}	2.51^{b}	2.80^{ab}	3.09^{a}	0.12	**	*	***
b^*_0	4.92	5.01	4.84	5.30^{a}	4.87^{ab}	4.60^{b}	0.18	ns	*	ns
b^*_{24}	5.29^{ab}	4.74^{b}	5.61^{a}	5.62^{a}	5.28^{ab}	4.74^{b}	0.18	**	**	**
Dropping loss (%)	2.87^{a}	2.17^{b}	1.46^{c}	2.12	2.21	2.17	0.07	***	ns	ns
Cooking loss (%)	19.44^{a}	15.34^{c}	17.54^{b}	17.81^{ab}	17.90^{a}	16.62^{b}	0.37	***	*	*
WBSF (kg)	2.37^{a}	1.61^{b}	1.33^{b}	1.87	1.76	1.68	0.09	***	ns	ns

S: Season, TD: Transport Distance, ns: Non significant (P>0.05)
a, b, c: Mean values in the same row with different letters differ significantly (P<0.05).
* P<0.05
**P<0.01
***P<0.001

Table 3. Means and significance levels of meat quality characteristics at different transportation distances according to the seasons ($\overline{X} \pm S\overline{x}$)

Characteristics	Summer				Autumn				Winter			
	Short	Medium	Long	Sig.	Short	Medium	Long	Sig.	Short	Medium	Long	Sig.
pH_{4h}	6.06±0.03	6.10±0.02	6.03±0.05	ns	6.45±0.05^{a}	6.22±0.05^{b}	6.37±0.05^{ab}	**	6.52±0.06^{ab}	6.61±0.05^{a}	6.38±0.06^{b}	*
L^*_0	47.10±0.20^{a}	47.25±0.59^{a}	45.09±0.59^{b}	**	41.37±2.70	44.59±0.48	44.14±0.25	Ns	45.07±0.36	43.86±0.27	44.61±0.61	ns
L^*_{24}	47.21±0.27^{a}	47.28±0.58^{a}	45.51±0.54^{b}	*	44.50±0.33	44.65±0.41	44.56±0.25	Ns	44.41±0.30	43.25±0.21	44.08±0.52	ns
a^*_0	2.16±0.13	2.17±0.27	3.44±0.26	***	2.94±0.13	2.99±0.17	3.45±0.24	Ns	2.89±0.15	3.34±0.24	2.86±0.22	ns
a^*_{24}	2.12±0.15	2.13±0.27	3.49±0.28	***	2.57±0.12	2.76±0.16	3.02±0.18	Ns	2.83±0.18	3.51±0.27	2.75±0.21	ns
b^*_0	5.75±0.28	4.71±0.24	4.30±0.40	**	4.82±0.39	5.30±0.22	4.89±0.31	Ns	5.32±0.32	4.60±0.24	4.59±0.31	ns
b^*_{24}	6.25±0.27	5.05±0.28	4.55±0.35	***	4.40±0.34	5.25±0.23	4.51±0.30	Ns	6.21±0.31	5.44±0.31	5.04±0.32	*
Dropping loss (%)	2.78±0.12	3.07±0.09	2.77±0.15	ns	2.12±0.14	2.19±0.15	2.19±0.12	Ns	1.47±0.06	1.36±0.07	1.55±0.05	ns
Cooking loss (%)	19.45±0.55^{ab}	21.32±0.70^{a}	17.55±0.82^{b}	**	15.61±0.82	15.38±0.59	15.04±0.39	Ns	18.36±0.59	17.01±0.39	17.26±0.73	ns
WBSF (kg)	2.59±0.23	2.30±0.28	2.23±0.18	ns	1.54±0.06	1.72±0.13	1.57±0.08	Ns	1.49±0.11	1.25±0.07	1.25±0.08	ns

ns: Non significant (P>0.05)

a, b, c: Mean values in the same row with different letters differ significantly (P<0.05).

* P<0.05

**P<0.01

***P<0.001

Table 4. The incidence of DFD, normal and PSE meat by season

Traits	Summer (n=45)		Autumn (n=45)		Winter (n=45)		Overall (n=135)		Sig.
	N	%	n	%	N	%	n	%	
DFD	3	6.66^{c}	11	24.44^{b}	24	53.33^{a}	38	28.15	***
Normal	30	66.67^{b}	34	75.56^{a}	21	46.67^{c}	85	62.96	***
PSE	12	26.67^{a}	0	0^{b}	0	0^{b}	12	8.89	***

a, b, c: Incidence values in the same row with different letters differ significantly (P<0.05).
Sig.: Significance, *** (P < 0.001).

DISCUSSION

Poultry is more sensitive to stress than other animal species, especially ruminants. Therefore, meat quality problems are more common in poultry compared to cattle and sheep (Warriss et al. 1992). It has been reported that pre-slaughter stress factors such as pre-slaughter fasting period, environmental temperature and humidity, and pre-slaughter resting conditions may affect the meat quality of broiler chickens (Mitchell and Kettlewell 1998).

The Effect of Season on Meat Quality Characteristics

In this study, broilers transported in summer were more pale (+2.75 L^*_{24h} units), less red (−0.45 a^*_{24h} units), and less yellow (−0.32 b^*_{24h} units) compared to those transported in winter, WBSF value and drip loss increased breast meat. In most of the studies, it has been reported that broilers transported in summer were a decrease in pH, a decrease in red color (a*), drip loss, cooking loss and lightness (L*) compared to cold weather conditions. In addition, it has been reported by the researchers that the WBSF value increases when broilers are exposed to pre-slaughter heat stress (Bianchi et al. 2007). It has been reported that exposure of broilers to pre-slaughter heat stress accelerates the formation of rigor mortis and causes protein denaturation. It has been stated that if there is a rapid decrease in pH in the early postmortem period when the carcass temperature is high, undesirable results such as PSE meat (pale, soft and exudative) may occur (Mitchell et al. 2001). These results are in accordance with those reports by Petracci et al. (2001), Petracci et al. (2004), Bianchi et al. (2006) and Bianchi et al. (2007). However, it has been reported by some researchers that heat stress did not have a significant difference on meat quality characteristics of broiler chickens (Sandercock et al. 2001, Debut et al. 2003). Differences between studies may be due to differences in ambient temperature, resting period before slaughter or starvation period before slaughter. On the other hand, it has been stated that cold weather conditions before slaughter may also cause stress in broilers and adversely affect meat quality. It has been reported by many researchers that breast meat has less drip loss, more shear force, darker meat color and higher pH compared to optimum weather conditions of broilers exposed to cold stress (Dadgar et al. 2010, 2011). Similar results were also recorded in this study. Broilers exposed to seasonal cold stress during transport use some of the glycogen in the muscles to keep their body temperature constant. Therefore, it is thought that less glycogen is converted to lactic acid and the pH of meat remains high (Warriss et al. 1999).

Effect of Transport Distance on Meat Quality Characteristics

In this study, it was determined that meat quality characteristics were affected more in summer than other seasons in different transportation distances in different seasons. Three different transport times (15 km, 50 km and 150 km) and season (autumn, winter and summer) stress on broilers were investigated by Elsayed (2014). In this study, it was determined that as the transport distance increased, glucose and LDH, decreased, while H:L and corticosterone increased. In addition, it has been reported that corticosterone increased in all seasons at 50 km, especially in long transport (150 km). Stress of broiler chickens increased in summer season. Nijdam et al. (2005) reported an increase in plasma corticosterone level of broiler chickens after 3 hours of transportation. Zhang et al. (2009) reported that the plasma glucose level increased slightly in the first 45 minutes and then decreased within 3 hours. Oba et al. (2009) investigated the effect of three different transport distances (30 min, 90 min and 180 min) on meat quality characteristics under hot weather conditions (33 ^{o}C). At the end of the study, it was reported that the a* value increased while the L* value decreased with the increase in the transportation period. Yalçin and Güler (2012) investigated the effects of three different transport distances (65 km, 115 km and 165 km) on blood metabolites and meat quality. At the end of the study, it was reported that long-distance transport (165 km) has a negative effect not only on animal welfare, but also on meat quality as well as slaughter weight is an important factor in the occurrence of this negative effect. A study in turkeys (Owens and Sams 2000) reported that turkeys transported for 3 hours had a significantly lower breast meat L* value compared to turkeys that were not transported. It was reported that there was a negative correlation between L* value and breast meat pH when the correlation between meat quality characteristics was examined. The results found in this study regarding transportation distance and meat quality characteristics are similar to the research findings of Yalçın and Güler (2012). Transport is the most important source of environmental stress for broilers (Mitchell and Kettlewell 1998). The extent of this stress is highly dependent on transport distance and ambient temperature (Warriss et al. 1992, Kannan et al. 1997, Warriss et al. 2005). It was reported that transportation longer than three hours causes increased mortality, increased carcass injuries and decreased carcass quality (Warriss et al. 1992). Since poultry are more sensitive to stress than other animal species, it is recommended that the transport time should be shorter (Warriss et al. 1993, 1999). It is thought that deteriorate in meat quality parameters due to the decrease in muscle glycogen stores when transport distance increase.

On the other hand, in the study by Vosmerova et al. (2010), three different ambient temperatures (25-35 ^{o}C, 10-20 ^{o}C and -5-5 ^{o}C) and four different transport distances (0 km, 10 km, 70 km and 130 km) were compared according to stress conditions of broilers transported. As a result of the research, it was reported that broiler chickens in short distance and cold weather conditions had the highest corticosterone level. Yue et al. (2010) used two different transport distances and two different rest periods (45 min transport and 45 min rest, 45 min transport and 3 hours rest, 3 hours transport and 45 min rest, 3 hours transport and 3 hours rest). Biochemical properties and meat quality parameters were compared between the groups. As a result of the research, there were biochemical changes between the groups, but it was reported that there was no significant change in breast meat quality characteristics other than the a* value. In another study, the same transport times and the same rest periods were applied (45 min transport and 45 min rest, 45 min transport and 3 hours rest, 3 hours

transport and 45 min rest, 3 hours transport and 3 hours rest) (Zhang et al. 2009). It has been reported that short transport time and short resting time cause an increase in a* value. Bianchi et al. (2006), three different transport distances were applied (<40 km, between 40-210 km and >210 km) and meat quality characteristics were compared. It was reported that there was a significant difference only among a* values. The a* value was higher in broilers transported <40 km than in the other two transport groups. The results of current study on the effect of transport distance on meat quality characteristics are different from the results of some researchers (Bianchi et al., 2006; Zhang et al. 2009, Vosmerova et al. 2010; Yue et al. 2010). It is thought that the differences between the studies may be due to the differences in environmental temperature, resting period before slaughter, starvation period before slaughter, and transportation distances.

Incidence of DFD, normal and PSE meat

Bianchi et al. (2006) reported that broiler breast meats at <12°C were darker. The a* and b* values were higher than those in other groups from broiler chickens housed at three different ambient temperatures (<12°C, 12-18°C and >18°C). As a result of the study, it was reported that the incidence of PSE meat was 15.3% in housed at >18°C, 13.3% in broilers housed in the 12-18°C temperature range, and 2.8% in housed at <12°C. Petracci et al. (2004) reported that the incidence of PSE meat was 26.7% in broilers sent to slaughter in the summer season, while this rate was 5.9% in the winter season. Dadgar et al. (2010) reported that the incidence of PSE meat was 13% at 20°C ambient temperature while the incidence decreased to 4% at 0°C.

In this study, it was determined that DFD meat incidence was highest in winter and PSE meat incidence was highest in summer. The results of this study were similar by Bianchi et al. (2006), Dadgar et al. (2010) and Petracci et al. (2004). In addition, many researchers (Mc Kee and Sams 1997, Owens and Sams 2000, Van Laack et al. 2000, Petracci et al. 2004, Bianchi et al. 2005) reported that the incidence of PSE meat was high at high ambient temperature while the incidence of DFD meat was high in cold environmental conditions.

This rate in Europe was %10 (Petracci et al. 2009), 5% in Poland (Lesiow et al. 2007), in England 20%, 37-47% in the USA (Woelfel et al. 2002). In this study, the overall incidence of PSE meat was 8.89%. The current results in this study was similar to the results of Petracci et al. (2009), it was lower than the value reported by Wilkins et al. (2000) and Woelfel et al. (2002). It is thought that the reason for the differences among the results may be due to the environmental temperature, the different microclimate conditions of the transport vehicles in different studies, the differences in the slaughter age and weight of the broilers. On the other hand, Lesiow et al. (2007) reported that the incidence of DFD meat was between 18-34%. DFD meat incidence was reported by Dadgar et al (2010) as 8% in broilers transported between −8°C and 0°C. The results obtained for DFD meat incidence in this study were similar to the results of Lesiow et al. (2007), while it was higher than the results of Dadgar et al (2010). Differences may be due to differences in DFD meat classification created by Dadgar et al. (2010) (pH>6.1 and L*<46).

CONCLUSION

Poultry transport from the coop to the slaughterhouse is a multifactorial and extremely stressful process involving many traumatic factors for broilers. This stress is highly dependent on transport distance and ambient temperature. These factors bring about economic loss by causing meat quality problems such as PSE or DFD. In this study, the effect of the season on meat quality characteristics

was significant. It was determined that the meat obtained from broilers transported in the summer season was paler, less red, less yellow, higher drip loss and WBSF value than those transplanted in the winter season. In addition, it was determined that broiler chickens exposed to cold environmental conditions had a darker, less drip loss and less WBSF value due to the higher pH value of breast meat compared to other seasons. On the other hand, the highest PSE meat incidence was in summer, while the highest DFD meat incidence was in winter. At the same time, it was determined that the lowest normal meat incidence was in winter.

It was determined that meat quality characteristics were partially affected, as the transport distance increased, but the effect of transport distance was much more evident, especially in the summer season compared to other seasons. Broiler chickens are affected more in winter than other seasons because the DFD meat ratio is the highest and the normal meat incidence is the lowest. In addition, PSE meat incidence was highest in summer and the effect of transport distance was significant, especially in this season. In conclusion, In order to improve the meat quality transportation of broiler chickens should be carried out within thermal comfort zone ranges and avoided from long-distance transports especially in summer.

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RESEARCH ARTICLE

Retrospective Evaluation of Cases Admitted to the Internal Medicine Clinic of the Faculty of Veterinary Medicine, Aksaray University

Melek AYDEMİR[1*], Ali Evren HAYDARDEDEOĞLU[2], İlker ÇAMKERTEN[3], Hidayet Metin ERDOĞAN[4]

[1,2,3,4]Aksaray University, Veterinary Faculty, Department of Veterinary Internal Diseases, 68100, Aksaray, Turkey

ABSTRACT

In this study, demographic analysis of cases admitted to our newly established faculty hospital was aimed. For this purpose, casese reffered between 23.06.2015-30.06.2021 to the clinic of Internal Medicine, Faculty of Veterinary Medicine, Aksaray University, for diagnosis and treatment were included in the study. A total of 657 patients were evaluated retrospectively. The distribution of the cases according to year, month and species, and classification by the body systems involved were made. The highest number of cases referred was carnivores and the least was poultry between the evaluated years. The highest number of cases were recorded recorded in 2021 and the lowest in 2015. The number of cases peaked in the winter season, especially in February, and was the lowest in the summer season, particularly in July. In the majority of cases admitted, the digestive system was affected and the second most affected system was respiratory.
Keywords: Animal diseases, retrospective study, data analysis

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Aksaray Üniversitesi Veteriner Fakültesi İç Hastalıkları Kliniğine Getirilen Hastaların Retrospektif Değerlendirilmesi

ÖZ

Bu çalışmada yeni kurulan fakültemiz hastanesine getirilen hastaların demografik analizleri amaçlanmıştır. Bu amaçla 23.06.2015-30.06.2021 tarihleri arasında Aksaray Üniversitesi Veteriner Fakültesi İç Hastalıkları Kliniği'ne tanı ve tedavi amacıyla getirilen hastalar çalışmaya dahil edildi. Toplam 657 hastanın retrospektif değerlendirilmesi yapıldı. Hastaların yıl, ay ve türlere göre dağılımları ile hastalıkların yerleştiği sistemlere göre sınıflandırılması yapıldı. Söz konusu yıllar arasında en fazla karnivor, en az kanatlı hastanın getirildiği belirlenmiştir. Hasta yoğunluğunun en fazla olduğu yıl 2021 ve en az ise 2015 yılı olarak kaydedilmiştir. Hayvan sayısının kış mevsiminde, özellikle Şubat ayında en yüksek orana ulaştığı, yaz mevsiminde ise Temmuz ayında en az olduğu belirlenmiştir. Sistem hastalıkları yönünden değerlendirildiğinde, tüm türlerde en çok sindirim sistemi ikinci sırada ise solunum sisteminin etkilendiği vakalar tespit edilmiştir.
Anahtar kelimeler: Hayvan hastalıkları, retrospektif çalışma, veri analizi.

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ORCID ID; *MA: 0000-0002-4732-8279, AEH: 0000-0002-8473-0072, İÇ: : 0000-0002-6952-7703, HME: 0000-0003-1261-4352*
*Corresponding author e-mail: melekaydemir09@gmail.com

GİRİŞ

Veteriner fakülteleri, hastalıkların teşhisi, tedavisi ve korunması, zoonoz hastalıkların teşhis ve profilaksisinin yanı sıra ülke hayvancılığının gelişmesi ve ilerlemesine katkı sağlayacak veteriner hekimleri yetiştiren önemli eğitim kurumlarıdır (Aksoy ve ark. 2018). Veteriner Fakültesi'nde eğitim öğretim faaliyetleri, teorik, laboratuvar ve klinik uygulamaları kapsamaktadır.

Aksaray Üniversitesi Veteriner Fakültesi 2014-2015 yılında ilk eğitim ve öğretime geçmiştir. 2018-2019 yılında ilk veteriner hekim mezunlarını vermiştir (Anonim 2019).

Aksaray İl Tarım ve Orman Müdürlüğü'nün 2021 yılında yayınlamış olduğu brifinge göre büyükbaş hayvan sayısı 336.200 olup iller arasında Türkiye'de 15. sırada yer alırken, küçükbaş hayvan sayısı ise 991.397 olup Türkiye'de 20. sırada yer almaktadır. Aksaray ilinde 2020 yılında TÜİK verilerine göre 1.557.000 ton yonca, üretimi ile ülkemizde 2. sırada yer almaktadır. Aksaray ili, süt sığırları canlı hayvan sayısı kategorilerinde, Türkiye'de il sıralamasında ilk 15 arasında yer almaktadır. Hayvan yetiştiriciliğinde ve yem bitkileri üretiminde önemi bir ildir (Anonim 2021).

Aksaray ilinde hem yetiştiricilerin bilinçlendirilmesi için eğitimler ve gezilerin yapılması hem de kırsal kalkınma yatırımlarını destekleme programlarıyla da (2021-2025) ekonomik yönden tarım ve hayvancılık desteklenmektedir. Ayrıca Aksaray ilinin tarım ve hayvancılık olarak daha iyi bir seviyeye gelebilmesi amacıyla yatırım amaçlı teşvik ve kredi avantajları da sunulmaktadır (Sevimli 2020).

Büyük ve küçük baş hayvancılığın yanında İç Anadolu bölgesinde yetiştirilen ve genel olarak Aksaray iline özgü 'Aksaray Malaklısı' veya 'Malaklı Karabaş' olarak da bilinen çoban köpeği yetiştiriciliği de Aksaray halkı için önem arz etmektedir. Tarih boyunca birçok medeniyete ev sahipliği yapan Aksaray İli ve çevresinde yetiştiği için 'Aksaray Malaklısı' denilmekte ve bu ırkın anavatanı olarak kabul edilmektedir (Aslım ve Sinmez 2017).

Bu retrospektif çalışmada, 2015-2021 yılları arasında Aksaray Üniversitesi Veteriner Fakültesi İç Hastalıkları kliniğine getirilen hasta hayvanların türü, kliniğe getirildikleri ay ve yıl, tanısı konulan hastalık ve hastalığın etkilediği sistem dağılımlarının ortaya konulması amaçlanmıştır. Bu çalışma ile elde edilecek sonuçların daha sonraki bilimsel ve klinik çalışmalarla birlikte klinik hizmetlerinin de iyileştirilmesine temel oluşturabileceği düşünülmüştür.

MATERYAL ve METOT

Araştırmanın materyalini, 23.06.2015-30.06.2021 yılları arasında Aksaray Üniversitesi Veteriner Fakültesi İç Hastalıkları Ana bilim Dalı kliniğine muayene için getirilen toplam 657 hastanın protokol defterindeki kayıtlı veriler kullanılmıştır. Hastaların kliniğe getirildikleri ay ve yıl ile hayvan türlerine göre dağılımları belirlenmiş olup hastalığın etkilediği sistemlere göre dağılımları tablo ve grafiklerle gösterilmiştir. Aksaray ili ve çevresinde daha çok yetiştirilen 'Aksaray Malaklısı' için de ayrıca tablo ve grafiklerle kullanılmıştır. Sığır, koyun ve keçi hastalıklarının tanımlanmasında, Gül'ün 'Geviş Getiren Hayvanların İç Hastalıkları' isimli kitabı Gül (2016) ve kedi, köpek hastalıklarının tanımlanmasında Aytuğ'un 'Köpek ve Kedilerin İç Hastalıkları' isimli kitabı temel alınarak Aytuğ (2019) düzenlemeye çalışılmıştır.

Genel muayene sonucu bazı hastalıkların birden fazla sistem üzerinde etkili olması nedeniyle, kliniğe getirilen hasta hayvan sayısı ile türe özgü toplam sistem hastalıklarının sayısal verilerinde farklılık görülebilmektedir. Elde edilen verilerin betimleyici istatistik analizleri yapılmıştır.

BULGULAR

Kliniğimize muayene için getirilen toplam 657 adet hayvanın muayenesi yapılmış olup en çok hastanın 2021 yılında (210, %31.96), en az hastanın ise 2015 yılında (15, %2.28) getirildiği tespit edilmiştir. Kliniğe bu zamana kadar en çok karnivor (389, %59.21), en az ise kanatlı hayvan (8, %1.22) getirilmiştir. Kliniğe getirilen hayvan türleri çoktan aza doğru sıralandığında; karnivor (%59.21), küçük ruminant (%26.94), büyük ruminant (%12.63) ve kanatlı hayvan (%1.22) şeklinde sıralanmaktadır (Tablo 1). Tüm türlerin aylara göre dağılımı Tablo 2' de gösterilmiştir.

Kliniğe getirilen hayvanların aylara göre dağılımı incelendiğinde en yoğun şubat ayında (108, %16.44), daha sonra mart ayında (96, %14.61) ve en az temmuz ayında (19, % 2.89) getirildiği görülmektedir. Ruminantların en fazla kış aylarında getirildiği, yaz aylarında ise bu değerlerin azaldığı; karnivorlarda ise bu durumun aksi görüldüğü kış aylarında daha az vaka getirilirken yaz aylarında ise gelen vaka sayılarının arttığı görülmektedir (Şekil 1). Ruminantların ve karnivorların aylara göre sistem hastalıkları dağılımları Şekil 2'de gösterilmiştir.

Kliniğimize getirilen tüm hayvanların türlere göre ayrımı yapılmış ve hastalıkların etki ettiği sistemler grafikler üzerinde gösterilmiştir. Genel muayene sonucu bazı hastalıkların birden fazla sistem üzerinde etkili olduğu ve bu nedenle toplam hayvan sayısı ile hastalıkların etkilediği sistem hastalıkları arasında farklılık görülebilmektedir.

Büyük ruminantlarda en fazla sindirim (54, %58.06) ikinci sırada ise solunum problemleri (32, %34.41) ile karşılaşılırken en az iz element ve vitamin hastalıkları (1, % 1.08) ile karşılaşılmıştır (Şekil 2). Üriner sistem, karaciğer ve metabolizma hastalıkları, dolaşım sistemi ve sinir sistemine lokalize olan hasta hayvan kliniğe getirilmemiştir. Getirilen küçük ruminantlarda ise en fazla sindirim sistemi (106, %57.30) hastalığı ile karşılaşılırken en az metabolizma hastalıkları (2, % 1.08) ile karşılaşılmıştır (Şekil 2). Üriner ve dolaşım

sistemi hastalığı için kliniğe hasta hayvan getirilmemiştir. Getirilen karnivorlarda en fazla sindirim sistemi (169, %56.90) hastalığı ile karşılaşılırken en az iz element ve vitamin eksikliği (1, %0.34) hastalığı ile karşılaşılmıştır (Şekil 2). Metabolizma hastalığı şikayetiyle kliniğimize hasta hayvan getirilmemiştir. Bu zamana kadar toplam 105 kedi-köpek türü aşı için kliniğimize getirilmiş ve genel muayene sonucu herhangi bir hastalık bulgusu saptanmayan hayvanların aşı uygulamaları yapılmıştır. Kanatlı hayvanlarda ise üç sistem ile ilgili hasta hayvan getirilmiştir. Bunlar çoktan aza doğru sırasıyla; sindirim sistemi (5, %62.50), solunum sistemi (2, %25), en az iz element ve vitamin eksikliğidir (1, %12.50). Diğer sistemlerin etkilendiği hasta hayvanlar kliniğe getirilmemiştir.

Klinikte en çok karşılaşılan hastalıklar büyük ruminantlarda enteritis, pnömoni, buzağı septisemisi; küçük ruminantlar da ise en çok enterotoksemi, çiçek, kuzu septisemisi; karnivorlar için en çok üst solunum yolu enfeksiyonları, distemper, parvoviral enteritis, mix enfeksiyonlar iken; kanatlı hayvanlar içinde en sık enterit vakalarıyla karşılaşılmıştır.

Aksaray iline özgü 'Aksaray Malaklısı' olarak adlandırılan çoban köpeği ise kliniğimize en çok muayene için getirilen köpek ırkı arasında yer almaktadır. Bu retrospektif çalışmada, 2015-2021 Haziran ayına kadar ki süreçte kliniğimize getirilen toplam karnivor sayısı 389 olup, bunun 254' ü köpektir (Şekil 3). Köpekleri de kendi içerisinde Aksaray Malaklısı ve diğerleri olarak ayırdığımızda ise ; Aksaray Malaklısı'nın toplam sayısı 85, diğer köpek ırklarının sayısı ise 169 olarak kayıt edilmiştir (Tablo 3). Kliniğimize Aksaray Malaklısı en çok 2017 yılında (23, %43.4), en az ise 2019 yılında (5, %14.70) getirilmiştir. Kliniğe getirilen toplam Aksaray Malaklısı'nın aylara göre vaka dağılımı incelendiğinde en yoğun şubat ayında (14, %16.47), ikinci sırada mart ayında (13, %15.29) ve en az hastanın ise temmuz ve ağustos ayında (4, %4.71) getirildiği, 2015'ten 2021 Haziran ayına kadarki süreçte eylül ayında (%0) ise Aksaray Malaklısı getirilmediği görülmektedir (Şekil 4).

Kliniğimize 2015 yılından 2021 yılının haziran ayına kadar getirilen toplam Aksaray Malaklı (saf ve melez) sayısı 85 olarak kaydedilmiş olup, bunlardan 71 tanesinin kliniğimize hasta olarak getirilmiştir, on dört tanesinin ise genel muayenesi sonucu sağlıklı olduğu tespit edilip sonrasında aşı yapılmıştır (Tablo 3). Hasta olarak getirilen 71 hayvanın muayenesi sonucu hastalığın tek bir sistemi etkilemediği ve etkilenen sistemsel hastalıklarının toplam sayısının 79 olduğu tespit edilmiştir. Hastalıkların etkilediği sistemler grafiklere aktarılarak vakaların dağılımları gösterilmeye çalışılmıştır (Şekil 5).

Getirilen Aksaray Malaklı hayvanlarında en fazla sindirim (49, %65.33), ikinci sırada ise solunum problemleri (10, %13.33) ile karşılaşırken en az üriner (2, %2.67), dolaşım (2, %2.67), sinir sistemi (2, %2.67) ve karaciğer hastalıkları (2, %2.67) ile karşılaşılmıştır. Metabolizma hastalıkları ile iz element ve vitamin eksikliği şikâyeti olan hasta, kliniğe getirilmemiştir. Klinikte en çok karşılaşılan hastalıklar: Parvoviral enterit, gastirit ve trakeobronşittir.

Tablo 1. Kliniğe getirilen hayvanların türlere ve yıllara göre dağılımı.
Table 1. Distribution of cases admitted to the clinic by species and years.

	Büyük Ruminant	Küçük Ruminant	Karnivor	Kanatlı Hayvan	Toplam (%)
2015	-	1	14	-	15 (2.28)
2016	3	1	41	-	45 (6.85)
2017	3	-	62	-	65 (9.89)
2018	5	5	13	-	23 (3.50)
2019	12	28	51	1	92 (14.00)
2020	35	80	91	1	207 (31.51)
2021	25	62	117	6	210 (31.96)
Toplam (%)	83 (12.63)	177 (26.94)	389 (59.21)	8 (1.22)	657 (100.00)

Tablo 2. Kliniğe gelen hayvan türlerinin aylara göre toplam sayısı ve oranları.
Table 2. The total number and rates of animal species admitted to the clinic by months.

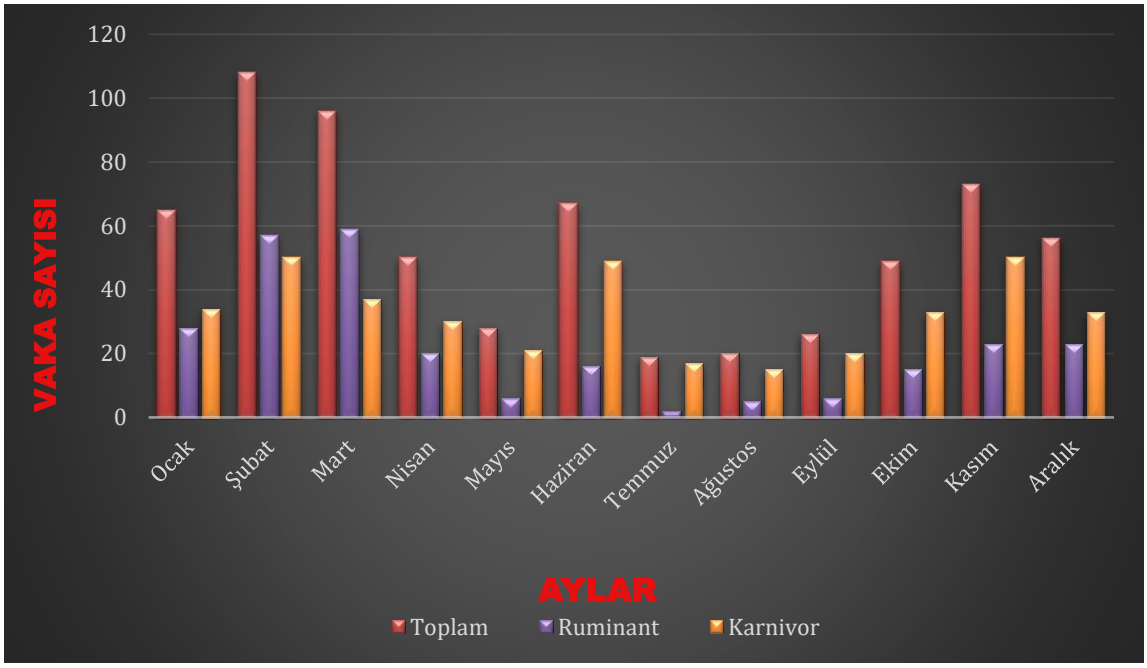
Aylar	Toplam	Ruminant %	Karnivor %	Kanatlı %
Ocak	65	**28 (43.08)**	**34 (52.31)**	**3 (4.62)**
Şubat	108	**57 (52.78)**	**50 (46.30)**	**1 (0.93)**
Mart	96	**59 (61.46)**	**37 (38.54)**	**0.00**
Nisan	50	**20 (40.00)**	**30 (60.00)**	**0.00**
Mayıs	28	**6 (21.43)**	**21 (75.00)**	**1 (3.57)**
Haziran	67	**16 (23.88)**	**49 (73.13)**	**2 (2.99)**
Temmuz	19	**2 (10.53)**	**17 (89.47)**	**0.00**
Ağustos	20	**5 (25.00)**	**15 (75.00)**	**0.00**
Eylül	26	**6 (23.08)**	**20 (76.92)**	**0.00**
Ekim	49	**15 (30.61)**	**33 (67.35)**	**1 (2.04)**
Kasım	73	**23 (31.51)**	**50 (68.49)**	**0.00**
Aralık	56	**23 (41.07)**	**33 (58.93)**	**0.00**

Tablo 3. Kliniğimize getirilen Aksaray Malaklısı ve diğer köpek ırklarının yıllara göre dağılımı.
Table 3. Distribution of Aksaray Malaklısı and other dog breeds admitted to our clinic by years

Yıllar	Malaklı * %	Diğer Irklar %	Toplam
2015	**7 (70)**	**3 (30)**	**10**
2016	**15 (42.86)**	**20 (57.14)**	**35**
2017	**23 (43.4)**	**30 (56.60)**	**53**
2018	**-**	**7 (%100)**	**7**
2019	**5 (14.70)**	**29 (85.30)**	**34**
2020	**16 (25)**	**48 (75)**	**64**
2021	**19 (37.25)**	**32 (62.75)**	**51**
Toplam	**85 (33.46)**	**169 (66.54)**	**254**

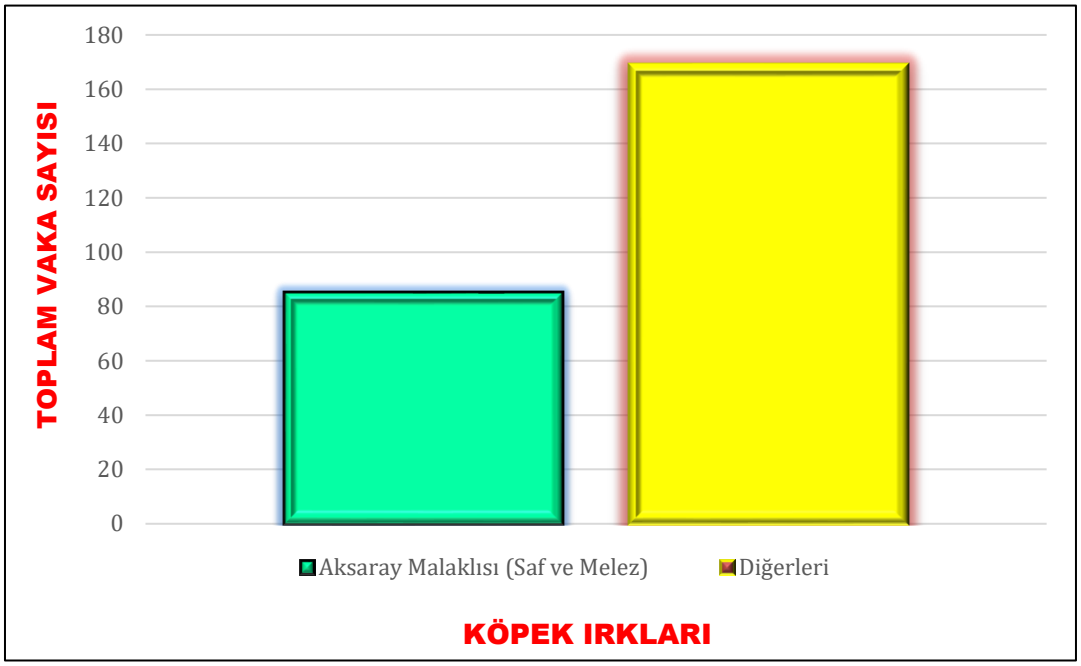
*Saf ve melez ırklar.

* Pure and mixed breeds.



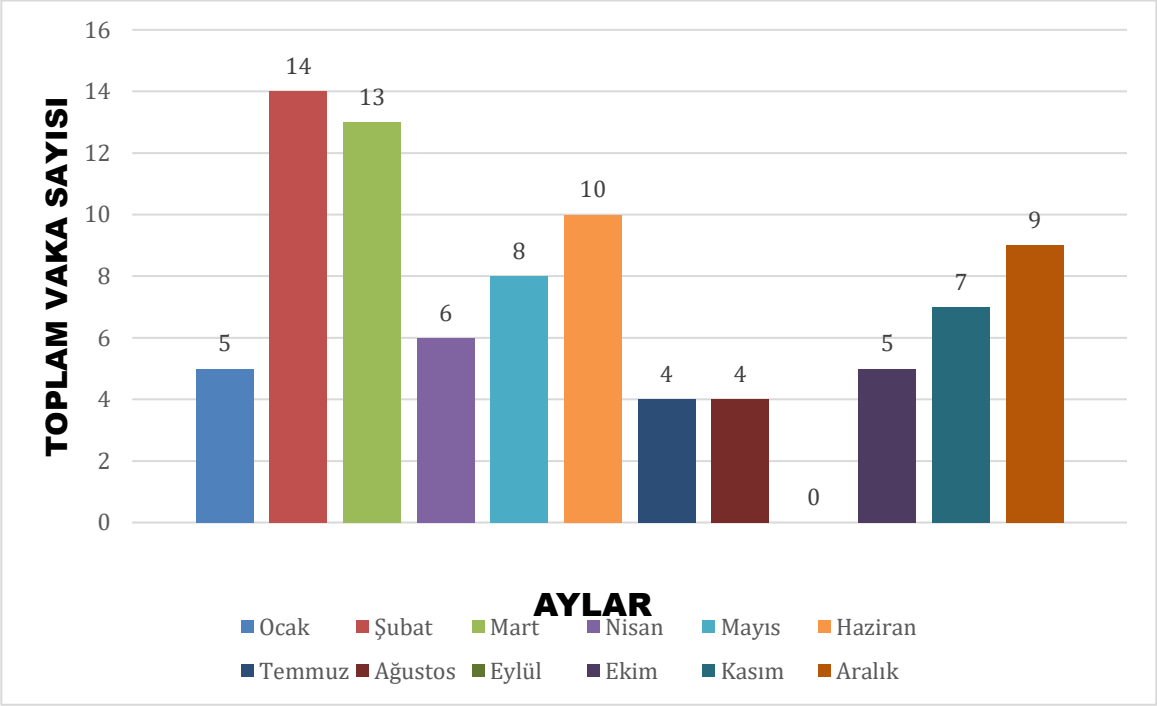
Şekil 1: Kliniğimize getirilen toplam vaka ile ruminant ve karnivor vakalarının aylara göre dağılımı.
Figure 1: Distribution of the total cases and carnivor cases admitted to our clinic by months.

Şekil 2: Kliniğe getirilen tüm vakaların etkilendiği sistem hastalıkları sınıflandırılmış ve bunların türler üzerinde dağılımı.
Figure 2: Distribution of disease of body system all cases admitted to the clinic by species.



Şekil 3: Aksaray Malaklısı (Saf ve Melez) ve diğer köpek ırklarının dağılımı.

Figure 3: Proportion of Aksaray Malaklısı (Pure and Crossbreed) and the other dog breeds.



Şekil 4: Kliniğe getirilen Aksaray Malaklısı'na ait tüm vakaların aylara göre dağılımı.

Figure 4: Distribution of all cases of Aksaray Malaklısı admitted to the clinic by months.

Şekil 5: Aksaray Malaklısı'na ait sistem hastalıklarının dağılımı.
Figure 5: Distribution of diseases located in the body system of Aksaray Malaklısı.

TARTIŞMA ve SONUÇ

Aksaray Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı kliniğimize 6 yılda toplam 657 hayvanın muayenesi yapılmıştır. Hastaların önemli bir kısmını karnivorlar oluşturmuştur. İkinci sırada ise küçük ruminant gelmektedir. Aksaray ilinde ruminant yetiştiriciliğinin büyük oranda merkezden uzak kırsal kesimlerde yapılması Sevimli (2020) ve ulaşım problemleri sebebiyle kliniğimizin daha az tercih edilmesi ile özel veteriner kliniklerin sayıca fazla olmasına istinaden daha çok tercih edilmesi, hasta sahiplerinin hayvan hastanesinde hasta bakıldığını bilmemesi gibi nedenlerden dolayı kliniğimize çok fazla ruminantın getirilemediği düşünülmektedir. Yıllara göre bakıldığında 2015 yılından 2021 yılına kadar olan sürede kliniğimize getirilen büyükbaş ve küçükbaş hayvanların sayılarındaki artışın akademik kadromuzun güçlenmesi ve fakültemizdeki hayvan hastanemizin 2020 ekim ayında ruhsat alması, hasta sahiplerinin hayvan hastanesi ile ilgili daha fazla bilinçlendirilmesi, yakın yıllarda daha fazla alet, ekipman ve cihaza sahip olarak daha detaylı muayene ve tedavi olanağının olmasıyla paralel olabileceği kanısına varılmıştır. Yıllara göre değerlendirme yapıldığında ise 2017-2018 yıllarındaki akademik personel yetersizliği, fakültenin yeni binaya geçişi sürecinde hasta kabul edilmesi, yasal süreçlerin tamamlanmasının zaman alması ve hayvan hastanesinde hasta bakıldığının bilinmemesi kaynaklı hasta sayılarında düşüş olduğu tahmin edilmektedir. Kliniğimize en çok getirilen hasta türünün kedi ve köpek olması ulaşımın diğer hayvan türlerine göre daha kolay olması ve pandemi sürecinde insanların daha çok pet hayvanı sahiplenmesi, özel veteriner kliniklerin daha çok ruminant ağırlıklı olması, Aksaray iline özgü Aksaray Malaklısı yetiştiriciliğinin artması nedeniyle yüksek olduğu düşünülmektedir.

Büyükbaş ve küçükbaş hayvan hastalıklarının daha çok sindirim sistemi hastalıkları ile ilişkili olduğu görülmektedir. Aksaray ilinde tarım arazilerinin fazla olmasına rağmen yetiştiricilerin tek tip besleme yapması ve yine yetiştiricilerin küçükbaş hayvanlarının aşılamalarına yeterince önem vermemesi, endo-ekto parazit uygulamalarının bilinçsizse yapması sindirim sistemi hastalıklarını arttıran nedenler arasında sıralanabilir. İstatiksel olarak baktığımız da ise kliniğimize getirilen sistem hastalıkları arasında daha çok sindirim sistemi hastalıkları ilk sırayı almaktadır. Can ve ark. (1989) Elazığ'da, Aslan ve Tiftik (1987) Konya'da yapmış olduğu çalışmalar sonucunda da aynı sebeplerden dolayı sindirim sistemi hastalıklarının en fazla olduğu görülmektedir. Barınma şartlarının iyileştirilmemesi, havalandırmaya yeterince önem verilmemesi, kapalı barınma yerlerindeki hayvan sayısının çok fazla olması, iklim şartları gibi nedenlerden dolayı da solunum problemlerindeki artış ikinci sırada yer almaktadır. Kedi ve köpekler içinde sistem hastalıklarında ilk sırayı sindirim sistemi, ikinci sırayı ise solunum sistemi hastalıklarının yer aldığı görülmektedir. Bunun nedeninin ise kedi ve köpeklere yönelik uygun besleme yapmadıkları, aşılama programlarının düzensiz veya hiç yapılmaması, yine iç ve dış parazit uygulamalarının kontrollü bir şekilde yapılmaması, sokaktaki hasta hayvanlarla temaslarının olması, bilinçsiz hayvan sahiplenmeleri gibi nedenlerin olduğu düşünülmektedir.

Şekil 1'e bakıldığında kliniklerimize en fazla sayıda hastanın şubat-mart aylarında getirildiği görülmektedir.

Sekin ve ark. (1996) Van ve çevresinde yapmış olduğu retrospektif çalışmada da hasta hayvanların en çok mart ayında getirildiği ve en fazla sindirim sisteminin daha sonra ise solunum sisteminin etkilendiği sonucuna varılmış olup ve farklı bölgelerde olunmasına rağmen çalışmamız ile paralellik gösterdiği görülmüştür. Bu aylarda özellikle doğum olaylarının artması ve septisemia neonatorum grubu hastalıkların sık görülmesi, hayvanların kış süresince depo edilmiş besin kaynaklarını tüketmeleri ve hastalıkların (enfeksiyöz ve paraziter) daha fazla yayılma imkânı bulması hastalık artışında etkili olmaktadır (Aslan ve Tiftik 1987, Can ve ark. 1989, Sekin ve ark. 1996). Temmuz-Ağustos aylarında ise en az sayıda hasta hayvanın muayene edilmesi, hayvanların uzak yaylım alanlarına götürülmesi, hasat zamanı olmasıyla beraber tarım işlerine daha fazla vakit ayrılması olabilir (Sekin ve ark 1996). Aynı zamanda bu aylarda eğitim-öğretime ara verilmesi de hasta sayısında bir düşüşe neden olabileceğini akla getirmektedir. Bu bahsedilen hususlar, Sekin ve ark. (2005) Diyarbakır ve Aksoy ve ark. (2018) Elazığ'da yapmış oldukları retrospektif çalışma sonuçları ile elde ettiğimiz veriler paralellik göstermektedir. Karnivorlar için ise bu durum tam tersi olmuştur. Kış aylarında vaka sayılarının düştüğü, yaz aylarında ise arttığı görülmüştür. Kediler için kızgınlık mevsiminin kış aylarında arttığı ve bunun sonucunda ise yavruların bahar ve yaz mevsimlerinde doğduğu, bu zaman dilimlerinde doğmuş olan yavruların ve annelerin hastalanma oranını arttırdıkları düşünülmektedir. Köpeklerde ise mevsimsel kızgınlığın çok etkili olmadığı, genel olarak bakıldığında ise kedi ve köpekler için sıcak havaların en önemli stres faktörü olduğu, iklim değişiklikleri nedeniyle havaların giderek daha da ısındığı ve hayvanların bu iklim değişikliklerine adaptasyonda zorlandıkları düşünülmektedir. Sıcaklık stresinin sindirim ve solunum hastalıklarına predispoze etken olabileceği düşünüldüğünde, yaz mevsimlerinde kedi ve köpeklerde vaka sayısını da olumsuz yönde arttırabileceği kanısına varılmaktadır.

Kliniğimize 6 yıllık süreç içinde muayene için getirilen 657 hasta hayvanın yıl, ay, hayvan türü ve hastalıkların etki ettiği sistem dağılımları incelendiğinde, Aksaray ili ve çevresinde yapılan hem ruminant hem de kedi ve köpek türleri için doğru besleme yapılmadığı, bakım-hijyen şartlarının iyi olmadığı, en önemli unsurlardan biri olan aşılama ve ekto-endo paraziter uygulamaların zamanında ve doğru uygulanmadığı, tarım alanlarının iyi kullanılamadığı sonucuna varılmıştır.

Bu retrospektif çalışma ile kliniğimize 6 yıllık süre içinde getirilen 657 hayvanda teşhis edilen hastalıkların toplu olarak değerlendirilmesi yapıldığında, hayvan türleri ve hastalıklarla ilgili yapılacak epidemiyoloji çalışmalar ile kliniğimize muayene için getirilecek olan hasta hayvanların teşhis ve tedavisine daha verimli klinik hizmeti verilmesinde yardımcı olabilecek bir çalışma olabileceği kanısına varılmıştır.

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

Yazarların Katkı Oranı: Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan etmişlerdir.

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RESEARCH ARTICLE

Potential effects of punicalagin on New Zealand White rabbits exposed to bisphenol A

Özlem YILDIZ GÜLAY[1], Gülşah CANDAN[2], Mehmet Şükrü GÜLAY[1]*

[1]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Physiology, 15030, Burdur, Turkey
[2]Bucak State Hospital, 15030, Burdur,Turkey

ABSTRACT

The possible effects of punicalagin on some oxidant-antioxidant enzymes and biochemical parameters in bisphenol A (BPA)-treated rabbits were investigated. Animals were randomly divided into 4 groups, each containing 6 rabbits: Control (C; corn oil and distilled water), BPA (BPA; 20 mg/kg BPA in corn oil and distilled water), the punicalagin (PUN; corn oil and 2 mg/kg punicalagin in distilled water), and BPA-punicalagin (B+P; 20 mg/kg BPA in corn oil and 2 mg/kg PUN in distilled water) groups. Daily treatments continued for 9 weeks and doses were adjusted according to body weights measured for each week. At the end of the study, hematological, biochemical, and oxidant-antioxidant parameters were measured from blood and tissue samples. The difference in the levels of plasma bilirubin, albumin, total plasma protein, Mg, P, Ca, Na, K, and levels of glutathione peroxidase in plasma, liver, and kidney were non-significant ($p>0.1$). However, oral BPA administration adversely affected serum cholesterol, LDL, HDL, amylase, lipase, CRP, and GGT concentrations. Likewise, malondialdehyde, catalase, and superoxide dismutase levels in the kidney and liver were also negatively altered by BPA ($p<0.05$). Significant improvements in these parameters were apparent in the B+P group. The data generated here showed that punicalagin possessed a beneficial impact on potentially reducing the possible toxic effects of BPA in rabbits.

Keywords: Antioxidants, Cholesterol, Oxidant-Antioxidant enzymes, Polyphenols, Toxication

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Bisfenol A'ya maruz kalan erkek Yeni Zelanda tavşanlarında punikalajinin potansiyel etkileri

ÖZ

Çalışmada, BPA verilen Yeni Zelanda tavşanlarında punikalajininbazı oksidan-antioksidan enzimler ile bazı biyokimyasal parametreler üzerine olası etkilerin incelendi. Bu amaçla 2 hafta boyunca laboratuvar koşullarına alıştırılan tavşanlar, her grupta 6 tavşan olacak şekilde rastgele 4 gruba ayrıldı: Kontrol (C; mısır yağı ve distile su), BPA (BPA; mısır yağı içerisinde 20 mg/kg BPA ve distile su), punikalajin (PUN; mısır yağı ve distile su içerisinde 2 mg/kg punikalajin), ve BPA-punikalajin grubu (B+P; mısır yağı içerisinde 20 mg/kg BPA ve distile su içerisinde 2 mg/kg PUN). Uygulamalar 9 hafta boyunca günlük olarak yapıldı ve haftada bir kez yapılan tartımlara göre dozlar ayarlandı. Çalışma sonunda alınan kan ve doku örneklerinden hematolojik, biyokimyasal ve oksidan-antioksidan parametrelerin ölçümleriyapıldı. Analizler neticesinde plazma bilirubin, albümin ve toplam plazma protein düzeyleri ile Mg, P, Ca, Na, K, seviyelerinde herhangi bir istatistiki farka rastlanmazken, farklı gruplardaki plazma, karaciğer ve böbrek glutatyon peroksidaz değerleri de önemsiz bulundu ($p>0,1$). Oral BPA uygulamaları serum kolesterol, LDL, HDL, amilaz, lipaz, CRP, GGT seviyeleri ile karaciğer ve böbrek dokusundaki malonildialdehit, katalaz ve süperoksit dismütaz seviyelerini olumsuz etkiledi ($p<0,05$). B+P grubunda ise bu parametrelerde önemli ölçüde iyileşme gözlendi. Elde edilen sonuçlar, BPA'nın erkek tavşanlarda yol açtığı olası toksik etkilerin punikalajin tedavisi ile önemli ölçüde düzeltilebileceğini gösterdi.

Anahtar kelimeler: Antioksidanlar, Kolesterol, Oksidan-Antioksidan enzimler, Polifenoller, Toksikasyon

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ORCID ID; *ÖYG: 0000-0001-6218-3568, GC: 0000-0002-4626-5739, MŞG: 0000-0002-4960-1152*

*Corresponding author e-mail: msgulay@mehmetakif.edu.tr

INTRODUCTION

Antioxidants can be helpful in preventing the formation of reactive oxygen species (ROS) and reducing the potential damage caused by ROS. Thus, it is a major defense mechanism that regulates the detoxification process (Şener and Yeğen 2009). Antioxidants are either produced by the organism itself (endogenous) or taken from the diet or other outer sources (exogenous). When exposed to high levels of oxidants, the body's natural antioxidant mechanism may be insufficient. This phenomenon is called oxidative stress and occurs due to the imbalance between pro-oxidants and antioxidants reserves (Karabulut and Gulay 2016a). Substances such as superoxide, hydroperoxyl, and hydroxyl radical are some of the sources of oxidative stressors for the body. Such substances can cause both lipid peroxidation in cell membranes and DNA damage (Aydemir et al. 2009). Therefore, live organisms require certain levels of antioxidants to prevent the detrimental effects of free radicals on tissues (Karabulut and Gulay 2016b).

Over the last two decades, there has been a considerable scientific effort to evaluate the toxic effect of bisphenol A (BPA). People are exposed to man-made industrial chemical products, particularly plastics in daily life. It is thought that the intensive use of BPA resulted in environmental pollution that could adversely affect human and animal health, and lead to an increase in oxidative stress in the body (Hamed and Abdel-Tawwab 2017). BPA is closely associated with oxidative stress in different tissues and organs, and further cause a number of metabolic problems (Ogo et al. 2017, Karabulut 2019, Karabulut and Gulay 2020). Today, reducing the use of BPA and other external oxidative stress sources is extensively discussed elsewhere (ECHA, 2021). Moreover, it is evaluated that endogenous antioxidants alone may not be sufficient against increased oxidative stress (Suhendi et al. 2018).

Pomegranate (Punica granatum L.) is a fruit that is frequently consumed in our country and the Mediterranean region. This fruit has a very high antioxidant capacity and punicalagin is thought to be responsible for the most important part of the antioxidant properties in pomegranate juice (Gil et al. 2000). Due to its high antioxidant capacity, it is possible to see studies suggesting anti-inflammatory (Jean Gilles et al. 2013, Lin et al. 1999), antimicrobial (Machado et al. 2002, Silva et al. 2015), and tissue-protective effects (Lin et al. 2001, Yildiz-Gulay and Gulay 2019) of punicalagin. Moreover, it is possible that punicalagin can be used to reduce or prevent the possible toxic effects of BPA (Yildiz-Gulay et al. 2020). Therefore, our study aimed to investigate the potential protective effect of this natural antioxidant in male New Zealand rabbits given oral BPA.

MATERIALS and METHODS

The experiment was supported partly by TUBITAK (116O027) and Mehmet Akif Ersoy University Scientific Research Projects Unit (BAP-0474YL-17) and approved by the Ethics Committee of the Mehmet Akif Ersoy University (25.11.2015/159). Individually housed male New Zealand White rabbits (8 to 10 months old, n=24) were kept at 22 ± 2 ºC, 50-55% humidity, and 10 hours of dark - 14 hours of light cycle in the Experimental Animals Unit of Mehmet Akif Ersoy University, Faculty of Veterinary Medicine. Water was freely available at all times. The rabbits were fed ad libitum with commercial rabbit feed (0,49% calcium, 0.46% phosphorous, 3,67% crude oil, 6,93% crude ash, 12.68% crude cellulose, 17,0% crude protein; Korkuteli Food Company, Antalya, Turkey). The initial weights of the rabbits prior to the experiment were 2,8-3,7 kg. Bodyweight and feed intake measurements were made weekly. BPA and punicalagin amounts were adjusted weekly according to the weekly body weight changes of each individual rabbit. After the adjustment period of 2 weeks, the rabbits were randomly divided into 4 groups (n=6 per treatment group). Rabbits in the control group (C) received daily corn oil (1 mL corn oil for 1 kg live weight) + daily distilled water (1 mL distilled water for 1 kg live weight). Rabbits in the bisphenol A group (BPA) were treated with daily BPA (20 mg/kg live weight) in corn oil (1 mL corn oil contained 20 mg BPA) and daily distilled water (1 mL distilled water for 1 kg live weight), whereas Punicalagin group (PUN) received daily punicalagin (2 mg/kg live weight) in distilled water (1 mL distilled water contained 2 mg punicalagin) and daily corn oil (1 mL corn oil for 1 kg live weight). The last group (Bisphenol A+Punicalagin group: B+P) was given the same amounts of daily BPA (20 mg/kg live weight) in corn oil and daily punicalagin (2 mg/kg live weight) in distilled water. The current oral doses for BPA (Karabulut and Gulay 2020) and punicalagin (Yildiz-Gulay and Gulay 2019) were selected according to the previous studies. Daily BPA and punicalagin doses were administered orally before the morning feedings between 08:00 and 09:30 hours.

At the end of the experiment (day 63), no food was given to the rabbits for 12 hours. K3 EDTA and gel-activated blood collection tubes were used for a blood sample collection from the ear arteries. Hematologic parameters (such as red blood cells-RBC, white blood cells-WBC, hematocrit, hemoglobin, etc.,) were measured by using an autoanalyzer (Abacus Junior Vet SN-100702) right after blood collection. The remaining blood samples were centrifuged (20 min, at 1457 x g). Following centrifugation, serum and plasma samples were stored at -80 °C. Serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, and creatinine values were analyzed on the same day from fresh serum by an

autoanalyzer (Gesan Chem 200). Other biochemical parameters (Mg, P, Ca, Na, K, bilirubin, albumin, total plasma protein levels, serum cholesterol, LDL, HDL, Amylase, Lipase, gamma-glutamyl transferase-GGT, and C-reactive protein-CRP) were analyzed from the thawed serum samples (Achitech C8000).

The lungs, liver, kidney, brain, and spleen of each rabbit were collected immediately after sacrifice and washed with PBS chilled to 5ºC, and weights of the organs were recorded. The right kidney and the caudal lobe of the liver were stored at -80 ºC until used for oxidant and antioxidant parameters. Before the analysis for oxidant-antioxidant parameters with ELISA kits (SinoGeneClon Biotech Co., Ltd., China), the tissue samples were prepared according to the manufacturer's instructions. Superoxide dismutase (SOD; Cat No: SG-0061Rb; detection range of 30-1000 pg/mL; intra- and inter-assay precision of <10%), glutathione peroxidase (GPx; Cat No: SG-0120Rb; detection range of 33-3000 pg/mL; intra- and inter-assay precision of <10%), catalase (CAT; Cat No: SG-50185; detection range of 1-36 pg/mL; intra- and inter-assay precision of <10%), and malondialdehyde (MDA; Cat No: SG-50252; detection range of 0.3-7 mmol/L; intra- and inter-assay precision of <10%), concentrations of the tissue and serum samples were read at 450 nm.

Statistical analysis of the data was evaluated using the SAS statistical program. ANOVA test was used to determine the statistical differences among the groups. The Tukey test was used for the comparisons of the individual treatment groups.

RESULTS

During the experiment, no apparent health problems were encountered in any of the study groups. Data on feed consumption, body weights, and wet organ weights of rabbits are in Table 1. There was no statistical difference in feed consumption, body weights, and the weights of various organs among the groups.

Considering the hematological parameters, no significant changes were observed in the total WBC and platelet counts, WBC percentages, hematocrit, and MCH values. On the other hand, RBC, hemoglobin, MCV, and MCHC were affected significantly (Table 2). While RBC count did not differ in the C, PUN, and B+P groups, there was a significant decrease in the BPA group compared to the C and PUN groups. (Table 2). The MCV was also affected by BPA treatment. Moreover, MCHC was the highest in the PUN group. Although MCHC decreased significantly in the BPA group, the decrease in MCHC was corrected by punicalagin in the B+P group (Table 2).

The serum biochemical parameters from the treatment groups were in Table 3. Results indicated that orally administered punicalagin and BPA applications did not have any effect on serum minerals, glucose, and triglyceride levels. Similarly, punicalagin and BPA applications did not have any effect on plasma bilirubin, total bilirubin, albumin, and total protein levels. However, serum lipase, CRP, and GGT levels were significantly affected by the treatments. BPA treatment caused a significant increase in serum amylase, lipase, CRP, and GGT levels while punicalagin treatments were able to lower these parameters up to control levels when given with BPA in the B+P group. In addition, the administration of BPA alone caused a significant increase in serum urea, creatinine, AST, and ALT levels when compared with the C and PUN groups, while this increase was inhibited in the B+P group. A similar trend was apparent in serum cholesterol levels. Serum total cholesterol and LDL levels were higher in the BPA treated group than in the C and PUN groups. Conversely, HDL levels were not affected by the BPA and punicalagin treatments. On the other hand, BPA alone caused a decrease in the serum HDL/LDL percentages (Table 3).

The effects of BPA and punicalagin treatments on some antioxidant parameters are in table 4. In general, GPx levels in plasma, kidney, and liver tissues were not statistically affected by the treatments studied. However, the SOD enzyme was decreased due to BPA treatments at tissue and plasma levels, and punicalagin administration reduced this decrease to control levels in the B+P group (Table 4). A similar trend was evident for the CAT enzyme. CAT levels were decreased significantly in the BPA group, but the decrease in CAT levels was statistically inhibited in the B+PUN group when punicalagin was administered with BPA. In addition, the use of BPA increased MDA levels in all tissues and blood compared to the C group. However, punicalagin applications in the B+P group were effective in reducing the MDA levels to the levels of the C group and reduced the negative effect of BPA.

Table 1. The effects of oral administration of BPA and Punikalagin on body weight, feed consumption and organ weights in male New Zealand rabbits.

	C	BPA	PUN	B+P	P=
Body Weight (kg)	3.65 ± 0.49	3,61 ± 0.36	3.45 ± 0.42	3.39 ± 0.45	0.625
Feed Intake (g)	196.3 ± 39.3	168.5 ± 27.0	180.5 ± 38.0	173.9 ± 28.5	0.129
Lung (g)	14.9 ± 2.09	15.0 ± 1.62	13.9 ± 1.64	15.8 ± 2.06	0.395
Liver (g)	105.5 ± 15.5	98.4 ± 17.5	106.2 ± 18.1	99.9 ± 17.2	0.675
Right Kidney (g)	10.5 ± 1.20	9.59 ± 1.19	9.22 ± 1.53	9.98 ± 1.14	0.392
Left Kidney (g)	10.3 ± 1.21	9.58 ± 1.34	9.07 ± 1.60	10.0 ± 1.00	0.429
Spleen(g)	1.02 ± 0.32	1.12 ± 0.39	1.18 ± 1.41	1.13 ± 0.39	0.859
Brain (g)	7.18 ± 0.80	6.61 ± 0.35	6.75 ± 0.35	6.78 ± 0.67	0.398
Heart (g)	9.62 ± 1.07	10.6 ± 1.20	9.81 ± 1.39	9.65 ± 0.98	0.435

C= Control; BPA= 20mg/kg/day BPA; PUN= 2 mg/kg/day punicalagin; B+P= 20mg/kg/day BPA and 2 mg/kg/day punicalagin.Values have given as mean ± standard deviation.

Table 2. The effect of oral administration of BPA and Punikalagin on some blood parameters in male New Zealand rabbits.

	C	BPA	PUN	B+P	P=
WBC ($x10^9$/l)	7.16 ± 1.42	6.41 ± 1.80	7.69 ± 2.17	7.81 ± 1.92	0.470
Lymphocyte($x10^9$/l)	1.97 ± 1.20	2.34 ± 1.14	2.36 ± 1.91	2.74 ± 2.01	0.875
Monocyte ($x10^9$/l)	0.24 ± 0.18	0.42 ± 0.30	0.46 ± 0.36	0.50 ± 0.28	0.456
Granulocyte($x10^9$/l)	4.95 ± 1.09	3.64 ± 1.20	4.88 ± 2.07	4.55 ± 2.28	0.554
Lymphocyte (%)	26.6 ± 16.1	36.1 ± 16.6	30.0 ± 17.0	34.1 ± 20.4	0.789
Monocyte (%)	3.2 ± 2.56	6.8 ± 4.98	5.9 ± 4.25	7.1 ± 4.11	0.350
Granulocyte (%)	70.2 ± 0.49	57.1 ± 18.2	64.1 ± 17.8	58.8 ± 33.6	0.623
RBC ($x10^{12}$/l)	6.88^a ± 0.91	6.17^b ± 0.16	7.00^a ± 0.90	6.64^{ab} ± 0.21	0.035
Hemoglobin (g/dl)	13.5^a ± 0.45	11.9^b ± 0.34	13.4^a ± 1.34	12.9^{ab} ± 1.28	0.042
Hematocrit (%)	46.1 ± 3.68	47.2 ± 1.98	43.6 ± 3.87	44.7 ± 4.57	0.246
MCV (fl)	67.1^a ± 2.16	76.5^b ± 4.39	62.6^a ± 5.10	67.3^a ± 5.79	0.001
MCH (pg)	19.6 ± 0.39	19.3 ± 0.74	19.2 ± 1.00	19.3 ± 1.66	0.892
MCHC (g/dl)	29.3^{ab} ± 1.09	25.3^c ± 1.20	30.7^a ± 1.06	28.7^b ± 0.56	0.001
Platelet ($x10^9$/l)	418 ± 60	430 ± 99	351 ± 80	395 ± 109	0.457

C= Control; BPA= 20mg/kg/day BPA; PUN= 2 mg/kg/day punicalagin; B+P= 20mg/kg/day BPA and 2 mg/kg/day punicalagin.WBC= White blood cells, RBC=Red blood cells, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration. Values have given as mean ± standard deviation.

Table 3. The effect of oral administration of BPA and Punikalagin on some biochemical parameters in male New Zealand rabbits.

	C			BPA			PUN			B+P			P=
Chol (mg/dl)	41.2^{ab}	±	1.30	46.8^{c}	±	1.92	39.8^{a}	±	3.11	43.6^{bc}	±	2.70	0.002
LDL (mg/dl)	16.22^{a}	±	1.57	20.44^{b}	±	2.42	14.36^{a}	±	1.90	16.94^{a}	±	2.02	0.002
HDL (mg/dl)	10.56	±	1.59	9.64	±	1.62	10.46	±	0.77	10.54	±	0.89	0.627
H/LDL (%)	65.10^{a}	±	10.89	47.16^{b}	±	8.77	72.84^{a}	±	9.04	62.21^{ab}	±	5.99	0.036
Trig (mg/dl)	95.8	±	18.5	97.2	±	14.4	100.1	±	12.8	99.8	±	15.6	0.953
Glucose(mg/dl)	119.3	±	13.1	113.2	±	8.81	120.6	±	12.4	118.7	±	6.80	0.634
Mg (mmol/l)	1.72	±	0.33	1.35	±	0.17	1.46	±	0.21	1.52	±	0.40	0.284
P (mmol/l)	5.48	±	1.20	5.16	±	1.56	4.65	±	0.65	5.0	±	0.91	0.770
Ca (mg/dl)	13.22	±	0.68	12.06	±	2.31	12.96	±	1.69	12.92	±	0.41	0.639
Na (mmol/l)	139.2	±	4.20	138.6	±	2.50	140.4	±	4.77	139	±	2.23	0.873
K (mmol/l)	4.24	±	0.26	4.15	±	0.13	3.97	±	0.28	4.13	±	0.24	0.379
AST (IU/l)	19.8^{a}	±	4.94	34.6^{b}	±	12.6	17.3^{a}	±	4.20	21.9^{a}	±	9.93	0.012
ALT (IU/l)	58.3^{a}	±	12.9	82.5^{b}	±	16.9	51.5^{a}	±	5.54	60.5^{a}	±	19.9	0.009
ALP (IU/l)	70.2^{a}	±	26.9	123.3^{b}	±	33.7	78.3^{a}	±	21.6	93.8^{ab}	±	17.4	0.019
Urea (mmol/l)	29.3^{a}	±	3.82	40.1^{b}	±	7.96	31.7^{a}	±	7.44	31.8^{a}	±	3.76	0.029
Creat (mg/dl)	0.67^{a}	±	0.15	0.82^{b}	±	0.10	0.62^{a}	±	0.07	0.69^{a}	±	0.08	0.024
Amylase (IU/l)	155.6^{a}	±	50.9	228.8^{b}	±	52.4	148.0^{a}	±	41.1	169.6^{ab}	±	29.1	0.043
Lipase (IU/l)	119.6^{a}	±	47.5	173.2^{b}	±	22.9	112.8^{a}	±	21.3	136.8^{ab}	±	26.3	0.004
CRP (md/dl)	0.98^{a}	±	0.59	4.75^{b}	±	2.38	0.73^{a}	±	0.46	2.74^{a}	±	1.58	0.002
GGT (IU/l)	6.40^{a}	±	2.96	15.40^{b}	±	2.30	6.40^{a}	±	1.14	9.80^{a}	±	3.49	0.001
Bil (µg/dl)	0.12	±	0.04	0.18	±	0.13	0.12	±	0.04	0.14	±	0.09	0.654
TotBil (µg/dl)	0.51	±	0.38	1.04	±	0.64	0.42	±	0.24	0.69	±	0.26	0.167
Albümin (g/dl)	3.16	±	0.45	2.84	±	0.70	3.10	±	0.40	3.16	±	0.26	0.608
TotP (g/dl)	6.06	±	0.32	5.38	±	1.22	6.16	±	0.43	5.76	±	0.42	0.323

C= Control; BPA= 20mg/kg/day BPA; PUN= 2 mg/kg/day punicalagin; B+P= 20mg/kg/day BPA and 2 mg/kg/day punicalagin. Chol=Cholesterol, LDL=Low density lipoprotein, HDL=High density liploprotein, H/LDL=HDL/LDL ratio (HDL/LDLx100), Trig=Triglyceride, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, Creat=Creatinine, CRP= C reactive protein, GGT=Gamma glutamyl transferase, Bil=Billirubin, TotBil=Total billirubin, TotP=Total plasma protein. Values have given as mean ± standard deviation.

Table 4. The effect of oral administration of BPA and Punicalagin on some oxidant-antioxidant enzymes in blood and various tissues in male New Zealand rabbits.

	C			BPA			PUN			B+P			P=
SOD (nmol/L)													
Plasma	501.2^{ab}	±	99.2	410.4^{c}	±	76.2	567.8^{a}	±	44.5	458.4^{bc}	±	45.1	0.012
Kidney	386.2^{a}	±	98.4	272.6^{b}	±	78.1	420.6^{a}	±	64.7	347.4^{ab}	±	63.8	0.045
Liver	400.1^{ab}	±	52.4	280.8^{c}	±	64.8	437.2^{a}	±	66.0	335.2^{bc}	±	69.8	0.011
MDA (nmol/L)													
Plasma	3.37^{ab}	±	0.65	4.88^{b}	±	1.76	2.51^{a}	±	0.77	3.68^{ab}	±	0.87	0.028
Kidney	2.10^{ab}	±	0.80	3.24^{b}	±	1.37	1.16^{a}	±	0.44	2.36^{ab}	±	1.11	0.033
Liver	2.72^{ab}	±	0.96	4.84^{c}	±	1.91	1.69^{a}	±	0.54	3.78^{bc}	±	0.82	0.004
CAT (pg/ml)													
Plasma	4.81^{ab}	±	1.43	2.59^{c}	±	0.48	5.81^{a}	±	2.04	3.32^{bc}	±	1.45	0.013
Kidney	17.32^{a}	±	4.82	11.97^{b}	±	1.73	19.21^{a}	±	1.81	16.16^{a}	±	2.81	0.012
Liver	16.80^{a}	±	4.74	11.71^{b}	±	2.05	19.1^{a}	±	2.30	14.98^{ab}	±	2.86	0.014
GPx(pg/ml)													
Plasma	2742	±	171	2784	±	236	2764	±	262	2879	±	437	0.887
Kidney	2347	±	687	1880	±	618	2133	±	268	2183	±	404	0.573
Liver	2144	±	633	1779	±	316	2176	±	299	1988	±	394	0.470

C= Control; BPA= 20mg/kg/day BPA; PUN= 2 mg/kg/day punicalagin; B+P= 20mg/kg/day BPA and 2 mg/kg/day punicalagin. SOD=superoxide dismutase; MDA=malondialdehyde; CAT= catalase; GPx= glutathione peroxidase.Values have given as mean ± standard deviation.

DISCUSSION

In the present study, BPA applications had no effect on body weight and feed consumption in male rabbits. There are different results regarding the effects of BPA on body weight in laboratory animals (Liu et al. 2013). Unlike our findings, Moghaddam et al. (2015) reported that BPA administered intraperitoneally up to 2mg/kg/day for 4 weeks caused an increase in body weight in adult mice. However, there are also findings that BPA given at a dose of 5000µg/kg/day in adult mice had no effect on body weight (Marmugi et al. 2012). Similarly, Avcı et al. (2016) stated that BPA administered at a dose of 25 mg/kg/day had no effect on body weight in rats. It has been reported that there may be a relationship between the increase in blood BPA levels and obesity in humans and rodents and that high BPA may cause an increase in body weight by accelerating fat storage due to oxidative stress (Moghaddam et al. 2015). However, Marmugi et al. (2014) reported that there was no change in body weight in 6-week-old mice exposed to BPA for 31 weeks. This difference in the literature can be explained by the duration of application of BPA, the age of the animals, and the different species.

Red blood cells are the most abundant cell type in the blood and have very important physiological functions. Many xenobiotics are carried by our blood and therefore it is possible for RBC to be affected by these xenobiotics (Stasiuk et al. 2009). In our study, oral BPA had a negative effect on the RBC, hemoglobin amount, and MCHC. Similarly, Macczak et al. (2016) reported that BPA could affect RBC levels and cause hemolysis. In a different study, BPA triggered the formation of free radicals in the RBC membrane (Suthar et al. 2014). It has been reported that BPA was cytotoxic to RBC due to its lipophilic property. It is suggested that BPA might bind to the iron in hemoglobin and cause the iron to dissociate from hemoglobin. Then, the free iron could pass into the RBC cytoplasm and cause lipid peroxidation. Free radical formation and peroxidation cause damage to RBCs, shortening their lifespan and causing early hemolysis (Macczak et al. 2016). The literature reveals that phenolic compounds such as BPA cause the formation of superoxide radicals and thus the oxidation of iron in the heme molecule, leading to methemoglobin conversion (Bukowska and Kowalska 2004). Consequently, RBCs that contain methemoglobin will rapidly be removed from the circulation.

Punicalagin applied at different doses in our study did not have a positive or negative effect on the blood parameters measured in this study. In a study in rats, punicalagin administered orally at a dose of 4800 mg/kg/day for 37 days had no effect on hematological parameters (Cerda et al. 2003). Moreover, pomegranate extract at doses of 60, 200 and 600 mg/kg/day given orally in rats did not have a negative effect on the number of RBCs and the amount of hemoglobin (Patel et al. 2008). Although oral administration of punicalagin to rabbits alone did not affect blood parameters in our study, when applied together with BPA, it improved the negative effect of BPA on RBC. The favorable effects of punicalagin, when administered with BPA, may be due to the superb antioxidant properties of this phytochemical. As previously shown, punicalagin was responsible for more than 50% of the high antioxidant properties found in pomegranate juice (Gil et al. 2000). Exogenous antioxidants, together with endogenous antioxidants, are a vital system that works both outside and inside the cell to prevent the deterioration of the oxidant-antioxidant balance. Thus, the number of free radicals was reduced and oxidative stress was suppressed, thereby increasing resistance to diseases (Berger 2005).

The results of the current study suggested that oral BPA negatively affected cholesterol levels in male rabbits. Studies examining the effects of BPA on cholesterol levels are limited. Parallel to our study, oral BPA at a dose of 2.72 mg/kg body weight in female mice increased the cholesterol levels (Miyawaki et al. 2007). On the other hand, Dodge et al. (1996) reported that BPA given orally for 4 days (0.1, 1, and 10 mg/kg body weight) did not affect total cholesterol levels in rats. Similarly, Seidlova-Wuttke et al. (2005) did not find any effect on total cholesterol levels of BPA given with feed at doses of 0.033 and 0.333 mg/kg body weight for 3 months. However, in these last two studies, either BPA duration (4 days) or doses (0.033 and 0.333 mg/kg body weight) were kept at limited levels, so BPA might not have had any effect on serum cholesterol.

In different studies examining the effects of punicalagin on cholesterol levels, punicalagin protected macrophage cells from lipid deposition and foam cell formation (Aviram et al. 2002, Kaplan et al. 2001, Rosenblat and Aviram 2011). Similarly, when given along with a cholesterol-lowering drug (statins), punicalagin reduced the required statin dose, strengthened the effect of statin, and inhibited cholesterol biosynthesis (Reiner, 2014; Rosenblat et al., 2013). In atherosclerotic mice supplemented with 6.25 mL/L pomegranate juice in drinking water, macrophage lipid peroxidation, cellular cholesterol accumulation, and the development of atherosclerosis was reduced (Kaplan et al. 2001). It is assumed that punicalagin binds to ApoB100 in close proximity to the LDL receptor binding site. Upon binding, punicalagin could change the conformation of the protein and increase the affinity of LDL for the LDL receptor. Thus, punicalagin might lower serum LDL levels, possibly by interacting with the lipid portion of LDL and protein and by accelerating LDL transport

to macrophages (Atrahimovich et al. 2016). Although the punicalagin dose we applied in our study did not have a statistical effect on cholesterol or LDL levels, it contributed positively by reducing the negative effects of BPA, in line with the information in the literature.

Serum Mg, P, Ca, Na, and K levels were within the normal physiological limits suggested for rabbits (Jones 1975). Furthermore, the oral BPA administration did not significantly alter the levels of these minerals in serum. Although there is no study on the Mg, P, Na, and K levels of BPA in serum, it is thought that it may affect the plasma Ca level due to its potential estrogenic nature (Suzuki et al. 2003). In an 8-day experiment on goldfish, BPA increased the serum Ca level for the first 4 days and decreased it for the next four days. In addition, the serum calcitonin level was also affected in the last 4-day period when the Ca level decreased in these fish (Suzuki et al. 2003). Studies have reported that this was due to the effect of BPA not indirectly on estrogen, but directly by changing osteoblastic and osteoclastic activity (Suzuki and Hattori 2003).

Serum ALT, AST, and ALP are important enzymes that provide information about liver functions. An increase in serum levels of these enzymes is observed when there is a degeneration of liver tissue. (Henderson and Moss 2005). In our study, the daily 20 mg/kg BPA increased the serum levels of these liver enzymes in male rabbits. In similar studies in rats, it was stated that BPA increased liver enzymes (Avcı et al. 2016, Korkmaz et al. 2010). The degree of elevation of these enzymes increases in direct proportion to the loss of hepatocellular function. There are studies that BPA increases oxidative stress and may cause toxicity in organs (Korkmaz et al. 2010, Daidoji et al. 2003). It is thought that this situation occurs because it causes an increase in reactive oxygen products and free radical levels, disrupts the balance of prooxidants and antioxidants, and increases the risk of causing damage to the liver tissue (Videla 2009).

The punicalagin treatment during our study had no effect on the levels of ALT, AST, and ALP compared to control rabbits. In a similar study conducted on humans, it was reported that ellagitannin-enriched polyphenols were given 1420 mg/day orally for 28 days caused no negative effect on serum ALT, AST, or ALP levels (Haber et al. 2007). Supplementing rats at a dose of 600 mg/kg/day for 90 days did not cause an increase in these enzymes (Patel et al. 2008). In our study, punicalagin reduced the ALT, AST, and ALP levels to normal levels in rabbits treated with BPA. Similar effects of punicalagin were observed in rats given carbon tetrachloride (Vora et al. 2015). There are indications that pomegranate juice protects liver tissue in rats with hepatitis induced by D-galactosamine (Amal et al. 2012). Punicalagin may have shown this effect as an antioxidant indirectly by protecting the antioxidant defense system, removing ROS from the system, or suppressing lipid peroxidation.

In the current study, the levels of different tissue markers such as amylase, lipase, CRP, and GGT increased by BPA applications. An increase in blood levels of these markers suggests the possible negative effects of BPA in organs such as the liver, kidney, and pancreas. In addition, in previous studies in humans, there was a link between the increase in BPA levels and inflammation indicators, and the increase in BPA levels caused an elevation in serum CRP levels (Tarantino et al. 2013). Thus, the increase in serum levels of CRP seen in the current study due to BPA treatments suggests that there was a general inflammation related to BPA. On the other hand, it has appeared that punicalagin applications may be beneficial in alleviating the negative effects of BPA in these organs. The fact that BPA administration caused a raise in all these parameters suggests that this chemical alters the oxidative stress in the main organs and causes tissue damage related to it, and the positive effect of punicalagin on the parameters examined implies that the antioxidant capacity of punicalagin can be effective in overcoming increased oxidative stress.

The intracellular enzymatic antioxidants such as SOD, CAT, and GPx are vital because of their role in scavenging hydrogen peroxide and superoxide radicals. Furthermore, SOD forms the first line of defense against oxygen species (Sen et al. 2010). In the current study, the concentrations of SOD and CAT in the tissues and plasma of the BPA-treated rabbits were lower than for the rabbits in the C group. This may typically be the result of increased lipid peroxidation as a result of oxidative stress.

In different studies, BPA caused oxidative stress in fish and rodents (Andersen 2004, Geetharathan and Peera 2018, Kabuto et al. 2004, Qiu et al. 2016). In carp fish, BPA augmented the lipid peroxidation and suppressed SOD, CAT, and GPx activities when added to the water tanks at the doses of 10 - 1000 μg/L (Qiu et al. 2016). The increase in oxidative stress in rat heart tissues treated with BPA was accompanied by a decrease in the activity of SOD and CAT (Geetharathan and Peera 2018). Furthermore, BPA exposure increased lipid peroxidative damage in neurons by suppressing the enzymatic antioxidant defense, thus, negatively affecting the functional and structural development of the central nervous system (Andersen 2004). The increase in tissue MDA levels due to BPA can also be observed due to the production of ROS more than normal levels (Yonar et al. 2014). Therefore, BPA could boost the formation of additional ROS by inducing free radical formation in rabbits and causing oxidative stress (Chitra et al. 2003).

The data from the current study shows that the increase in MDA activity and the decrease in SOD and CAT concentrations due to BPA approached the levels of the C group with the addition of punicalagin.

Our results with punicalagin on antioxidant parameters are similar to the results obtained with different flavinoids and pomegranate juice in previous studies. In an in vitro study on goat liver tissues, punicalagin showed significant antioxidant activity exposed to oxidative stress (Yaidikar 2001). Similarly, Sun et al. (2017) showed that the application of punicalagin increased the effect of SOD. It shows that these antioxidants obtained from various plants and fruits are powerful superoxide molecule scavengers (Coballase-Urrutia et al. 2011). Various studies have shown that the pharmacological effects of tannins and flavonoids have major antioxidant activities, which may result from their ability to scavenge superoxide, chelate metal ions, and exert synergistic effects with other antioxidant metabolites (Manna et al. 2006, Niki et al. 2005, Raja et al. 2007). Our results suggest that punicalagin can directly or indirectly reduce oxidative damage by preventing the increased formation of free radicals that may occur due to BPA.

CONCLUSIONS

The current study suggests that punicalagin applications have potential ameliorating effects in the blood and various tissues against the oxidative stress caused by BPA in male New Zealand White rabbits. The protective effects of punicalagin may be due to both an increase in the activity of the antioxidant defense system and the inhibition of lipid peroxidation. These findings imply that punicalagin may be effective in preventing the possible negative effects of BPA on various tissues.

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RESEARCH ARTICLE

Effect of Glukagon-Like Peptide-1 Analog Liraglutide On Neural Tube Development In Chick Embryo Model

Hava AÇAR KAYA[1], Mehmet Aydın AKALAN[1*], İsmail TÜRKMENOĞLU[1]

[1]*Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Anatomy, 03200, Afyonkarahisar, Turkey*

ABSTRACT

The aim of this study is to scan different doses of Liraglutid on the neural tube in a chick embryo model, which is similar to first month development in mammals. 100 eggs of 61 ± 5 gr, specific pathogen-free 0 day white fertilized chicken eggs were used. Incubation of 28 hours was maintained at a constant temperature of 37.5 ± 0.5°C, humidity in the range of 60 – 68%. They were divided into 4 groups of 25 eggs each. Liraglutid was administered subblastodermically with a Hamilton micro-injector in 3 different doses. The control group was injected with 0.9% sterile saline, and the experimental groups were injected with liraglutide at doses of 1.5, 7.5 and 15 µg/egg. After the injection, the windows were closed with sterile drape and left for incubation. At the end of 48 hours of incubation, all eggs were hatched and evaluated on the basis of Hamburger-Hamilton scale. In the study, neural tube patency, somite numbers, and Hamburger-Hamilton stages were examined, it was determined that the dose-related difference between the control and experimental groups was not statistically significant ($p>0.05$). It was determined that the difference between the high dose group and the other groups in terms of fore-aft lengths was statistically significant ($P<0.05$). As a result, there was no significant relationship between the doses of Liraglutide and neural tube patency and somite counts, but differences were found between fore-aft measurements. Further research is recommended for a clearer understanding of the effects of liraglutide on embryo development.

Keywords: chick embryo model, incretins, liraglutide, neural tube, obesity.

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Glukagon Benzeri Peptid-1 Analoğu Liraglutid'in Tavuk Embriyo Modelinde Nöral Tüp Gelişimi Üzerine Etkisi

ÖZ

Bu çalışmada tip 2 diyabet ve obezite tedavisinde kullanılan inkretin bazlı glukagon benzeri peptid-1 (GLP-1) reseptör agonistlerinden liraglutid'in tavuk embriyo modelinde nöral tüp üzerine etkilerinin incelenmesi amaçlandı. Çalışmada 100 adet 61 ± 5 gr ağırlıkta, spesifik patojen içermeyen fertil yumurta 37.5 ± 0.5°C'de ve % 60 - 68 nem ortamında 28 saat inkübe edildi. Bu süre sonunda pencereleme tekniği ile açılan yumurtalar 4 gruba (n=25) ayrılarak subblastodermik olarak uygulama yapıldı. Kontrol grubuna % 0.9 steril serum fizyolojik, deney gruplarına ise 1.5, 7.5 ve 15 µg/yumurta dozda liraglutid enjekte edildi. Enjeksiyon sonrası pencereler steril drape ile kapatılarak inkübasyona bırakıldı. 48 saat inkübasyon sonunda tüm yumurtalar açılıp Hamburger-Hamilton skalası temel alınarak değerlendirildi. Çalışmada nöral tüp açıklığı, somit sayıları, Hamburger Hamilton skalasına göre gelişim evreleri incelendiğinde kontrol grubu ile deney grupları arasındaki doza bağlı farkın istatistiksel olarak anlamlı olmadığı tespit edildi ($p>0.05$). Baş-kıç uzunlukları bakımından yüksek doz grubu ile diğer gruplar arasındaki farkın istatistiksel olarak anlamlı olduğu tespit edildi ($P<0.05$). Sonuç olarak Liraglutid'in dozları ile nöral tüp açıklığı ve somit sayıları arasında anlamlı bir ilişki saptanmazken baş-kıç ölçümleri arasında farklılıklar saptandı. Liraglutid'in embriyo gelişimi üzerine etkilerinin daha net anlaşılması için ileri araştırmalar önerilmektedir.

Anahtar Kelimeler: tavuk embriyo modeli, inkretinler, liraglutid, nöral tüp, obezite.

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ORCID ID; *HAK: 0000-0002-7572-7525, MAA: 0000-0001-9924-2920, İT: 0000-0002-0775-2622*

*Corresponding author e-mail: makalan@aku.edu.tr

GİRİŞ

Nöral tüp defektleri (NTD) konjenital malformasyonlar olup çevresel ve genetik etkenler yanında gebelikte ilaç ve kimyasallara maruziyet durumuyla da ilişkilidir. NTD kliniğinde parapleji, ciddi parezi ve idrar-gaita inkontinansı ön plandadır. Tedavisi genellikle cerrahi olup, tedavinin prognozuna ciddi bir katkısı yoktur. Koruyucu önlemler daha ön plana çıkmaktadır (Kürtül, 2018; Akosman, 2020).

Dünya'da canlı doğumlarda bebeklerin yaklaşık olarak % 2'sinde ciddi doğumsal anomaliler saptanmaktadır. Anomalilerin de yaklaşık olarak % 60'ını santral sinir sistemi anomalileri oluşturmaktadır (Baker, 1994). NTD yenidoğan döneminde en sık rastlanan doğumsal anomalilerden birisidir (Robert, 2000). Nöral tüp erken dönemde kapanmaz ise anensefali, ensefalosel, spina bifida okülta, meningomyelosel, meningosel, miyeloşizis, sirengomiyeli, dermal sinüs ve gerginspinalkord gelişmektedir (Rowland ve ark., 2006).

NTD coğrafi açıdan farklı yayılımlar gösterir. Avrupa ülkelerinde % 0.1 sıklıkta görülürken Türkiye'de bu oran, çeşitli çalışmalardan elde edilen verilere göre %0.3 - 0.5 arasında değişmektedir. Ülkemizde her yıl yaklaşık 5000 çocuk NTD ile doğmaktadır (Tunçbilek ve ark., 1999). İngiltere'nin kuzey ve batı bölgelerinde yüksek insidans (% 0,8) görülürken, güney ve doğusunda düşük insidans (% 0,2 - % 0,3) görülmektedir (Milunsky, 1986). Amerika ve Kanada'da insidans oldukça düşüktür (% 0,1). Bölgesel faktörlerin yanında siyah ırkta beyaz ırka göre daha az, kız bebeklerde ise erkek bebeklere göre daha sık NTD'ye rastlandığı da bildirilmiştir (Brocklehurst, 1976). NTD oluşmasında yetersiz beslenme, yüksek ateş, hipertansiyon, diabetes mellitus, obezite, kullanılan ilaçlar, çevresel kirleticiler, geçmişte NTD öyküsü olması, annenin 20 yaşından küçük 35 yaşından büyük olması, primipar olma, dört ve daha fazla çocuk doğurma, düşük sosyoekonomik düzeye sahip olma ve folik asit eksikliği gibi birçok faktör rol oynamaktadır (Daly ve ark., 1997; Akan, 2002; Yıldız ve Akbayrak, 2008). Gebelikte bazen zorunlu olarak kullanılması gereken ilaçlar bu tip konjenital malformasyonlara neden olmakta fakat özellikle günümüzde yeni geliştirilen ilaçların gebelik kategorisi ve ne tür malformasyonlara neden olabileceği yapılan çalışmaların azlığı nedeniyle tam olarak bilinmemektedir (Tureci ve ark., 2011; Song ve ark., 2012).

Liraglutid; kandaki glukoz seviyesini düşüren metabolik hormonlar ailesinin bir üyesi olan Glukagon Benzeri Peptit-1 analoğu olarak tanımlanmaktadır (Baggio ve Drucker, 2007). Gıdanın alımından sonra insülin salınımını stimüle ederken glukagon salınımını inhibe eder (Özaçmak ve Bayraktaroğlu, 2017). Deri altı uygulandığında normoglisemik bireylerde kan glukoz düzeylerini etkilediğinden tip 2 diyabet tedavisinde kullanılmaktadır (Lovshin ve Drucker, 2009; Vella ve ark., 2002). Liraglutid'in antidiyabetik etkisinin yanında antioksidatif ve antiapoptotik etkilerinin varlığı da rapor edilmiştir (Inoue ve ark., 2015; Briyal ve ark., 2014).

Böbrek (Zavattaro ve ark., 2015), kalp (Inoue ve ark., 2015), mide (Jelssing ve ark., 2012), bağırsak (Candeias ve ark., 2015), beyin (Briyal ve ark., 2014), pankreas (Kimura ve ark., 2015), ovaryum (Rasmussen ve ark., 2014), karaciğer (Gao ve ark., 2015) ve adipoz doku (Cantini ve ark., 2015) gibi çeşitli organlarda da çalışılan liraglutid'in, nöral tüp gelişimi üzerine etkisini gösteren spesifik tavuk embriyo çalışmasına rastlanmamıştır. Bu çalışmada liraglutid'in tavuk embriyolarında nöral tüp (orta hat) kapanmasına olan etkilerini makroskobik, mikroskobik ve histolojik olarak inceleyerek ortaya koymayı amaçladık.

MATERYAL ve METOT

Bu çalışma için Afyon Kocatepe Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan (AKÜ HADYEK) 24/02/2020 tarih ve 49533702/216 sayılı kararı ile etik kurul izni alındı. Çalışmanın deney aşaması Afyonkarahisar Sağlık Bilimleri Üniversitesi Tıp Fakültesi Anatomi Anabilim Dalı'nda makroskobik ve mikroskobik incelemeleri ise Afyon Kocatepe Üniversitesi Veteriner Fakültesi Anatomi Anabilim Dalı'nda gerçekleştirildi.

Çalışmada ağırlıkları 61±5 gr arasında; 100 adet, beyaz, fertil ve 0 günlük SPF (specific patogen free) tavuk yumurtaları kullanıldı. Yumurtalar Afyonkarahisar Sağlık Bilimleri Üniversitesi Tıp Fakültesi Anatomi Anabilim Dalı Laboratuvarındaki, standardizasyonu yapılmış otomatik inkübatörde 37.5±0.5°C sabit ortam sıcaklığı ve %60±5 bağıl nem oranında, her 2 saatte bir 45° açıyla otomatik olarak döndürülerek inkübe edildi. Liraglutide (Saxenda 6 mg/3 ml pre-filled-pen, novo nordisk), suda steril şartlarda çözülerek uygun solüsyonlar hazırlandı (Tablo 1).

Tablo 1. Deney grupları ve uygulanan ilaçların dozları

Gruplar	Kullanılan Etken Madde ve Dozları
Kontrol (n=25)	% 0.9 Serum Fizyolojik
Düşük Doz (n=25)	Liraglutid 30 mg/kg (1.5 µg/yumurta)
Orta Doz (n=25)	Liraglutid150 mg/kg (7.5 µg/yumurta)
Yüksek Doz (n=25)	Liraglutid 300 mg/kg (15 µg/yumurta)

Çalışmanın 24 ile 28. saatleri arasında döllü yumurtalara steril şartlar altında pencereler açılarak Hamilton mikroenjektörüyle yaklaşık 45° açı ile tüm gruplara 30 mikrolitre subblastodermik enjeksiyon yapıldı. Kontrol grubuna ise % 0.9 steril serum fizyolojik uygulandı. (Resim 3.4). Enjeksiyon sonrası tüm yumurtalardaki delikler steril drape ile kapatıldı ve kapatılan yumurta 180° çevrilerek kuluçka makinesine tekrar yerleştirildi. İnkübasyonun 44-48. saatinde SPF yumurtalar açıldı, elde edilen embriyoların gelişim evreleri Hamburger-Hamilton skalasına göre ışık mikroskobu altında değerlendirildi. Daha sonra nöral tüpün açık veya kapalı olması, somit sayıları, kranio-kaudal uzunlukları ve embriyolojik gelişim durumları belirlendi.

Verilerin istatiksel analizi SPSS 20 (Statistical Package for the Social Sciences) programı kullanılarak yapıldı. Ortalama değerleri arasındaki farklılıkların analizinde tek yönlü varyans analizi (ANOVA), gruplar arası farkın önemlilik kontrolü için ise "Duncan" testi uygulandı. $P<0.05$ olan değerler istatistiksel olarak anlamlı kabul edildi.

BULGULAR

Çalışmada düşük, orta ve yüksek olmak üzere farklı dozlarda liraglutid ile % 0.9 serum fizyolojik uygulanan toplam dört temel grup bulunmaktaydı.

İncelenen tüm parametrelerin gruplara göre dağılımı gösteren çizelge Şekil 1'de gösterilmiştir. Kontrol grubunda 21 embriyoda nöral tüpün kapalı olduğu (%84), 1 embriyoda nöral tüpün açık olduğu (%4), 1 embriyoda gelişim geriliği olduğu (%4), 2 embriyoda diskin oluşmadığı (%8) ve açma hatası olmadığı (%0) gözlendi (Tablo 2).

Düşük doz (1,5 µg/yumurta) grubunda 20 embriyoda nöral tüpün kapalı olduğu (%80), 1 embriyoda nöral tüpün açık olduğu (%4), 1 embriyoda gelişim geriliği olduğu (%4), 1 embriyoda diskin oluşmadığı (%4) ve 2 embriyoda ise açma hatası oluştuğu (%8) görüldü (Tablo 2).

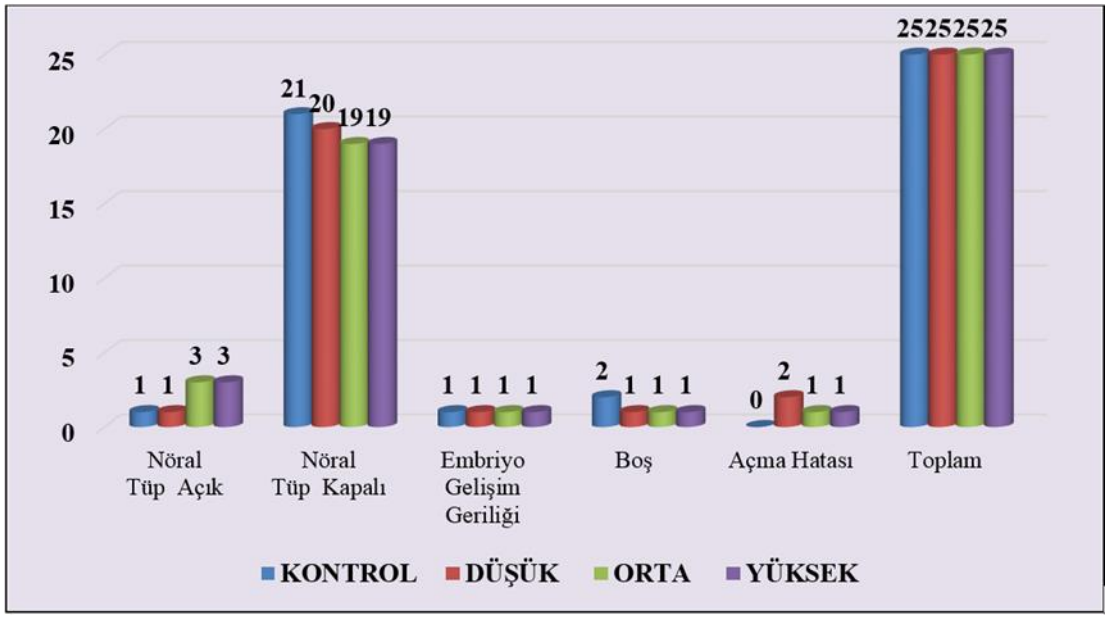
Orta doz (7,5 µg/yumurta) grubunda 19 embriyoda nöral tüpün kapalı olduğu (%76), 3 embriyoda nöral tüpün açık olduğu (%12), 1 embriyoda gelişim geriliği olduğu (%4), 1 embriyoda diskin oluşmadığı (%4) ve 1 embriyoda ise açma hatası oluştuğu (%4) görüldü(Tablo 2).

Yüksek doz (15 µg/yumurta başı) grubunda ise 19 embriyoda nöral tüpün kapalı olduğu (%76), 3 embriyoda nöral tüpün açık olduğu (%12), 1 embriyoda gelişim geriliği olduğu (%4), 1 embriyoda diskin oluşmadığı (%4) ve 1 embriyoda ise açma hatası oluştuğu (%4) görüldü (Tablo 2).

Tüm gruplarda embriyoların Hamburger Hamilton skalasına göre 11-12 evre aralığında olduğu saptandı.

Tablo 2. Gruplara göre embriyo gelişim geriliği ve nöral tüp açıklığı

Gruplar	**Embriyo Gelişim Geriliği**	**NT Açık**	**NT Kapalı**
Kontrol	1 (% 4)	1 (% 4)	21 (% 84)
Düşük Doz	1 (% 4)	1 (% 4)	20 (% 80)
Orta Doz	1 (% 4)	3 (% 12)	19 (% 76)
Yüksek Doz	1 (% 4)	3 (% 12)	19 (% 76)



Şekil 1: İncelenen tüm parametrelerin gruplar arasında dağılımı

Tablo 3 incelendiğinde somit sayısı bakımından kontrol grubu ile deney grupları arasındaki farkın istatistiksel olarak anlamlı olmadığı tespit edildi ($p>0.05$). Aynı tabloya göre baş-kıç uzunlukları ortalaması kontrol grubu (670.50 μm) ile düşük doz (660.37 μm) ve orta doz (660.20 μm) gruplarında benzer iken yüksek doz grubunda 631.62 μm'ye düştüğü ve yüksek doz grubu ile diğer gruplar arasındaki farkın istatistiksel olarak anlamlı olduğu tespit edildi ($P<0.05$).

Tablo 3. Gruplara göre somit sayısı ve baş-kıç ölçümlerinin dağılımı

Gruplar	Somit Sayısı MEANS ±SE	Baş-Kıç(μm) MEANS ±SE
Kontrol	16,27 ± 0,26	670,50 ± 6,43 [a]
Düşük Doz	15,95 ± 0,31	660,37 ± 4,58 [a]
Orta Doz	15,45 ± 0,29	660,20 ± 6,48 [a]
Yüksek Doz	15,50 ± 0,31	631,62 ± 6,65 [b]
P Değeri	0,159	0,000

TARTIŞMA ve SONUÇ

Merkezi sinir sistemi embriyolojik hayatta ilk fonksiyon göstermeye başlayan yapılardan biridir. NTD oluşumu multifaktöriyel olup, birçok genetik ve çevresel faktör rol alır. Oluşma ya da tekrarlama riski yalnızca anensefali, egzensefali, ensefalosel ve açık spina bifida gibi major anomalilerde gösterilmiştir. Bununla birlikte toplumdaki genel insidans, ailede daha önce NTD tanısı sayısına, hastaların yakınlık durumuna da bağlıdır. NTD, oluşmakta olan sinir dokusunun tüm ya da parçalı olarak kapanamaması nedeniyle ortaya çıkmaktadır. NTD modeli oluşturma yöntemleri arasında; memeli, kanatlı, amfibiler ve bilgisayar modellemeleri oluşturma seçenekleri sayılabilir. Bu örneklerin birbirlerine göre avantaj ve dezavantajları söz konusudur. Memeli modellemeleri olan rat ve fareler daha karmaşık ve uzun süreli çalışma gerektirirken, kanatlı ve amfibilerdeki modellemeler ise daha kolay ve pratik yöntemlerdir. Erken dönem tavuk embriyosu modellemesi (ilk 48 saat) memelilerde embriyonel gelişimin ilk ayına uyan ve kimyasalların embriyonel gelişim üzerine etkilerinin incelendiği uygun bir modelleme örneğidir (Tureci ve ark., 2011).

Yapılan çalışmalarda ortalama 8-10 yumurta kullanılırken deneyimizde 25 yumurta kullanılmıştır. Kanatlı yumurtalarının kullanıldığı çalışmalarda test maddesinin uygun çözücüde çözünmesi oldukça önemlidir. Bu nedenle en uygun steril bidistile su çözücü olarak kullanılmıştır. Literatür taramalarında da görüldüğü gibi deneyde önceki deneyler ile paralellik göstermektedir.

NTD merkezi sinir sisteminin en yaygın doğumsal anomalisi olup embriyonik gelişimin 3. ve 4. haftalarında nöral tüpün uygunsuz veya eksik kapanmasının bir sonucu olarak ortaya çıkar (Wang ve ark., 2019). Tavuk embriyo modelinin kullanıldığı nöral tüp gelişimi üzerine birçok çalışma vardır (Yerby, 2003; Ertekin ve ark., 2019; Dady ve Duband,

2017; Mete ve ark., 2016). Tavuk embriyosunun özellikle ilk 48 saatlik gelişimi memeli omurgasının embriyonik gelişiminin ilk ayına benzer. Bu nedenle NTD'ye neden olabileceği düşünülen maddeler için tavuk embriyolarının kullanımı oldukça uygundur (Atay ve ark., 2020; Emon ve ark., 2015). Yapılan çalışmalarda teratojenik maddelerin nöral tüpün açık veya kapalılık durumlarına etkileri incelenmiştir (Yıldız ve Akbayrak, 2008; Song ve ark., 2012).

Erken tavuk embriyo modelinde sitokalazinler, ionofor, papaverin, diazepam, verapamil, lokal anestetikler, etanol, metotreksat ve aminopterin gibi folat antagonistleri, fenitoin ve valproik asit gibi antiepileptikler, meloksikam benzeri non steroidal antienflamatuar ilaç etken maddesine sahip ağrı kesicilerin NTD'ye sebep olduğu rapor edilmiştir (Özer ve ark., 2012). Cetinkal (2010) çalışmasında yüksek doz meloksikamın erken tavuk embriyo modelinde NTD'yi arttırdığını saptamıştır. Vatansever ve ark. (2003) metotreksatın 48-72 saatlik inkübasyondan sonra embriyolarda nöral tüpün kapanmasında kusurlara neden olduğunu bildirmiştir. Lee ve ark. (1982) erken dönem tavuk embriyolarında 500 μg/ml kafein maddesinin NTD gelişme sıklığını belirgin şekilde arttırdığını belirtmişlerdir. Çalışmamızda liraglutid'in tavuk embriyolarında nöral tüp açıklık / kapalılık durumuna etkisi olmadığı belirlenmiştir.

Whitsel ve ark. (2002), epilepsi tedavisinde kullanılan valproik asitin teratojenik etkilerini incelemek için embriyoya 24. saatinde blastositin altına farklı dozlarda enjeksiyonunu yapmış, makroskobik olarak büyümede gerileme, göz dokusu ve iskelet sisteminde ait anomaliler tespit etmişlerdir. Ertekin ve ark. (2019) çalışmalarında non steroid antiinflamatuvar bir ilaç olan diklofenak sodyumun, tavuk embriyosunda NTD'ye neden olmasının yanında baş-kıç uzunluğu ve somit sayısını önemli ölçüde azalttığını ve nöron gelişimini etkilediğini göstermişlerdir. Bu çalışmada sadece yüksek doz liraglutid'in baş-kıç uzunluğunu düşürdüğü ve bu farkın istatistiksel olarak anlamlı olduğu ($P<0.05$) belirlenmiştir. Bu bulgu Liraglutid'in yüksek dozunun embriyoda gelişim geriliğine neden olabileceğini düşündürmüştür.

Sonuç olarak kandaki glikoz seviyesini düşüren, kilo vermek için kullanılan ve liraglutid etken maddesini içeren ilacın tavuk embriyolarında NTD'ye sebep olmadığı tespit edilmiştir. Ancak hamileliklere obezite ve tip 2 diyabetin eşlik ettiği durumlarda liraglutid kullanımı ile ilgili yapılacak çalışmalarda sinir dokusuna ait histokimya ve immünohistokimyasal boyamalarda daha nitelikli sonuçlar elde edilebileceği düşünülmektedir. Bununla birlikte yüksek doz verilen grupta baş-kıç uzunluğunda saptadığımız düşüşün, ileriki çalışmalarda liragulitid'in gelişim geriliğine olan etkilerinin daha iyi anlaşılmasına katkı sağlayacağı düşünülmektedir.

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RESEARCH ARTICLE

The Effects of Nutritional Periods on Oxidative Stress Levels in Lambs During Birth-Weaning Period

Duygu BUDAK[1]*, Besime DOĞAN DAŞ[2], Güzin ÖZKURT[3], Yavuz KAL[4]

[1]Aksaray University, Faculty of Veterinary Medicine, Zootechnical and Animal Nutrition Department, 68100, Aksaray, Turkey
[2]Harran University, Faculty of Veterinary Medicine, Zootechnical and Animal Nutrition Department, 63200, Şanlıurfa, Turkey
[3]Aksaray University, Faculty of Veterinary Medicine, Department of Veterinary Biochemistry, 68100, Aksaray, Turkey
[4]Bahri Dagdas International Agricultural Research Institute, 42070, Konya, Turkey

ABSTRACT

In this study were used 40 newborn singleton Akkaraman and Merino lambs with 4 trial groups consisting of selected 10 lambs homogeneous according to race and gender. Trial was carried out 100 days, covering 5 feeding periods with 21-day periods the first application is on the 14th day from the birth of lambs. The lambs were fed with lamb starter feed for the first two months, then with lamb grower feed *ad-libitum* and 250 g/day/head dry alfalfa hay. At the end of the study; it was determined that the lowest total antioxidant status (TAS) was in Akkaraman male lambs, the highest was in Merino's female, and it was found higher in Merino females than males ($P<0.05$). There was no difference between the groups in terms of total oxidant status (TOS) and native thiol (NTL), during the trial ($P>0.05$). Oxidative stress index (OSI) obtained from Akkaraman lambs was found to be higher than Merino, lower OSI was reached in female Merino's, and total thiol (TTL) were increased in Merino male lambs compared to Akkaraman ($P<0.05$). It was concluded that the Merino had better adaptation to feeding after weaning than the Akkaraman.

Keywords: Lamb, oxidative stress, ruminant, thiol/disulfide balance

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Doğum-Sütten Kesim Dönemi Aralığında Besiye Alınan Kuzularda Beslenme Dönemlerinin Oksidatif Stres Düzeyleri Üzerine Etkileri

ÖZ

Bu çalışmada, ırk ve cinsiyete göre homojen seçilmiş 10 kuzudan oluşan 4 deneme grubu ile 40 adet yeni doğan tekiz Akkaraman ve Merinos kuzular kullanılmıştır. Deneme, kuzuların doğumundan itibaren, ilk uygulama 14. günde olmak üzere 21 günlük periyotlar ile 5 besleme periyodunu kapsayan 100 gün sürede gerçekleştirilmiştir. Kuzular ilk iki ay kuzu başlangıç yemi, sonrasında deneme süresince kuzu büyütme yemi ile *ad-libitum* ve 250g/gün/baş kuru yonca otu ile beslenmişlerdir. Araştırmanın sonunda; en düşük total antioksidan seviye (TAS)'nin Akkaraman erkek kuzularında, en yüksek değerinin ise Merinos ırkı dişi kuzularda olduğu, Merinos ırkı dişilerde de erkeklerine göre daha yüksek bulunduğu tespit edilmiştir ($P<0.05$). Deneme süresince total oksidan seviye (TOS) ve nativ tiyol (NTL) değerleri bakımından gruplar arasında farklılık olmadığı gözlenmiştir ($P>0.05$). Akkaraman ırkı erkek ve dişi kuzulardan elde edilen oksidatif stres indeksi (OSI), Merinos ırkı erkek ve dişi kuzulara ait OSI'nden yüksek bulunmuş, dişi Merinoslarda daha düşük OSI'ne ulaşıldığı ($P<0.05$), total tiyol (TTL) düzeylerinin Merinos ırkı erkek kuzularda Akkaraman ırkı erkek kuzulara göre arttığı tespit edilmiştir ($P<0.05$). Merinos ırkının Akkaraman ırkına göre sütten kesim ardından yeme adaptasyonunun daha iyi olduğu sonucuna varılmıştır.

Anahtar kelimeler: Kuzu, oksidatif stres, ruminant, tiyol/disülfit dengesi

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ORCID ID; *DB: 0000-0001-9327-3830, BDD: 0000-0003-2163-2632, GÖ; 0000-0003-2732-9490, YK; 0000-0002-3672-0048.*
*Corresponding author e-mail: budakduygu@aksaray.edu.tr

INTRODUCTION

The health, growth rate and performance of the lambs from the moment they born, are closely related to their nutritional status and are an important criterion for increasing the quality and productivity, which is the main purpose of animal production. The growth rate and yield power of lambs are directly proportional to the level of feed efficiency. Poor care and feeding conditions are the obvious cause of stress factors (Serin 2015, Altınçekiç 2016).

Oxidative stress can be defined as the deterioration of molecular and cellular functions as a result of the loss of the balance between the body's antioxidant defense and the production of free radicals that cause peroxidation of the lipid layer of the cells (Yokuş and Çakır 2002, Celi 2011). Hydroxyl, superoxide, nitric oxide, lipid peroxide, reactive oxygen and nitrogen species (ROS and RNS) from free radicals produced by normal cell metabolisms as a result of oxidative stress cause tissue damage by lipid peroxidation (Mercan 2004, Tabakoğlu and Durgut 2013, Karabulut and Gülay 2016). The resulting oxidative damage; it is seen as the main cause and indicator of diseases that occur with the deterioration of tissue functions such as cancer, cardiovascular diseases, immune system diseases, degenerative diseases (Pratic`o 2005). Reactive oxygen species (ROS) are primary molecules that cause oxidative damage when they rise above physiological levels. Increased production of free radicals leads to damage to cell membrane lipids and weakening of cellular protein functions (Valko et al. 2004, Devasagayam et al. 2014). In the antioxidant defense system of the organism against free radicals, primarily enzymatic or non-enzymatic antioxidant mechanisms in the cells come into play. The damage caused by radicals is prevented by the enzyme systems of superoxide dismutase, catalase and glutathione S-transferase in the body, as well as important biological thiols such as glutathione, cysteine, homocysteine, N-acetylcysteine. Thiols, also known as mercaptans, are organic chemical compounds containing hydrogen and sulfur atoms and sulfhydryl (-SH) groups attached to the carbon atom, which show antioxidant properties in preventing the formation of any oxidative stress state (Sen and Packer 2000, Erel and Neşelioğlu 2014).

The plasma thiol level consists mostly of albumin and protein thiols, with small amounts of low molecular weight cysteine, homocysteine, glutathione, N-acetylcysteine and ɣ-glutamylcysteine biothiols (Turell et al. 2013). Thiols participate in oxidation reactions via oxidants and form covalent disulfide bonds. These are defined as S-S bonds formed between the sulfhydryl groups of two cysteine amino acids in proteins (Huber and Parzefall 2007, Cremers and Jakob 2013). Under oxidative stress conditions, lead to the formation of reversible disulfides between oxidative residues of cysteine, low molecular thiol stacks and protein thiol groups. The disulfide bonds formed can be reduced back to thiol groups. Thus, dynamic thiol/disulfide balance can be achieved. Dynamic thiol/disulfide balance has a critical role in many cellular activities such as, antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and cell growth. Today, it is a very topical indicator in the field of medicine and is associated with many diseases (Erel and Neşelioğlu 2014). The thiol groups of sulfur-containing amino acids (cysteine, methionine..) in proteins are the primary target point of ROS. The oxidation of thiol groups in the environment with ROS to reversible disulfide bonds is the earliest manifestation of radical-mediated protein oxidation. Biological importance of thiols and disulfides; can be explained by the stabilization of the structures of proteins, the regulation of the functions of proteins, the regulation of enzyme functions, their roles in receptors, transporters, Na-K channel and transcription (Ateş et al. 2015).

The primary immune response to pathogens, stable oxidant/antioxidant balance and vitality ability of lambs born and growing with insufficient immune system capacity depend on the nutritional level during growth and development stages from birth. On the other hand, it is expected that the transition from intrauterine to extrauterine environment with birth, that is, the increase in the oxygen requirement during the adaptation at birth and then the feeding conditions that require high energy for many body functions, increase the level of oxidative stress. Disruption of dynamic thiol-disulfide balance plays a role in the development of many diseases. By measuring the dynamic thiol-disulfide balance, can be obtained a lot of information related to the animal's health and nutritional status.

In this study, it was aimed to determine the effects of feeding on the oxidative stress level of female and male lambs of Anatolian Merino and Akkaraman offspring, which are the most preferred growing in our country (TUIK 2020), by using the thiol/disulfide balance measurement method at the developmental stages from birth.

MATERIAL and METHOD

This study was carried out with the decision of the local ethics committee of animal experiments in Bahri Dağdaş International Agricultural Research Institute with the code 293286/90.

In the study, a total of 40 newborn singleton heads; Akkaraman (20 head; 10 male and 10 female) and Anatolian Merino (20 head; 10 male and 10 female) lambs were used. Healthy and homogeneous animals

with the same birth weight were selected by taking birth weights and necessary records (single birth type, ear number, gender, breed) from birth. The experiment was continued for a total of 100 days from the birth of the lambs, covering the 3-week period in 21-day periods after the first two weeks (0-14. days) when they were fed only with milk. The experiment was continued for a total of 100 days from the birth of the lambs, covering the first two weeks (0-14. days) when they were fed only with milk, and the 4 week period in 21-day periods. In the study, 4 experimental groups were formed, each of which consisted of 10 lambs and the lambs with the same weight average of each group were randomly distributed. Lambs housed in group compartment, each group is arranged so that there are 10 animals in each group; Akkaraman female lambs (AFL) were kept in the 1st group compartment, Akkaraman male lambs (AML) were kept in the 2nd group compartment, Anatolian Merino female lambs (MFL) were kept in the 3rd group compartment, and Anatolian Merino male lambs (MML) were kept in the 4th group compartment. Fattening lambs, according to the feeding period; the period when they are fed only with milk from birth (0-14 days), the period of adaptation to solid feed in addition to milk feeding (15-36 days), the period when they are fed with milk and solid feed (36-57 days), the weaning period (58-78 days) and only solid feeding period (79-100 days) by considering 5 periods (5 feeding periods).

Group feeding was applied in the study, and lambs were fed with only milk until the 14th day from birth, and with milk from the 15th day to the 36th day, as well as with ad-libitum lamb starter feed (including 2.80 Mcal/kg dry matter, 17% crude protein). At the same time, while starting solid feed, dry alfalfa hay was started to be given to the lambs as roughage. After lambs had been get used to consuming 250 g of dried alfalfa hay per animal daily, it was kept constant by giving 2.500 g daily to each group throughout the trial. Starting from the 58th day of the trial, it was pass to lamb grower feed (including 2.75 Mcal/kg dry matter, 16% crude protein). By adding daily increasing amounts to the reduced lamb starter feed, the transition was ensured with practice. Up to the 78th day, they were fed with milk as well as ad-libitum lamb grower feed. Thus, the lambs which were provided with sufficient solid feed consumption for weaning and reached 4 times their birth weight, were weaned on the 78th day and after the 79th day fed only with feed until the 100th day which corresponds to the end of the trial. Fresh and clean drinking water was always available in front of the lambs.

AOAC 1984 was used as the method of determining the amounts of dry matter (DM) crude protein (CP), crude cellulose (CC), ether extract (EE) and crude ash (CA); Van Soest, 1994 procedure was followed for determining the amounts neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Metabolic energy (ME) (Anonymous 2016), organic matter (OM), non-nitrogen extract (NNE), cellulose (CL) and hemicellulose (HCL) values were derived from the analysis results on feed materials through calculation (Table 1).

During the trial, the live weights of the lambs were started to be determined together with their birth weights and they were recorded on the first 15th day, then once every three weeks at the same time (08:00) in the morning. Lambs were hungry for 12 hours before weighing in order to prevent variation (hungry-full) in live weight.

Table 1. The chemical composition of feed materials used in the study

Nutrients	Lamb started feed	Lamb grower feed	Alfalfa hay
ME, Mcal/kg DM	2.80	2.75	1.38
DM,%	90.44	88.73	93.44
*OM,%	82.74	81.08	82.86
CP,%	17.00	16.00	9.57
CC,%	7.14	8.10	34.96
EE,%	3.70	2.89	0.92
CA,%	7.70	7.65	10.58
*NNE,%	53.94	54.55	37.41
NDF,%	-	29.24	50.09
ADF,%	-	9.70	42.43
ADL,%	-	0.90	9.11
*CL,%	-	8.8	33.32
*HCL,%	-	19.54	7.66

*OM=DM-CA, NNE=DM-(CP+CA+EE+CC), CL=ADF-ADL, HCL=NDF-ADF

Blood samples were taken from animals 4 hours after feeding at the beginning of each feeding period (5 periods). These blood samples were collected in flat gel tubes (Becton Dickinson and Company, New Jersey, USA), all samples were centrifuged at 3000 rpm for 10 minutes and stored at -80°C until analysis. Then, enzymatic and non-enzymatic measurements of all antioxidant and oxidant molecules were made in these blood samples with native thiol (--SH), total thiol (--SH+-- S--S--), Total antioxidant status (Total Antioxidant Level -TAS), Total oxidant status (Total Oxidant Level -TOS) kits (RelAssay Diagnostic, Turkey) (Erel and Neşelioğlu 2014). The disulfide level was calculated with the formula (serum total thiol - serum native thiol)/2. All results are reported as micromoles per liter (μmol/L), TAS millimoles (mmol/L) (George and Hero 1979).

For total thiol measurement, 10 μl of reagent 1 (R1) (10μl of R1' is used for free thiol measurement) and 10 μl of sample were mixed. Afterwards, R2 and R3 were added and the first absorbance (A1) reading was made spectrophotometrically at 415 nm wavelength (Schimadzu UV-1201 spectrofotometer, Kyoto, Japan). The second absorbance (A2) reading was taken at the same wavelength at the 10th minute when the reaction peaked, and the measurement was completed by obtaining the A2-A1 absorbance difference. It was used 14.100 mol/L-1 cm-1 which is the molar extinction coefficient of 5-thiol-2-nitrobenzoic acid (TNB) for the calculation of total and free thiol levels.

Antioxidants in the sample convert the dark blue-green ABTS (3-ethyl-benzothiazoline 6 sulfonate) radical solution to the colorless ABTS form. The change in absorbance at 660 nm is related to the total amount of antioxidants. The kit has been calibrated with a stable antioxidant standard called Trolox Equivalent, similar to vitamin E. Oxidants in the sample oxidize the ferrous ion-clamp integrated with the ferric ion. The oxidation reaction is prolonged by the amplifying molecules present in the reaction medium. Ferric ion forms a colored compound with chromogen in acidic medium.

The total amount of oxidant molecules in the sample was determined in relation to the darkness of the color measured in the spectrophotometer. The kit was calibrated with hydrogen peroxide, the results were given as micromoles of hydrogen peroxide per liter (μmol H2O2 Equi v./L) (Erel and Neşelioğlu 2014). By taking the percentage of the ratio of TOS level to TAS level; Oxidative Stress Index (OSI) was calculated according to TOS (μmol H2O2 equiv./L) / TAS (mmol Trolox eqiv./L) formula (Erel 2005).

Analysis of the data; The data of variance was used to determine the differences between the groups, and Duncan test was used to control the significance of the differences. T-Test was used to analysis the significance of the difference between the means of two independent groups for each parameter studied (Duncan 1955, Düzgüneş et al. 1983). Variance analyzes were performed using Duncan Tests SPSS statistical package programs.

RESULTS

The average live weights (kg) of the lambs obtained during the feeding periods (with 21-day periods) from their birth are given in Table 2. Accordingly, in terms of live weight characteristics was observed no statistically significant difference between the group averages in the 1st, 2nd and 5th periods during the fattening period from the birth of the lambs ($P>0.05$). However, in the 3rd and 4th periods, the body weight differences of AML and MML were found to be significant ($P<0.05$) and the weights of MML were lower.

Table 2. Average live weight (kg) of the lambs obtained during the feeding periods (in 21-day periods) from their birth

Feeding periods	Groups				SEM	P
	AFL	AML	MFL	MML		
1	5.335	5.241	5.335	5.320	0.092	
2	14.830	15.718	14.517	14.415	0.267	
3	22.525	24.336[a]	22.685	20.745[b]	0.457	<0.05*
4	29.790	31.891[a]	29.470	28.509[b]	0.585	<0.05*
5	35.450	37.291	34.140	37.291	0.607	

SEM: Standart error of the mean

*Difference among the averages shown with different letters on the same line are significant

Intra-group TAS (mmol/L), TOS (μmol./L) and OSI values obtained during the feeding periods of the lambs from the beginning of the study are given in Table 3. Accordingly, no statistical difference was observed between the 1st, 2nd, 3rd and 4th feeding periods of AFL in terms of TAS (P>0.05). However, TAS values obtained in the 1st and 4th periods were found to be lower compared to the 5th period (P<0.05). In AML, while no statistical difference was observed in the 1st, 2nd, 3rd and 5th periods (P>0.05), the decrease detected in the 3rd period compared to the 1st and 5th periods was significant (P<0.05). Intra-group mean of TAS values of MFL did not change (P>0.05). TAS values obtained from MML during feeding periods were found to be lower in the 1st period compared to the 3rd and 5th periods (P≤0.001). Similarly, it was determined that there was a significant decrease in the 1st period compared to the other periods (P<0.05) and in the 4th period compared to the 1st, 3rd and 5th periods (P<0.05). As in AFL, it was observed that TOS values were highest in the 1st and 2nd periods and the lowest in the 5th period (P<0.05) in MML, but the difference in the 3rd and 4th periods was insignificant when compared to the other periods (P>0.05). In AML and MFL, the decrease observed in TOS values in the 5th periods compared to the 3rd and 4th periods was found to be statistically significant (P<0.05), a significant decrease was detected in the 5th period compared to the 1st and 2nd periods (P≤0.001). OSI was found to be higher in the 1st period compared to the 3rd and 4th periods in AFL (P<0.05), and it showed a significant decrease in the 5th period compared to the 1st and 2nd periods (P≤0.001). It was determined that the OSI value, which was highest in the 2nd period from AML, was the lowest in the 5th period (P≤0.001). This decrease observed in the 5th period was also detected in merino female and male lambs. The decrease in MFL in the 5th period compared to the 1st (P≤0.001), 2nd and 3rd (P<0.05) periods was found to be statistically significant. OSI values obtained from MML were significantly higher in the 1st period than in the 2nd period (P<0.05) and the 3rd 4th and 5th periods (P≤0.001). It was determined that this value was quite low in the 5th period compared to the 1st and 2nd periods (P≤0.001).

Intra-group TTL (μmol/L), NTL (μmol/L) and disulfide values obtained during the feeding periods of the lambs from the beginning of the study are given in Table 4. A decrease was observed in the TTL level obtained from AFL in the 5th period when the lambs were fed only with solid feed, compared to the other periods, and in AML compared to the 1st and 2nd periods (P<0.05). TTL values obtained in the 2nd and 3rd periods of MFL were found to be higher compared to the 5th period (P<0.05), and it was determined that the TTL values obtained in the 1st and 5th periods in MML compared to the other periods decreased significantly (P≤0.001).

Table 3. Intra-group TAS (mmol/L), TOS (μmol./L) and OSI values obtained during feeding periods of lambs

		Properties							
Groups	Period	**TAS**	**SEM**	**TOS**	**SEM**	**OSI**	**SEM**	**P**	
	1	1.348^{a}	0.218	13.308^{a}	1.054	1.110^{aA}	0.110	*	**
	2	1.640^{ab}	0.168	14.946^{a}	1.832	0.920^{A}	0.083	*	**
AFL	3	1.627^{ab}	0.127	11.669^{ab}	1.221	0.765^{b}	0.074	*	
	4	1.396^{a}	0.179	10.694^{ab}	0.889	0.785^{b}	0.087	*	
	5	1.918^{b}	0.119	10.029^{b}	0.825	0.545^{aB}	0.049	*	**
	1	1.766^{a}	0.207	13.331^{A}	1.000	0.800^{a}	0.104	*	**
	2	1.287^{ab}	0.159	14.208^{A}	1.738	1.136^{bA}	0.079	*	**
AML	3	1.112^{b}	0.120	11.706^{a}	1.159	1.048^{bA}	0.070	*	**
	4	1.281^{ab}	0.170	11.124^{a}	0.843	0.951^{A}	0.083	*	**
	5	1.472^{a}	0.113	7.827^{bB}	0.783	0.528^{bB}	0.047	*	**
	1	2.043	0.218	13.393^{A}	1.118	0.717^{aA}	0.110		**
	2	2.486	0.168	13.930^{A}	1.943	0.571^{a}	0.083	*	**
MFL	3	2.532	0.127	11.754^{a}	1.296	0.468^{a}	0.074	*	
	4	2.406	0.179	10.380^{a}	0.943	0.463	0.087	*	
	5	2.508	0.119	6.761^{bB}	0.875	0.282^{bB}	0.049	*	**
	1	1.370^{aA}	0.197	12.287^{a}	0.953	1.080^{aA}	0.099	*	**
	2	2.005^{bc}	0.152	13.598^{a}	1.657	0.702^{bAB}	0.076	*	**
MML	3	2.243^{bB}	0.115	11.119^{ab}	1.105	0.504^{BC}	0.067		**
	4	1.897^{c}	0.162	10.513^{ab}	0.804	0.613^{aBC}	0.079	*	**
	5	2.335^{bB}	0.108	8.939^{b}	0.747	0.398^{bC}	0.045	*	**

SEM: Standart error of the mean

*Difference among the averages shown with different lower case in the same group column are significant (P<0.05).

**Difference among the averages shown with different upper case in the same group column are significant (P≤0.001).

Table 4. Intra-group TTL (µmol/L), NTL (µmol/L) and disulfide values obtained during feeding periods of lambs

		Properties							
Groups	**Period**	**TTL**	**SEM**	**NTL**	**SEM**	**Disülfide**	**SEM**	**P**	
AFL	1	476.444^{a}	23.138	233.66^{aA}	15.683	121.38^{A}	10.186	*	**
	2	531.889^{a}	26.594	282.33^{b}	16.128	124.77^{aA}	12.652	*	**
	3	485.556^{a}	24.038	302.22^{B}	17.237	91.66^{bc}	9.198	*	
	4	482.444^{a}	29.031	272.1^{b}	12.470	105.16^{ab}	13.845	*	
	5	431.556^{b}	19.702	292.333^{b}	12.123	69.61^{cB}	9.056	*	**
AML	1	420.400^{a}	21.951	201.00^{A}	14.878	109.70^{aA}	9.663	*	**
	2	490.900^{b}	25.230	271.60^{Ba}	15.300	109.65^{aA}	12.003	*	**
	3	463.200	22.804	316.20^{Bbc}	16.352	73.50^{bc}	8.726	*	**
	4	453.300	27.542	286.20^{Bab}	11.830	83.55^{ab}	13.135	*	**
	5	417.800^{a}	18.691	319.50^{Bc}	11.501	49.15^{cB}	8.591	*	**
MFL	1	442.222	23.138	187.55^{A}	15.683	127.33^{aA}	10.186	*	**
	2	490.556^{a}	26.594	301.33^{B}	16.128	94.61^{ab}	12.652	*	**
	3	494.000^{a}	24.038	312.55^{B}	17.237	90.72^{b}	9.198	*	
	4	448.778	29.031	292.66^{B}	12.470	78.05^{bc}	13.845	*	
	5	403.222^{b}	19.702	286.11^{B}	12.123	58.55^{cB}	9.056	*	**
MML	1	413.364^{A}	20.929	200.72^{A}	14.186	106.31^{abc}	9.214	*	**
	2	530.273^{B}	24.056	277.54^{caBC}	14.588	126.36^{a}	11.445	*	**
	3	525.000^{B}	21.743	334.90^{bB}	15.591	95.04^{bc}	8.320	*	**
	4	522.182^{B}	26.260	286.90^{aBC}	11.279	117.63^{ab}	12.523	*	**
	5	448.727^{A}	17.821	263.81^{cC}	10.965	92.45^{c}	8.191	*	**

SEM: Standart error of the mean

*Difference among the averages shown with different lower case in the same group column are significant (P<0.05).

**Difference among the averages shown with different upper case in the same group column are significant (P≤0.001).

The highest NTL level of AFL was observed in the 3rd period, this level increased statistically significantly compared to the 1st period when they were fed only with milk (P≤0.001). It was determined that the NTL detected in the 1st period in AML and MFL decreased significantly compared to the other periods. In MML, the NTL value was found to be the lowest in this term (P≤0.001), and it decreased significantly in the 2nd period compared to the 3rd period (P<0.05). In terms of disulfide values, a significant decrease was observed in AFL and AML compared to the 1st and 2nd periods, and in the 5th periods compared to the 1st period in MFL (P≤0.001). In MML, the decrease observed in the 3rd period compared to the 2nd period and in the 5th period compared to the 2nd and 4th periods were found to be statistically significant (P<0.05).

In terms of mean values between groups, TAS level obtained from lambs in all feeding periods and during the experiment was found to be at the highest level in MFL and later in MML (Table 5). While no difference was observed between Akkaraman female and male lambs in terms of this value (P>0.05), the increase observed in MFL can be attributed to the decrease in TOS in the 5th period. However, there was no significant difference between the groups in terms of TOS during other feeding periods and during the trial (P>0.05). Depending on these changes, it was determined that OSI decreased significantly in merino female and male lambs.

The intergroup TTL (µmol/L), NTL (µmol/L) and disulfide values obtained during the feeding periods of the lambs are given in Table 6. According to the results obtained regarding the TTL levels in the blood, there was no statistically significant difference between the groups in each feeding period of the lambs (P>0.05). However, it was determined that TTL levels did not differ in females of both offspring during the trial (P>0.05), but increased in MML compared to AML (P<0.05). On the other hand, the mean NTL value, which did not differ between the groups during the experiment, was found to be higher in AFL than MFL in the first feeding period (P<0.05). The mean NTL (µmol/L) value of AML was found to be higher in the 5th period compared to MML (P≤0.001). It was observed that disulfide values of MML were higher than females and higher than AMLs (P<0.05).

Table 5. Intergroup TAS (mmol/L), TOS (µmol./L) and OSI obtained during feeding periods of lambs

Period	Groups				SEM	P	
	AFL	AML	MFL	MML			
TAS(mmol/L)							
1	1.348^{a}	1.766^{ab}	2.043^{b}	1.370^{a}	0.105	*	
2	1.640^{acA}	1.287^{aA}	2.486^{bB}	2.005^{c}	0.081	*	**
3	1.627^{aA}	1.112^{bA}	2.532^{B}	2.243^{B}	0.061	*	**
4	1.396^{aA}	1.281^{aA}	2.406^{aB}	1.897^{b}	0.086	*	**
5	1.918^{aA}	1.472^{bA}	2.508^{B}	2.335^{cB}	0.058	*	**
1-5	1.586^{a}	1.384^{a}	2.395^{b}	1.970^{c}	0.045	*	
TOS(µmol/L)							
1	13.308	13.331	13.393	12.287	0.516		
2	14.946	14.208	13.930	13.598	0.898		
3	11.669	11.706	11.754	11.119	0.599		
4	10.694	11.124	10.380	10.513	0.436		
5	10.029^{a}	7.827	6.761^{b}	8.939	0.405	*	
1-5	12.129	11.639	11.244	11.291	0.214		
OSI							
1	1.110^{a}	0.800^{bc}	0.717^{b}	1.080^{ac}	0.053	*	
2	0.920^{a}	1.136^{aA}	0.571^{bB}	0.702^{abB}	0.040	*	**
3	0.765^{a}	1.048^{bA}	0.468^{bB}	0.504^{bB}	0.036	*	**
4	0.785^{ac}	0.951^{aA}	0.463^{bB}	0.613^{cb}	0.042	*	**
5	0.545^{bcA}	0.528^{acA}	0.282^{aB}	0.398^{a}	0.024	*	**
1-5	0.825^{a}	0.892^{a}	0.500^{b}	0.659^{c}	0.018	*	

SEM: Standart error of the mean

*Difference among the averages shown with different lower case on the same line are significant ($P < 0.05$).

**Difference among the averages shown with different upper case on the same line are significant ($P \leq 0.001$).

Table 6. Intergroup TTL (µmol/L), NTL (µmol/L) and disulfide values obtained during feeding periods of lambs

Period	Groups				SEM	P	
	AFL	AML	MFL	MML			
TTL(µmol/L)							
1	476.444	420.400	442.222	413.364	11.154		
2	531.889	490.900	490.556	530.273	12.820		
3	485.556	463.200	494.000	525.000	11.588		
4	482.444	453.300	448.778	522.182	13.995		
5	431.556	417.800	403.222	448.727	9.498		
1-5	481.578	449.120^{a}	455.756	487.909^{b}	5.713	*	
NTL(µmol/L)							
1	233.66^{a}	201.00	187.55^{b}	200.72	7.560	*	
2	282.330	271.60	301.33	277.54	7.775		
3	302.220	316.20	312.55	334.90	8.309		
4	272.100	286.20	292.66	286.90	6.011		
5	292.333	319.50^{A}	286.11	263.81^{B}	5.844		**
1-5	276.533	278.900	276.044	272.782	4.458		
Disulfide							
1	121.38	109.70	127.33	106.31	4.910		
2	124.77	109.65	94.61	126.36	6.099		
3	91.66	73.50	90.72	95.04	4.434		
4	105.16	83.55	78.05^{a}	117.63^{b}	6.674	*	
5	69.61	49.15^{A}	58.55^{a}	92.45^{bB}	4.365	*	**
1-5	102.52^{ac}	85.11^{b}	89.85^{ab}	107.56^{c}	2,516	*	

SEM: Standart error of the mean

*Difference among the averages shown with different lower case on the same line are significant ($P < 0.05$).

**Difference among the averages shown with different upper case on the same line are significant ($P \leq 0.001$).

DISCUSSION

In general, no research has been found in ruminants on the evaluation of oxidative stress level in the field of animal nutrition by thiol/disulfide balance measurement in the literature. Therefore, the comparison of the results obtained is limited.

Growing performance is under the effect of factors such as breed, gender, age, care and feeding style, amount and quality of feed and feed consumption increases in parallel with the age and live weight of lambs (Esen and Yıldız 2000). In this study expected was a situation that the live weights obtained from the groups by periods will increase in male lambs compared to female lambs. However, it can be said that the decrease observed in MML in the lambs feding solid feed together milk and reduced milk periods compared to females and Akkaraman offspring is related to the need for higher protein in MML's fattening, as can be understood from the results of some studies (Esen and Yıldız, 2000, Sawal et al. 2011).

Insufficient, excessive or unbalanced nutrition is a factor in the deterioration of the oxidant-antioxidant balance of the organism and the formation of oxidative stress. This causes a decrease in the growth performance of newborn and developing young ruminant (Serin 2015, Altınçekiç 2016).

Serum or plasma concentrations of different types of oxidant (malondialdehyde, nitric oxide) and antioxidant concentration (superoxide dismutase, glutathione peroxidase, catalase, vitamin E and selenium) can be measured separately by direct or indirect methods. However, measuring each parameter individually has advantages and disadvantages. These measurements do not provide an overall cumulative measure of oxidative and antioxidant status. Individual measurements require time consuming, costly and complex techniques. Therefore, the measurement of TAS, TOS and OSI reflects this situation and is more economical (Harma et al. 2005). There are studies reporting that the TAS level, which shows the total activity of all substances with antioxidant properties in the serum, is high in healthy sheep or lambs (Heidarpour et al. 2013, Mert et al. 2019, Garret et al. 2021). The decrease in the in-group TAS values of AML in the 3rd period was found to be significant compared to the 1st and 5th periods. This situation is related to the decrease of antioxidant molecules in the blood of Akkaraman male lambs during this period. The increase observed until the 5th period is related to the antioxidant defense mechanism that develops in response to the high oxidant level, especially in the period until the adaptation to solid feeds. Similar situations were encountered in the males of the Merino. Although merino male lambs had higher compliance with the feed, it showed a decrease in the 4th period, and the antioxidant level increased significantly during the weaning period. This finding, which is valid for TAS, is also similar to the results of research showing that Roman lambs develop high antioxidant capacity during the suckling period (Mialon et al. 2021). In related to mean TAS values between groups obtained from lambs in all feeding periods and during the experiment at the highest level in MFL and later in MML can be explained by the fact that Merino lambs are better adapted to feed and reach higher antioxidant capacity than Akkaraman lambs during their developmental stages. There was no significant difference between the groups in terms of TOS during other feeding periods and during the trial. Depending on these changes, it was determined that OSI decreased significantly in merino female and male lambs. Accordingly, it can be said that the immunity of the groups develops better especially when the lambs consume milk and solid feed together. When the results obtained from this study are considered, similar to the results of the research, which stated that Norduz sheep developed better resistance to diseases due to the higher TAS level and lower OSI than Morkaraman offspring, the same can be said for Merino compared to Akkaraman in this study (Mis et al. 2018).

Thiol or sulfhydryl groups (SH), which form the most active and functional form of the sulfur atom, as well as antioxidant defense are of great importance in enzyme function, protein functionality, detoxification, regulation of transcription factors, signal transduction, apoptosis and cellular stimulation mechanisms (Akkuş 2021). It can be said that the highest TTL values obtained from lambs increase due to rising of thiol compounds, which show antioxidant properties in the blood, especially during the periods when they consume solid feed with milk in MML. It is understood that the antioxidant defense mechanism develops depending on the total thiol level in lambs in this process where solid feed is consumed with milk. As at the TTL level, intra-group differences in NTL have been observed to a significant size. It was concluded that the disulfide value, which increased more significantly in the Merino race, developed in parallel with the high level of TTL and NTL in all groups. In other words, the thiol/disulfide balance was found to be weaker in Akkaraman offspring, but it was observed that the balance achieved by the high blood total thiol level in male lambs shifted towards thiol groups. In oxidative stress situations, while the native thiol and total thiol values are expected to decrease, the disulfide value is expected to increase (Erel and Neşelioğlu 2014). Accordingly, it can be said that the disulfide level tends to decrease in parallel with the periods when the antioxidant defense mechanism of all trial groups is activated.

CONCLUSION

When the results obtained from the study were evaluated in general, it was determined that the lowest antioxidant capacity was found in Akkaraman male lambs, the highest in Merino female lambs, and it was found higher in Merino females than males. It has been observed that the total antioxidant capacity can increase in lambs consuming solid feed together milk. The highest TAS value was observed in the 5th period, which is the weaning period, in Merino male lambs as well as in Merino females. On the other hand, it was determined that oxidant parameters TOS, OSI and disulfide decreased gradually with adaptation to solid food in all 4 groups. The fact that the oxidative stress index is higher in Merino lambs, especially in females, is largely a result of higher blood antioxidan molecules and total thiol levels in Merino lambs than in Akkaraman lambs. This situation explained that the lambs of both breed were able to compensate for oxidative stress with the adaptation developed in the transition from milk to solid feed. According to the results, it can be said that the oxidative stress caused by the transition from milk to solid food and weaning is sufficiently tolerated by the antioxidant systems of the lambs. When all oxidant antioxidant parameters were evaluated, it was concluded that the adaptation of Merino lambs after weaning to feed was better than Akkaraman lambs.

By measuring the dynamic thiol-disulfide balance, which plays a role in the development of many diseases, a lot of information related to the health and nutritional status of the animal can be obtained. However, there is a need for research on the determination of oxidative stress by thiol/disulfide balance measurement method in ruminants.

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

Ethical Approval: This study was approved by the Animal Experiments Local Ethics Committee of Konya Bahri Dağdaş International Agricultural Research Institute with the date and number of 31.01.2019/90.

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RESEARCH ARTICLE

Evaluation of Triage and Glasgow Coma Scale Findings in Cats in Trauma Emergencies

Oğuzhan PINAR[1], Mustafa ARICAN[1*]

[1]Selçuk University, Faculty of Veterinary Medicine, Department of Surgery, 42130, Konya, Turkey

ABSTRACT

In the study, it was aimed to understand the traumas which was observed in cats and the pathological conditions that may occur due to these traumas by using animal trauma triage score (ATT) and modified Glasgow coma score scales (mGCS), and to reveal the preparation stage for them, the prognosis of the patient and the results of objective evaluation. The study material consisted of 30 cats of various ages, breeds and genders, brought to the Department of Surgery of the Faculty of Veterinary Medicine Selçuk University in a 10-month period between 2020-2021. The mean age of the cases was between 6 months and 2 years. Skeletal and neurological system examinations were performed, and mGCS and ATT scores were determined and noted. Clinical, radiological, ultrasonographic examinations and blood gas analyzes of the cases were performed. According to the scoring system findings, 30 cats; triage color coding was done with 6 of them green code (20%), 14 of them yellow code (46,6%), 10 of them red code (33,3%). ATT score distribution of thirty cats; 3,3% to 0 points, 6,6% to 1 point, 23,3% to 2 points, 33,3% to 3 points, 6,6% to 4 points, 13,3% to 5 points, 3,3% to 6 points, 6,6% to 8 points and 3,3% to 12 points were observed. Trauma distributions in cats; It was determined that it was due to falling from a height in 20 cases, traffic accidents in 4 cases, and being bitten in 3 cases, while the cause is unknown in 3 cases. The distribution of fracture cases is; 3 tibia, 6 femur, 2 humerus, 2 phalanx, 2 hernia diaphragmatica, 5 sacroiliac luxation, 1 calcaneal luxation, 2 antebrachial fractures, 2 vertebral fractures, 1 multiple coxa fracture, 1 metatarsal fracture, 2 soft tissue injuries and 2 cases in various regions determined as wound formation. As a result, it has been determined that the modified Glasgow coma scale and animal trauma triage scoring will be beneficial for the clinician in terms of prognosis and provide a more objective evaluation of the patient.

Keywords: Cat, Trauma, Trauma Triage Score, Modified Glasgow Coma Scale

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Kedilerin Travmaya Bağlı Acil Durumlarında Triyaj Ve Glasgow Koma Skalası Bulgularının Değerlendirmesi

ÖZ

Çalışmada kedilerde gözlenen travmalar ve bu travmalara bağlı oluşabilecek patolojik durumların hayvan travma triaj skoru (ATT) ve modifiye Glasgow koma skoru skalaları (mGCS) kullanılarak, önceden anlaşılması ve bunlara yönelik hazırlık aşaması, hastanın prognozu ve objektif değerlendirilme sonuçlarının ortaya konulması amaçlanmıştır. Çalışma materyalini Selçuk Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı Kliniği'ne 2020-2021 yılları arasında 10 aylık periyotta travma nedeniyle getirilen çeşitli yaş, ırk ve cinsiyetteki 30 kedi oluşturdu. Olgular 6 ay ile 2 yıl yaş ortalamalarındaydı. İskelet ve nörolojik sistem muayeneleri yapılarak mGCS ve ATT skorları belirlendi ve not edildi. Olguların klinik, radyolojik, ultrasonografik muayeneleri ve kan gazı analizleri yapıldı. Skorlama sistemi bulgularına göre 30 kedinin; 6 tanesi yeşil kod (%20) 14 tanesi sarı kod (%46.6), 10 tanesi kırmızı kod (% 33.3) ile triaj renk kodlaması yapılmıştır. Otuz kedinin ATT puan dağılımı; %3,3' ünün 0 puana, %6,6'sının 1 puana, %23,3'ünün 2 puana, %33,3'ünün 3 puana, %6,6'sının 4 puana, %13,3'ünün 5 puana, %3,3'ünün 6 puana, %6,6'sının 8 puana ve %3,3'ünün 12 puana sahip olduğu gözlemlenmiştir. Kedilerdeki travmaya; 20 olguda yüksekten düşme, 4 olguda trafik kazası, 3 olguda ısırılmaya bağlı olduğu belirlenirken, 3 olguda neden bilinmemektedir. Kırık olgularının dağılımı ise; 3 tibia, 6 femur, 2 humerus, 2 phalanx, 2 hernia diyaframatika, 5 sakroiliak luksasyon, 1 kalkaneus luksasyonu, 2 antebrachium kırığı, 2 vertebral kırık, 1 çoklu koksa kırığı, 1 metatarsus kırığı, 2 yumuşak doku zedelenmesi ve 2 olguda çeşitli bölgelerde yara oluşumu şeklinde belirlenmiştir. Sonuç olarak modifiye Glasgow koma skalası ve hayvan travma triaj skorlamasının hekime prognoz yönünden yararlı olacağı ve hastayı daha objektif değerlendirmeyi sağladığı belirlenmiştir.

Anahtar Sözcükler: Kedi, Travma, Travma Triaj Skorlaması, Modifiye Glasgow Koma Skalası

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ORCID ID; *OP: 0000-0001-5019-1624, MA: 0000-0001-8180-135X*

*Corresponding author e-mail: marican@selcuk.edu.tr

GİRİŞ

Türkiye'de 85 milyon nüfusun %10'u evinde bir kedi beslemektedir. Bu oran aynı nüfus da Avrupa ülkelerinde % 25 'dir. Rusya da ise (144 milyon nüfus) % 59, ABD'de (325 milyon nüfus) % 43 oranındadır. Nüfusa oranda ise en fazla Romanya'da kedi beslenmektedir (% 47). 2019 yılı verilerine göre; Türkiye'de ise 3,8 milyon kedi popülasyonu bulunmaktadır (Adams 2020). İstanbul Büyükşehir Belediyesi Veteriner Hizmetleri sahipsiz 125 bin de kedi bulunduğunu tahmin ettiklerini belirtmiştir (Anonymous 2020).

Kedilerin özgür ruhlu karakteristik özelliklerinden dolayı, başına buyruk tavırları travmaya uğrama oranlarını arttırmaktadır. Sürekli olarak aynı ortamda bulunmaktan sıkılmaları sebebiyle hareket etme ihtiyaçları vardır. Bu durum travmaya uğrama sıklığını arttırmaktadır. Ülkemizde ulaşım etkinliklerinin % 90'ından fazlası karayolu ile yapılmaktadır ve karayollarında meydana gelen kazalar insan ve kedi/köpekte önemli ölüm nedenleri arasında değerlendirilmektedir (Sungur ve ark. 2014). Travmaların ise başlıca sebepleri başta trafik kazaları olmak üzere; hayvan kavgaları, kesici cisim yaralanmaları, yüksekten düşme, olarak sınıflandırılabilir (Parlak ve Arıcan 2015, Klainbart ve ark. 2021).

Bu açıdan travma sebebiyle yaralanan kediler kliniklere başvurduğu zaman erken ve doğru travma protokolünün hızlı bir şekilde uygulanması gerekir. Temelde bu protokol iki ana aşamaya ayrılmaktadır. Birinci aşama, solunum ve kardiyovasküler sistemlerin değerlendirilmesi ve ardından merkezi sinir sistemi ve üriner yolu sisteminin değerlendirilmesidir. İkinci aşama, birinci aşama sırasında tespit edilen ve hayvanın yaşamını tehdit eden sorunların en kısa sürede müdahale edildikten sonra geriye kalan tüm sistemlerin değerlendirilmesidir. Travmalı hastalar klinik veya hastaneye geldiğinde hekim temel travma triyaj skorlama sistemi (ATT), gerçekleştirir ve tıbbi ihtiyaca göre tedaviye başlar. Triyaj, hasta stabilitesini hızlı bir şekilde değerlendirmek ve hayatı tehdit eden sorunları anında belirlemek için yapılır ve prosedür 30-60 saniyeden fazla sürmemelidir. Triyaj tam bir tıbbi geçmişin alınmasını veya tam bir fiziksel muayene yapılmasını içermez (Drobatz ve ark. 2011, Murgia ve ark. 2019).

Travma triyaj puanlama sistemi, veteriner literatüründe yayınlanan travma triyaj kılavuzlarından ve deneyimli acil personellerinin önerilerinden geliştirilmiştir. Triyaj skorlama sistemin'de listelenen önceden belirlenmiş kriterler kullanılarak 0-3 ölçekte altı kategori (perfüzyon, kardiyak, solunum, göz/kas/deri, iskelet ve nörolojik) puanlanır, (0 hafif yaralanmayı veya hiç yaralanma olmadığını belirtir, 3 ciddi yaralanmaları gösterir) puanlama tablosu fizik muayene bulguları birden fazla kriterleri karşılıyorsa, en yüksek puan verilir. Toplam bir ATT puanı vermek için altı puan bir araya getirilir. Mümkün olan en yüksek ATT puanı 18'dir (Rockar ve Drobatz 1994, Lapsley 2019, Murgia ve ark. 2019).

ATT puanı ne kadar yüksekse, hastanın hayatta kalma olasılığı o kadar azdır ancak bu puan sadece triyaja yardımcı olarak kullanılmalıdır. Deneyimli bir veteriner hekim tarafından yapılan fiziksel muayenenin ve ayrıntılı bir geçmişin alınmasının yerini alamaz, sadece daha az deneyimli ekip üyelerinin karar verme süreçlerine yardımcı olmada faydalı olabilir (Donnelly ve Leawis 2016, Lyons ve ark. 2020).

Triyajın değerlendirilmesinde; yeşil kod, 24 saat içerisinde müdahale edilebilecek pozisyondaki hastaları temsil eder (küçük sıyrıklar, kronik dermatolojik problemler, apse gibi). Sarı kod, birkaç saat içerisinde müdahale edilmezse kritikleşecek hastaları temsil eder (kapalı kırıklar, aktif kusma, diare, güç doğum gibi). Kırmızı kod hayati riski en yüksek gruptur ve dakikalar içinde müdahale edilmez ise hastanın kaybedeceğini temsil eder (kardiyak arrest, solunum distresi, anafilaktik şok, trakeal kollaps, aktif nöbetler, ağır kanamalar, zehirlenmeler gibi). Siyah kod, hastanın size ulaşana kadar yolda ex olma durumunu temsil eder (Uzun 2019, Nascimento ve ark. 2021).

Travma skoru, travmatik bir olayda bir hastanın maruz kaldığı yaralanmaların sayısal bir karakterizasyonudur. Çok heterojen bir hasta popülasyonu için nesnel bir sınıflandırma aracı sağlar. Klinik araştırma ve dahili hastane incelemesi için hasta popülasyonlarının sınıflandırılması için veya bireysel travma hastaları için sonuçların triyajı ve tahmini için bir travma skorlama sistemi kullanılabilir. İlk triyaj sırasında üç ana vücut sistemi değerlendirilir; Solunum, kardiyovasküler ve nörolojik sistem. Sistematik bir yaklaşım için acil bakımın (A) hava yolu, (B) solunum (C) dolaşım, (D) merkezi sinir sistemi disfonksiyonu ve (E) hipotermi/hipertermi, kritik deri muayenedir. Bu alanlardan herhangi birinde bir anormallik derhal harekete geçmenin gerekli olduğunu ve kardiyopulmoner arrestin yakın olabileceğini gösterir (Donnelly ve Leawis 2016).

Modifiye Glasgow koma skorunun (mGCS) belirlenmesi, merkezi sinir sisteminin hasarının ciddiyetine ilişkin objektif bilgi sağlanmasına yardımcı olur. mGCS'nin tekrarlanan değerlendirmesi, hastanın prognozunun daha objektif olarak belirlenmesine izin verir. Dört ekstremitede da derin ağrı algısının varlığı, istemli motor fonksiyonun varlığı ve refleksler değerlendirilmesini kapsar (Drobatz ve ark 2011, Hall ve ark. 2018, Lapsley ve ark. 2019).

Sunulan çalışmada, kedilerde gözlenen travmalar ve bu travmalara bağlı oluşabilecek patolojik durumların travma triaj skoru ve modifiye Glasgow koma skoru skalalarının kullanılarak, önceden anlaşılması ve bunlara yönelik hazırlık aşaması, hastanın prognozu ve objektif değerlendirilme sonuçlarının ortaya konulması amaçlanmıştır.

MATERYAL ve METOT

2.1. Çalışma Materyali

Çalışma materyalini, Selçuk Üniversitesi, Veteriner Fakültesi, Küçük Hayvan Hastanesi'ne 2020-2021 yılları arasında 10 aylık periyotta travma nedeniyle getirilen çeşitli yaş, ırk ve cinsiyetteki 30 kedi oluşturdu. Olguların yaş ortalamalarını 6 ay ile 2 yıl arasındaydı.

Çalışmaya Selçuk Üniversitesi Veteriner Fakültesi Deney Hayvanları Üretim ve Araştırma Merkezi Etik Kurulu (SÜVDAMEK) 17.11.2021 tarihli 2021/10 toplantı sayılı, 2021/119 nolu kararı ile etik kurul onayı alınarak başlandı.

2.2. Çalışma Metodu

Acil kliniğine getirilen hastanın, anemnezi alınarak, muayeneye başlandı. Triaj renk kodlaması yapılarak vakalar arasındaki aciliyet durumuna göre öncelik verildi. İlk yardımın temel prensibi olan ABC (A (Airway) Havayolu Kontrolü, B (Breathing) Solunum Kontrolü C (Circulation) Dolaşım) sistematiğine uygun olarak solunum, dolaşım ve sinir sistemleri sırasıyla muayene edildi. Hastaların solunum sıklığı ve düzeni değerlendirildi. Hastanın travmatik olayı takiben ayakta durup duramayacağını, tüm uzuvlarına ağırlık taşıyıp taşıyamayacağını görebilmek için yürütülmeye çalışıldı, duruşu ve bilinç seviyesi değerlendirildi. Kapiller dolum süresi, nabız sayısı ve kalp ritmine bakıldı. Olguların tam kan sayımı (Melet Schloesing, MS4E model, Fransa) ve kan gazı analizleri (Radiometer, ABL90 flex model, Danimarka) yapılarak enfeksiyon durumu, hematokrit, oksijenizasyon, baz açığı, mineral eksikliği, pH veriler kaydedildi. Abdominal organlarının değerlendirilmesi ve herhangi bir komplikasyon gerçekleşip gerçekleşmediğini görebilmek, herhangi bir patolojik durum varsa uygun tedavi protokolü oluşturabilmek için ultrason ile muayene edildi (Mindray, DC-6VET model, Çin) ve elde edilen bütün veriler vakalar için hazırlanan formlara not edildi.

Olguların iskelet ve nörolojik sistem muayeneleri yapılarak mGCS'. (Tablo 1) ve hayvan ATT sonuçları not edildi (Tablo 2). Genel durumu iyi olan, palpasyonda ve oskültasyonda lezyon tespit edilen olguların sedasyon ile ya da sedasyona alınmadan, ilgili bölgelerinin (cranium, abdomen ve ektremite) dorso-ventral (D/V), ventro-dorsal (V/D), latero-lateral (L/L) ve medio-lateral (M/L) pozisyonda olmak üzere radyografileri alındı (Siemens marka 4803404 model, İtalya). Alınan radyografilerin değerlendirilmesiyle lezyonun lokalizasyonu ve durumu saptanarak, uygulanacak sağaltım şekli belirlendi. Hemodinamik stabilite sağlandıktan sonra ilgilenilmesi gereken yaralar ve kırıklar gibi yaşamı tehdit etmeyen acil durumlara müdahale edildi. Kırık olguları, cerrahi müdahale veya bandaj yapılıncaya kadar stabilize edildi ve daha sonra operasyona alındı.

Tablo 1: Çalışmada kullanılan modifiye Glasgow Koma skalası

Modifiye GLASGOW Koma Skalası		
		SKOR
Motor Aktivitesi	Yürüyüş normaldir,omurga refleksleri normal	6
	Hemiparesis, tetraparesis veya decerebrate sertliği	5
	Hayvan yatar vaziyette, aralıklı ekstansör kaslarında sertlik (rijidite)	4
	Hayvan yatar vaziyette, aralıklı ekstansör kaslarında sürekli sertlik (rijidite)	3
	Opisthotonus ile sürekli sabit ekstansör kasların sertliği	2
	Hayvan yatar vaziyette, kaslarda hipotoni, depresif veya omurga refleksleri yok	1
Beyin sapı refleksleri	Pupillar ışık refleksi ve okülosefali refleksleri normal	6
	Pupillar ışık refleksi yavaş ve normalden azalmış okülosefalik refleksleri	5
	Normal ila azalmış okülosefali refleksleri olan bilateral yanıt vermeyen miyoz	4
	Nokta şeklinde miyosis, okülosefali reflekslerin ortadan kalkması	3
	Okülosefali refleksleri azalmıştır, tek taraflı yanıt vermeyen midriyazis	2
	Bilateral yanıt vermeyen midriyazis ve okülosefalik reflekslerinde azalma veya yok	1
Bilinç Seviyesi	Arasıra uyanıklık ve çevreye duyarlılık	6
	Depresyon veya delirium tepkilere cevap verebilir. Ancak cevap uygun olmayabilir.	5
	Yarı koma hali, görsel uyarıcılara cevap verir.	4
	Yarı koma hali, işitsel uyarılara cevap verir.	3
	Yarı koma hali, sadece tekrarlanan zararlı uyaranlara cevap verir.	2
	Koma hali, tekrarlanan zararlı uyaranlara cevap vermez.	1
mGSC		**Skor**
3-8		Önemli
9-14		Orta
15-18		İyi

Tablo 2: Çalışmada kullanılan hayvan travma triyaj skorlama sistemi

Çoklu Travma Triyaj Skor Sistemi	
Perfüzyon	**Skor**
Mukus membranlar pembe/nemli, CRT<2 sn, vücut ısısı >37.8^{0}C, femoral nabız güçlü ve alınabilir.	0
Mukus membranlar hiperemik veya soluk pemb, CRT<2sn, vücut ısısı >37.8^{0}C, orta seviyede femoral nabız	1
Mukus membran çok soluk pembe ve yapışkan hissi var, CRT 2-3 sn, vücut ısısı >37.8^{0}C femoral nabız, hissedilebilir ama zayıf	2
Mukus membran gri/mavi/beyaz, CRT>3 sn, vücut ısısı <37.8^{0}C, femoral nabız alınamıyor	3
Solunum	**Skor**
Respirasyon sayısı normal, stridor yok, solunum da abdominal komponent yok	0
Respirasyon sayısında hafif artış ve efor+ abdominal komponent, hafif şiddetli üst solunum yolu sesleri	1
Respirasyon sayısında orta şiddette artış ve efor abdominal komponent, orta şiddetl üst solunum yolu sesleri	2
Respirasyonda ciddi efor veya zorlanma/agoni/obstrüksiyon	3
Kardiyak	**Skor**
HR:köpek 60-140bpm,kedi 120-200 bpm;normal sinüs ritmi	0
HR:köpek 140-180bpm,kedi 200-260 bpm;normal sinüs ritmi veya ventriküler prematüre kontraksiyon<20/dk	1
HR:köpek >180bpm,kedi >260 bpm; sürekli aritmi	2
HR:köpek<60bpm,kedi <120 bpm; değişken aritmi	3
Göz/kas/deri	**Skor**
Abrasyon/laserasyon:yok veya kısmi çok az/ Göz:fluresein negatif	0
Abrasyon/laserasyon:komple kalınlık, derin doku karışıklığı yok/ Göz:korneal laserasyon, perfore değil	1
Abrasyon/laserasyon:komple kalınlık, derin doku karışması, arter,sinir,kas bozulmamış / Göz:korneal perforasyon, proptosis	2
Abdomen penetrasyon/Toraks abrasyon/laserasyon:derin doku karışması, arter, sinir, kas bütünlüğü bozulmuş	3
İskelet	**Skor**
Vücut ağırlığı üç ya da dört bacakta; palpe edilebilir kırık yok/eklemlerde laksite yok	0
Bacaklarda kapalı /kostal veya mandibular kırık; tek eklemde laksite/çıkık (sakroiliak), pelvik kırık/karpus ve Tarsus kemiklerde kırık	1
Çoklu grade1 kondüsyon;karpus/Tarsus üzerinde tek uzun kemik kırığı,kortikal kemik korunmuş, mandibular ve/veya kafatası kırığı yok	2
Vertebra kırığı/koksigeal hariç çıkığı, karpus/Tarsus üzeri çok uzun kemik kırığı,karpus/Tarsus açık kırığı	3
Nörolojik	**Skor**
Sentral tetikte veya az uyuşuk dışarıdan gelen seslere ilgili/Periferal:normal spinal refleks amaçlı hareket,tüm bacaklarda nosisepsiyon	0
Sentral: uyuşuk/depresif/içine kapanık/ Periferal: amaçsız hareket, bacaklarda intakt nosisepsiyon	1
Sentral: anksiyete, uyarıcılara yanıt veriyor/Periferal:amaçlı hareket yoksunluğu bir ya da iki bacakta nosisepsiyon, anal ya da kuyruk tonusunda azalma	2
Sentral:tüm stimulanlara karşı tepkisiz, felç/Periferal: bir ya da daha fazla bacakta nosisepsiyon yok, anal tonus yok	3

2.3. İstatistiksel Analiz

Bulgular % de olarak ifade edilmiştir. modifiye Glasgow Koma skalası ve travma triyaj skorlamasının mean değerleri alınmıştır.

BULGULAR

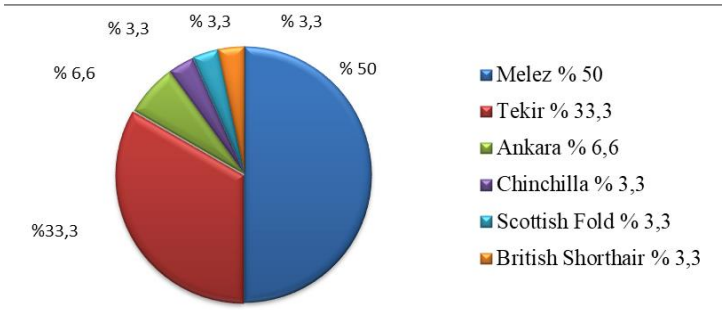
Olgularda travma sonrası oluşan lezyonların dağılımı Tablo 3 de verilmiştir.

Tablo 3: Kedilerin, ırkı, cinsiyeti, travma nedenleri ve lezyonların dağılımı

Olgu No.	Irk	Cinsiyet	Travmatik Neden	Lezyonlar
1	Tekir	Erkek	Sebebi bilinmiyor	Sağ tibia'da diyafizer oblik kırık
2	Melez	Dişi	Sebebi bilinmiyor	Plöral efüzyon
3	Tekir	Dişi	Sebebi bilinmiyor	Hernia diyaframatika
4	Tekir	Dişi	Yüksekten düşme	Sağ falanksda proksimal kırık
5	Sarman	Erkek	Yüksekten düşme	Plöral efüzyon
6	Chinchilla	Erkek	Yüksekten düşme	Sağ tibiada distal transversal kırık ve plöral efüzyon
7	Melez	Dişi	Yüksekten düşme	Vücudun çeşitli bölgelerinde yumuşak doku zedelenmesi
8	Melez	Dişi	Isırılma vakası	Lumbal bölgede açık yara
9	Melez	Erkek	Trafik kazası	Lumbal vertebra kırığı
10	Ankara	Dişi	Yüksekten düşme	Sağ humerus'un distalinde oblik kırık
11	Tekir	Dişi	Trafik kazası	Sol femur diyafizer parçalı kırık ve sağ femur distal transversal kırık
12	Melez	Erkek	Trafik kazası	Sol kalkeneusda çıkık ve sakroiliak ayrılma
13	Melez	Dişi	Yüksekten düşme	Çoklu koksa kırığı
14	Melez	Erkek	Yüksekten düşme	Sol falankslarda kırık
15	Melez	Erkek	Yüksekten düşme	Sol femurda suprakondiler kırık
16	Sarman	Erkek	Yüksekten düşme	Sol antebrachiumda
17	Melez	Erkek	Trafik kazası	Bilateral sakroiliak ayrılma
18	Tekir	Dişi	Isırılma vakası	C1-C2 servikal omur lezyonları
19	Melez	Erkek	Isırılma vakası	Vücudun çeşitli bölgelerinde yumuşak doku zedelenmesi ve yara
20	Melez	Erkek	Yüksekten düşme	Sol femur distal transversal kırık
21	Melez	Erkek	Yüksekten düşme	Plöral efüzyon
22	Melez	Erkek	Yüksekten düşme	Plöral efüzyon, sol sakroiliak ayrılma,sol kollum femoris kırığı ve sağ asetabulum kırığı
23	Ankara	Dişi	Yüksekten düşme	Plöral efüzyon
24	Melez	Erkek	Yüksekten düşme	Sol metatarsus da açık kırık ve sağ sakroiliak ayrılma
25	British Shorthair	Dişi	Yüksekten düşme	Plöral efüzyon
26	Tekir	Dişi	Yüksekten düşme	Plöral efüzyon ve sağ sakroiliak ayrılma
27	Scottisch Fold	Erkek	Yüksekten düşme	Plöral efüzyon
28	Melez	Dişi	Yüksekten düşme	Sağ tibia diyafizer oblik kırık ve sağ humerus'da distal transversal kırık
29	Tekir	Dişi	Yüksekten düşme	Sağ tarsal eklemde çıkık, sol sakroiliak ayrılma ve plöral efüzyon
30	Tekir	Erkek	Yüksekten düşme	Hernia diyaframatika ve sağ kaput femoris kırığı

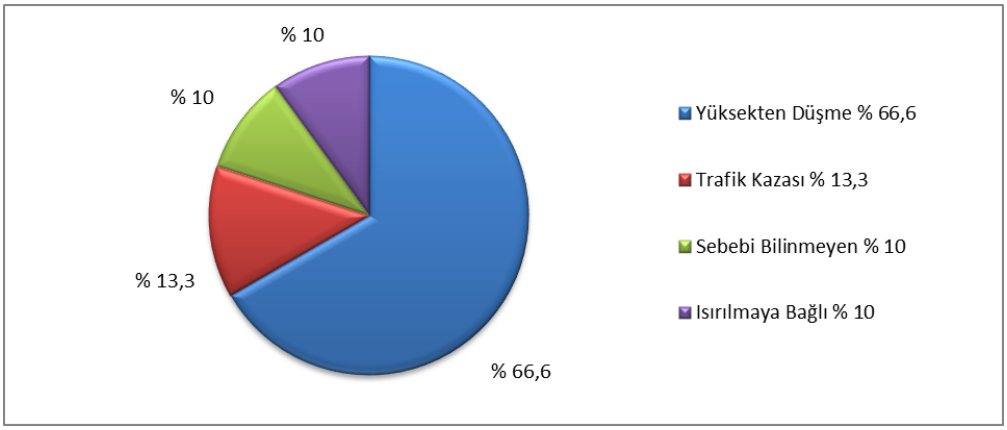
3.1. Klinik değerlendirme bulguları

Çalışma materyalini oluşturan 30 kedinin ırk dağılımında; 15 olgu melez (%50), 10 olgu Tekir (%33,3), 2 olgu Ankara (%6,6), 1 olgu Chinchilla (%3,3), 1 olgu Scottish Fold (%3,3) ve 1 olgu da British Shorthair (%3,3) olarak saptandı (Şekil 1). 30 kedinin; 16 tanesini erkek, 14 tanesini dişi olduğu belirlendi.



Şekil 1: Kedilerin ırk dağılımı.

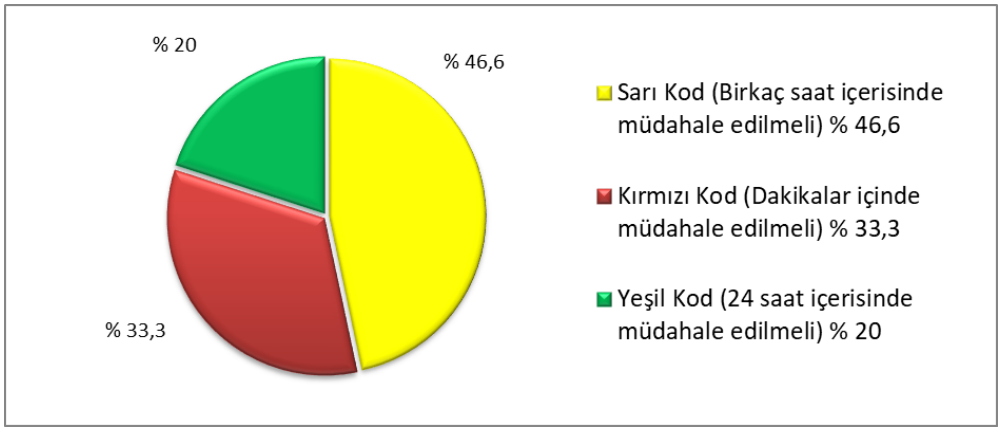
Kedilerdeki 20 tanesinde yüksekten düşme (%66,6), 4 tanesinde trafik kazası (%13,3), 3 tanesinde kavga (%10) ve 3 tanesinde sebebi bilinmeyen travmalara bağlı (%10) oluştuğu gözlendi. Olgulara ait klinik bilgiler (Şekil 2) de sunulmuştur. Otuz kedinin 3 tanesinin tedavi sonrası (%10) öldüğü belirlendi.



Şekil 2: Kedilerin travma dağılımları.

3.1.1. Triaj ve travma triyaj skorlama sistemi (ATT) bulguları

30 kedinin; 6 tanesi yeşil kod (% 20) (Olgu no. 4, 5, 7, 14, 15, 17), 14 tanesi sarı kod (% 46,6) (Olgu no. 1, 2, 10, 12, 13, 16, 19, 20, 21, 23, 24, 25, 26, 27), 10 tanesi kırmızı kod (% 33,3) (Olgu no. 3, 6, 8, 9, 11, 18, 22, 28, 29, 30) ile triaj renk kodlaması yapıldı. Çalışma materyalini oluşturan 30 kedinin ATT puan dağılımı; % 3,3' ünün 0 puana, %6,6'sının 1 puana, %23,3'ünün 2 puana, %33,3'ünün 3 puana, %6,6'sının 4 puana, %13,3'ünün 5 puana, %3,3'ünün 6 puana, %6,6'sının 8 puana ve %3,3'ünün 12 puana sahip olduğu gözlemlendi (Şekil 3). Olgulardaki travma triaj skorlaması sonuçları (Tablo 4) de verilmiştir.



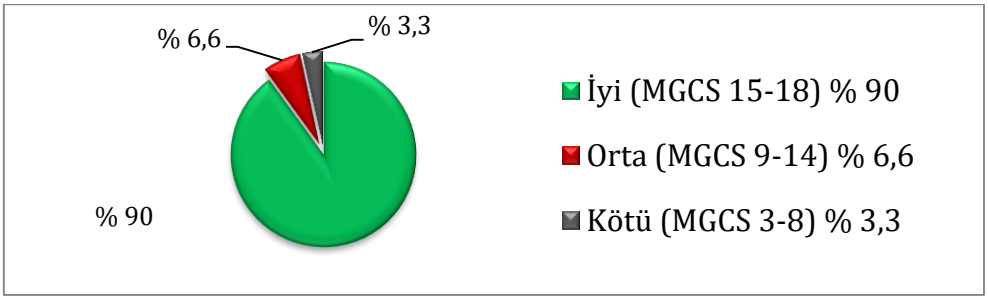
Şekil 3: Kedilerin triaj renk kodu dağılımı.

Tablo 4: Olgulardaki travma triaj skorlaması değerleri

Olgu No.	Travma Triyaj Skorlama					
	Perfüzyon	Solunum	Kardiyak	Göz/Kas/Deri	İskelet	Nörolojik
1	1	0	0	0	2	1
2	1	1	0	0	0	0
3	1	2	1	0	0	0
4	1	1	1	0	1	0
5	1	1	0	0	0	0
6	1	1	1	0	1	0
7	1	1	0	1	0	0
8	1	1	0	1	0	0
9	2	2	1	1	3	3
10	1	1	0	0	1	1
11	1	1	1	1	2	0
12	1	1	0	0	1	1
13	1	2	1	0	1	0
14	0	0	0	0	1	0
15	1	1	0	0	2	1
16	1	1	0	0	2	1
17	1	1	0	0	1	1
18	2	2	1	0	3	3
19	1	0	0	2	0	1
20	1	1	1	0	2	1
21	1	1	0	0	0	0
22	1	2	1	1	2	1
23	1	1	0	0	0	0
24	1	1	0	1	2	1
25	1	1	0	0	0	0
26	1	1	0	0	1	1
27	1	1	0	0	0	0
28	1	1	0	0	2	1
29	1	1	1	0	2	1
30	1	1	1	0	2	1

3.1.2. Modifiye Glasgow koma skalası (mGCS) bulguları

Modifiye Glasgow koma skalasına göre 30 kediden; 27 tanesi (%90) iyi (Olgu no. 1, 2, 3, 4, 5, 6, 7, 10, 11, 12,13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30) 2 tanesi orta (% 6,6) (Olgu no. 9, 11) ve 1 tanesi kötü (%3,3) (Olgu no. 8) olarak skorlandı (Şekil 4) Olgulardaki Modifiye Glasgow Koma skalasına sonuçları (Tablo 5) de verilmiştir.



Şekil 4: Kedilerin mGCS ile dağılımı.

Tablo 5: Olgulardaki modifiye Glasgow Koma skalası değerlendirmesi

Olgu No.	Modifiye Glasgow Koma Skalası		
	Motor Aktivitesi	**Beyin Sapı Refleksler**	**Bilinç Seviyesi**
1	5	6	6
2	6	6	6
3	6	6	6
4	5	6	6
5	6	6	6
6	5	6	6
7	6	6	6
8	2	6	5
9	1	5	5
10	5	6	6
11	3	6	6
12	5	6	6
13	5	5	5
14	5	6	6
15	5	6	6
16	5	6	6
17	5	6	6
18	3	4	5
19	6	6	6
20	5	6	6
21	6	6	6
22	5	5	5
23	6	6	6
24	5	6	6
25	6	6	6
26	5	6	6
27	6	6	6
28	5	6	6
29	5	5	5
30	5	6	5

3.1.3. Kan muayenesi bulguları

Kan muayenesi sonuçlarına göre 7 olguda (%23,3) enfeksiyon olduğu (Olgu no. 6, 11, 12, 19, 21, 22, 26), 1 olguda (%3,3) hematokrit değerinin normal sınırlardan yüksek (Olgu no. 21), 1 olguda (%3,3) hematokrit değerinin normal sınırların altında olduğu (Olgu no. 28), 16 olguda (%53,3) oksijen satürasyonunun normal sınırlarının altında olduğu (Olgu no. 1, 2, 6, 10, 11, 12, 19, 20, 21, 22, 23, 24, 26, 28, 29, 30) ve 6 olguda (%20) kan pH'sının asidoza kaydığı (Olgu no. 1, 6, 11, 12, 28, 30) gözlendi.

3.1.4. Ultrason bulguları

Çalışmayı oluşturan hayvanların abdominal ultrasonografik muayene sonuçlarına göre; 30 kedinin 12' sinde (%40) sistit başlangıcı ve idrar kesesin de tortu birikimi olduğu gözlemlendi.

3.2. Radyolojik değerlendirme bulguları

Çalışmayı oluşturan 30 kedideki lezyonların dağılımında; 3 tibia kırığı (% 10) (Olgu no. 1, 6, 28), 10 plöral efüzyon (% 33.3) (Olgu no. 2, 5, 6, 21, 22, 23, 25, 26, 27, 29), 6 femur kırığı (%20) (Olgu no. 'bilateral' 11, 15, 20, 22, 30), 2 hernia diyaframatika (% 20) (Olgu no. 3, 30), 2 humerus kırığı (%20) (Olgu no. 10, 28), 2 falanks kırığı (%20) (Olgu no. 4, 14), 5 sacroiliak ayrılma (%16,6) (Olgu no. 12, 'bilateral' 17, 22, 24, 26, 29), 1 calcaneus luksasyonu (3,3) (Olgu no. 12), 2 antebrachium kırığı (% 6,6) (Olgu no. 16, 22), 2 vertebral lezyon (%6,6) (Olgu no. 9, 18), 1 multiple coksa kırığı (% 3,3) (Olgu no. 13), 1 metatarsus kırığı (% 3,3) (Olgu no. 24), 2 tane yumuşak doku zedelenmesi (% 6,6) (Olgu no. 7, 19) ve 2 olguda yara (% 6,6) (Olgu no. 8, 19) oluşumu belirlenmiştir.

TARTIŞMA ve SONUÇ

Travma triyajı ve modifiye Glasgow koma skala skorları, travmaya özgü hastalık şiddeti ve yaralanma şiddetinin objektif olarak ölçülmesine izin vererek, mortalite oranının aşağıya düşmesini sağlamaktadır (Lapsley 2019). Hayvan travma triaj skoru, travmaya uğrayan kedi popülasyonunda hayatta kalma için ideal öngörücü performans oluşturmuştur. Yaralı kedilerde seri muayenede travma triyajı skorlama sistemi ile olgulardaki perfüzyon, solunum, kardiyak, göz/kas/deri, iskelet ve nörolojik alt kategorilerinin değerlendirilmesinin gözden kaçmasının önüne geçmektedir. Böylece klinisyenlerin teşhis koyarken herhangi bir konuyu atlamama kolaylığını sağlamaktadır (Lapsley 2019).

Modifiye Glasgow koma skoru ve travma triaj skorlarına bakılan 711 kedi çeşitli çalışmalarda, değerlendirilmiştir (Goggs 2019, Cameron ve ark. 2021). Glasgow koma skorlamasına göre puanlamada durumun kritik olduğu (3-8 puan), orta (9-14 puan) ve iyi (15-18 puan) olarak değerlendirilmektedir. Bu kedilerin % 26'sının travma triaj skor puanı 1, %17'si 2 puan ve %13'ü 3 puan olarak belirlenmiştir. Sadece 9 kedi, '>10' triaj puanı aldığı gözlemlenmiştir. Kafa travması geçirmeyen 66 kedide (%9,3) ise anormal modifiye Glasgow koma skor puanı '<18' olarak kaydedilmiştir. Kedilerin %71'i, normal bir muayeneye karşılık gelen '18' modifiye Glasgow koma skoru puanı aldığı gözlemlenmiştir (Goggs 2019, Cameron ve ark. 2021). Sunulan çalışmada modifiye Glasgow koma skalası puanlamasına göre 30 kediden; 27 tanesi (%90) iyi, 2 tanesi orta (% 6,6) ve 1 tanesi kötü (%3,3) olarak skorlanmıştır. Modifiye Glasgow koma skoru puanı ile kedilerin %3,3'ünün kötü (3-8), %6,6'sının orta (9-14) ve %90' nın iyi (15-18) olduğu belirlenmiştir. Hasta skalasının kriterlerine göre değerlendirildiğinde, hastaya 3 (derin bilinç kaybına işaret eder), 9-14 (orta dereceli hasarı gösterir), 15-18 (motor hareket fonksiyonlarını kullanabilme kapasitesiyle ölçülür.) arasında puanlar verilir. Orta ve kritik olguların 2'sinde vertebral lezyon ve diğer olguda ise sağ ve sol femur'da bilateral kırık tespit edilmiştir. Orta ve kötü olarak skorlanan 3 olguda ex olmuştur. Bu durum teşhisde kötü skorlaması olan olguların daha dikkatli gözetim altında tutulması gerekliliğini ortaya koymuştur.

Kedi ve köpeklerde travmatik yaralanmaların etiyolojisini etkileyecek etmenler, başta trafik kazaları olmak üzere; hayvan kavgaları, ateşli silah ve kesici cisim yaralanmaları, yüksekten düşme, güneş çarpması, donma ve kimyasal zehirlenmeler olarak sınıflandırılabilir (Parlak ve Arıcan 2015, Conroy ve ark. 2019). Kedilerde kırık oluşumunda ırk, yaş ve cinsiyet predispozisyonu bulunmamaktadır. Kırık oluşumundaki en yaygın olan neden yüksekten düşmelerdir. Yaşam alanlarına göre trafik kazaları da nedenler arasında yer almaktadır (Boysen ve Lisciandro 2013, Fossum 2007, Harris ve ark. 2018). Bu çalışmada da kedilerde yüksekten düşmeye bağlı yaralanmalar ilk sırada yer almıştır.

Çalışmada değerlendirilen olgularda yaralanma sebeplerinin ilk sırasında yer alan, yüksekten düşme bir sendrom olarak kabul edilmiştir. Özellikle kedilerde binaların balkon veya pencerelerinden düşmesi sonrasında oluşan travmatik lezyonları tanımlar. Yüksek binaların çok olduğu şehirlerde daha sık karşılaşılmaktadır. Bu sendrom, iki veya daha yüksek kattan düşen kediler için söz konusu olup, düşmeye bağlı üç travma bölgesi etkilenir. Bunlar; baş, toraks ve ekstremitelerdir. Düşmeye neden olan etkenler; genellikle bir kuş veya böceği kovalama sırasında balkon ya da pencereden atlama durumu olup, bazen de pencere ve balkon parmaklığı kenarında yürürken kayıp düşmedir. Yüksekten düşme sendromu, bir veya daha yüksek kattan düşen köpekler için de tanımlanmıştır. İnsanlarda ise; "yüksekten düşme sendromu", "yüksekten uçan sendromu" ve "atlayıcı sendromu" gibi tanımlamalar kullanılır (Vnuk 2004, Liehmann ve ark. 2012, Zimmermann ve ark. 2013).

Kedilerle yapılan retrospektif bir çalışmada 185 travma olgusunun nedenleri değerlendirmiş; bunlardan 104 trafik kazası (%56,2), 49 sebebi bilinmeyen olgular (%26,5), 18 yüksekten düşme (%9,7), 6 köpek saldırısı (%3,2), 2 ateşli silah yaralanması (%1,1), 2 küt travma (%1,1), 2 kuyruktan çekilme (%1,1), 1 at çifte darbesi (%0,5) ve 1 de çarpma (%0,5) sonucu oluştuğu rapor etmiştir. Bu olgulardan 116'sı çoklu travma yaralanmalarından etkilenirken 69'unun sadece bir travmatik etkiye maruz kaldığı belirtilmiştir (Hernon ve ark. 2018). Simpson ve Streeter, (2009)'ın yaptığı benzer bir çalışmada 100 kedi değerlendirilmiştir. Olguların yaşların ortalaması (2,8 yıl) olarak belirtilmiştir. Yapılan çalışmada, 32 olguda travmanın nedeni tam olarak bilinmemekte, 30 olguda trafik kazası, 25 olguda düşme, 7 olguda ısırık yaralanması, 4 olguda küt cisim yaralanması, 2 olguda derin yaralanma olarak sınıflandırılmıştır. Bu olgulardaki radyografik değerlendirmede 53 thorasik, 39 abdomen, 34 pelvik, 26 kolumna vertebralis, 28 periferal sinir yaralanması olarak gözlenmiştir. Bu olgulardan 23 tanesi ötenazi edilmiş, 4 tanesi ölmüş ve 73 tanesi yaşamını sürdürdüğü bildirilmiştir. Ali (2013)'nin travmaya uğrayan 28 kedide yaptığı bir diğer çalışmada, 10'u yüksekten düşme (%35,7), 6'sı trafik kazası (%21,4), 4'ü evde yaşanan bir travma (%14,2), 4'ü hayvan ısırığı (%14,2), 3'ü insan kaynaklı şiddet (%10,7) ve 1'i de bilinmeyen bir neden (%3,5) olarak aktarılmıştır.

Sunulan çalışma kapsamında değerlendirilen kedilerde 30 olgunun 20' sinin yüksekten düşme (%66,6), 4' ü trafik kazası (%13,3), 3'ü ısırılmaya bağlı (%10) ve 3'ü de sebebi bilinmeyen (%10), nedenlere bağlı oluştuğu belirlenmiştir. Bu dağılım ile literatür veri ile paralellik gösterdiği gözlemlenmiştir. Fakat sunulan çalışmada

travma nedenini ilk sırada yüksek düşme olmasıyla literatür veri ile farklılık göstermiştir ancak süre ve olgu sayısı daha fazla olduğu takdirde literatüre benzer sonuçlar alınabileceği düşüncesi oluşmuştur.

Yüksekten düşme mesafesi ile oluşan lezyonun şiddeti arasındaki ilişkiyi açıklayan bir çalışmada; 119 kedide, düşüş yüksekliğinin artmasıyla oluşan lezyonun şiddetinin de orantılı olarak arttığı belirlenmiştir. Çalışmada, değişik yükseklikten düşen 43 kedide farklı lezyonların oluştuğu belirlenirken, bu lezyonların düşüş yüksekliği ile doğru orantılı olarak artış göstermediği saptanmıştır. Özellikle düşüş sırasında herhangi bir yere çarpmadan toprak zemine düşmesi yaralanma riskini azaltmaktadır (Vnuk 2004). Yüksekten düşen kediler erken dönemde müdahale edilirse yaşama şanslarının %90 olduğu bildirilmiştir. Çünkü kedilerin düşerken koordinasyon ve denge becerilerinin hayatta kalma ve daha az hasar almalarına neden olduğu bildirilmektedir. Özellikle yapılan çalışmalarda kedilerin yedi kata kadar olan düşmelerinde yaralanma oranlarının fazla olduğu ve yedi kat üzeri düşmelerde bu oranın azaldığı belirtilmiştir (Adams 1996). Buna örnek olarak da 46. kattan düşen kedilerinde yaşadıkları bildirilmiştir. Araştırıcılar bu konuyu kedilerin beş kat kadar saatte 60 millik bir terminal hıza (maksimum hız) ulaşarak düştüklerini belirtmişlerdir. Kedilerin 4. kattan daha yüksekten düşmesi yaralanma oranlarını azaldığı bildirilmiştir. Bunun açıklaması kedilerin düşerken vücut koordinasyonlarını sağlayabilmeleri ve uçan sincaplar gibi yayılma kabiliyetine sahip olmalarına bağlanmıştır. Bu spekülasyon artık yaygın bir gerçek olarak kabul edilmektedir (Adams, 1996). Fakat, düşmeye bağlı, çeşitli thorakal lezyonların olabileceği bildirilmiştir. Sunulan bu çalışmada düşen olgularda en sık gözlenen problemlerin başında plöral efüzyon, pneumothorax ve kırıklar gelmiştir. Radyolojik muayenelerle bu problemler teşhis edilmiştir.

Öztürk ve ark. 2006'da yaptıkları çalışmada travmaya maruz kalmanın cinsiyet ile ilişkisi olabileceği ve erkeklerde dişilere oranla travmatik lezyonların daha sık oluştuğu belirtilmiştir. Bu çalışmada ise olguların cinsiyete göre dağılımı incelendiğinde kedilerin %53,3' nün erkek, %46,6' sını dişilerin oluşturmasıyla literatür verilerle benzerlik göstermiş olmakla birlikte, yüzdelik dilimleri birbirine yakın bulunmuştur.

Sunulan çalışmada ise 30 kedinin travma triyaj puan dağılımı; % 3,3' ünün 0 puana, %6,6'sının 1 puana, %23,3'ünün 2 puana, %33,3'ünün 3 puana, %6,6'sının 4 puana, %13,3'ünün 5 puana, %3,3'ünün 6 puana, %6,6'sının 8 puana ve %3,3'ünün 12 puana sahip olduğu gözlemlenmiştir. Travma triaj puanları düşük olan olguların yaşama oranlarının düşük olduğu belirlenmiştir. 30 kedinin; 6 tanesi yeşil kod (% 20), (Standart bekleme, 120 dakika bekleme ve gözlem süresine sahiptir, 14 tanesi sarı kod (% 46,6), (Acil müdahale, 30-60 dakika bekleme ve gözlem süresine sahiptir), 10 tanesi kırmızı kod (% 33,3) (Hemen müdahale, 0 dakika bekleme süresi) triaj renk kodlaması yapıldı.

Travmalara bağlı oluşabilecek lezyonların önceden bilinmesi ve bunlara yönelik hızlı bir hazırlık yapılması amacıyla, acil kliniklerinde veya acil olgularda Travma Triyaj Skorlama sistemi ve Glasgow Koma Skorlama sisteminin sistematik olarak yapılması önerilmiştir. Travma geçiren bütün olguların genel yaşam fonksiyonları, acele edilmeden fakat seri bir şekilde yapılmalıdır. Özellikle travmalı hastaların torakal radyografilerinin incelenmesi gereklidir. Ayrıca klinik muayenenin yanında ultrasonografik muayeneler de abdominal organ hasarları için önemli bilgiler vermektedir. Karaciğer, dalak, idrar kesesi başta olmak üzere ultrasonografik muayeneler teşhisin doğrulanması açısından çok önemlidir. Travma ile gelen hastalarda karaciğer, dalak ve idrar kesesinde oluşabilecek lezyonlar ultrasonografik muayenelerle anlaşılır.

Sonuç olarak; travma veya başka nedenlerle kliniğe getirilen olgularda ilk başta modifiye Glasgow koma skalası ve hayvan travma triaj skorlaması yapılmasının, klinisyenlere olguların acilliyet durumlarına göre hızlı sınıflandırılma, tedavi protokolü oluşturma ve olgunun prognozu yönünden yararlı ve objektif değerlendirme fırsatı sağladığı belirlenmiştir.

Proje Destek Bilgileri: Bu proje S.Ü.BAP Koordinatörlüğünü tarafından (Proje No. 21202133) desteklenmiştir.

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RESEARCH ARTICLE

Investigation of the Concentrations of Some Essential Elements in LPS-Induced Septicemic Sheep

Fulya ALTINOK YİPEL[1*], Mustafa YİPEL[2]

[1]Hatay Mustafa Kemal University, Samandağ Vocational School, Department of Veterinary Science, Hatay, 31060 Hatay, Turkey
[2] Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, 31060 Hatay, Turkey

ABSTRACT

Endotoxemia, which is defined as an organ disorder due to irregular immunological host response to infection, is a disease that causes serious economic losses as a result of high mortality and morbidity in sheep. In the study, macro (Ca, Mg, Na, K, P) and micro (Fe, Cu, Se, Zn) essential element concentrations of plasma and serum samples; taken from healthy (control) (n=6) and septicemic sheep (n=6) were determined. According to the results, the differences between the groups in terms of Ca, K, and Se concentrations were statistically significant ($p \leq 0.05$). Comprehensive studies on the concentrations and changes of elements using sensitive analysis methods such as ICP-MS are needed to successful diagnose and treat septic patients, identify new biomarkers, and explain their mechanisms.

Keywords: ICP-MS, Macro element, Micro element, Plasma, Septicemia, Serum.

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LPS ile Indüklenen Septisemik Koyunlarda Bazı Eser Element Konsantrasyonlarının İncelenmesi

ÖZ

Enfeksiyona karşı düzensiz immünolojik konak yanıtına bağlı meydana gelen organ bozukluğu olarak tanımlanan endotoksemi, koyunlarda yüksek mortalite ve morbidite sonucu ciddi ekonomik kayıplara neden olan bir hastalıktır. Çalışmada septisemi oluşturulmayan sağlıklı (kontrol) koyunlar (n=6) ile septisemi oluşturulan koyunlardan (n=6) alınan plazma ve serum örneklerinde makro (Ca, Mg, Na, K, P) ve mikro (Fe, Cu, Se, Zn) esansiyel element konsantrasyonları belirlendi. Sonuçlara göre Ca, K ve Se konsantrasyonları açısından gruplar arasındaki farklar istatistiksel olarak anlamlıydı ($p \leq 0.05$). Septik hastalarda başarılı teşhis ve tedavi, yeni biyobelirteç belirlenebilmesi ve mekanizmalarının açıklanabilmesi açısından ICP-MS gibi hassas analiz yöntemlerinin kullanıldığı elementler konsantrasyonları ve değişimleri üzerine kapsamlı çalışmalara ihtiyaç duyulmaktadır.

Anahtar kelimeler: ICP-MS, Makro element, Mikro element, Plazma, Septisemi, Serum.

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ORCID ID; *FAY: 0000-0001-8577-2031 MY: 0000-0002-6390-9313* *Corresponding author e-mail: fulyip@hotmail.com

INTRODUCTION

Bacterial infections are one of the major factors reducing farm animal productivity and raising morbidity and mortality rates. When the immune response, which is a defense mechanism against these infections, becomes unbalanced and cannot be successfully controlled, it can lead to the development of sepsis, which is defined as organ dysfunction resulting from the host's irregular immunological response to the infection (Riquelme et al. 2018, Türkmen 2017).

Lipopolysaccharide (LPS), a bacterial endotoxin, is a glycolipid molecule found in the outer membrane of gram-negative bacteria, such as Escherichia coli (E. coli), and plays a significant role in the pathophysiology of septic shock (Riquelme et al. 2018).

Animal models induced by LPS endotoxin injection have been widely used in sepsis research for many years. Endotoxin's ease of use, practical storage, applicability for controlled experiments, and suitability for standardization are some of the advantages of the endotoxic shock model. When administered to animals, LPS simulates many of the pathological symptoms of septicemia, such as fever, leukopenia, hemodynamic changes, coagulopathies, and complement activation (Redl et al. 1993).

Elements necessary for the maintenance of vital physiological functions for living organisms are structural components of enzymes and cofactors that prevent nutritional deficiencies and diseases, support antioxidant defense, and regulate immune functions and gene expression. They are classified as macro or micro depending on the amount required (Strachan 2010). Calcium (Ca), phosphorus (P), potassium (K), chlorine (Cl), sodium (Na), magnesium (Mg), and sulfur (S) are macro elements that are required in large quantities for normal physiological processes while micro elements such as zinc (Zn), selenium (Se), iron (Fe), manganese (Mn), copper (Cu), chromium (Cr), iodine (I), molybdenum (Mo), lithium (Li) and vanadium (V) are found in trace amounts in organisms. While many of these elements (Cu, Se, Zn, etc.) may have a toxic effect above tolerable concentrations, others can become toxic by interacting with one another. Some elements, such as aluminum (Al), mercury (Hg), and cadmium (Cd), are not required by the organism and can have toxic effects when they exceed tolerable concentrations (Patrashkov 2003, Nordberg et al. 2015, Ali and Khan 2018, Tatara et al. 2018, Zoroddu et al. 2019).

The prevention of the excess of all these elements that may cause toxic effects or the deficiencies that may cause different physiological or pathological effects is provided by the homeostatic mechanism. The regulation of element storage, absorption, and excretion is constantly maintained by homeostasis processes (Goyer, 2004). While this mechanism can be influenced by a variety of factors (age, nutrition, genetics, etc.), some homeostasis issues may arise (Strachan, 2010). Deficiencies or excess states of elements can result in many functional disorders. Blood (whole blood, serum, plasma) concentrations can also determine this deficiency or excess before clinical symptoms arise. Measurement of element concentrations can be helpful in identifying some diseases. Routinely measured biochemical and hematological blood parameters, as well as element profiles, can provide benefits such as obtaining additional information about disease pathogenesis, diagnosis, and prognosis, and assessing the suitability of element supplementation in treatment (Cedeño et al. 2004, Mert et al. 2008, Nordberg et al. 2015, Tuncer et al. 2020). As a result, the goal of this study was to establish how plasma and serum element concentrations change in the event of septicemia/endotoxemia, which causes considerable economic losses and has high death rates, as well as their association with infection.

MATERIAL AND METHODS

Animal Material

The study included 12 sheep (Awassi breed), both experimentally healthy control and septicemia-induced. The sheep had free access to feed and water throughout the study. Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee approved the study permit with the date 30/03/2022 and number: 2022/03-03

Blood samples were taken before septicemia was induced in sheep, and plasma (control plasma) and serum (control serum) were obtained (n=12). The sheep were then given 30 minutes of intravenous LPS toxin (E. coli O55:B5) in 30 ml 10 μg kg-1 0.9% NaCl to induce septicemia. Blood samples from sheep with septicemia (n=12) were used to obtain plasma (septicemia plasma) and serum (septicemia serum).

6 ml blood samples were taken into tubes with heparin and without anticoagulant to obtain plasma and serum from healthy controls and sheep with septicemia. After centrifuging the blood samples at 4000 x g for 10 minutes, the plasma and serum samples were stored at -80 C until analysis.

Element analysis

The concentrations of macro (Ca, Mg, Na, K, P) and micro (Fe, Cu, Se, Zn) elements were determined in serum and plasma samples collected from sheep with and without septicemia. Organic parts of serum and plasma samples were prepared for analysis by burning in a microwave system. P concentrations in prepared solutions were determined using ultraviolet visible light absorption spectroscopy (UV-VIS), Ca, Mg, Na, and K concentrations were determined using atomic absorption spectrometry (AAS), and Fe, Cu, Se, and Zn concentrations were determined using inductively coupled plasma-mass spectrometry (ICP-MS).

Statistical analyzes

The study's findings were evaluated using a statistical program at the end of the study (SPSS, IBM, USA). ANOVA and the Tukey HSD test were used to statistically analyze the data. Significance concentration was accepted as $p \leq 0.05$.

RESULTS

Macro (Ca, Mg, Na, K, P) and micro (Fe, Cu, Se, Zn) element concentrations in plasma and serum samples from healthy and septicemia-induced sheep were measured in the study. Tables 1 and 2 compare plasma and serum macro and micro essential element concentrations (mg L-1) (mean±SE) between groups.

Table 1. Plasma and serum macro-essential element concentrations of the groups (mg L-1) (mean±SE)

	Ca	Mg	Na	K	P
Control plasma (n=6)	100.42±21.85[a]	44.26±2.96	2771.67±148.64	184.58±10.58[a]	92.08±11.77
Control serum (n=6)	67.08±5.38[ab]	42.14±2.61	3620.83±462.76	182.50±5.16[a]	114.58±16.06
Septicemia plasma (n=6)	58.33±5.83[b]	42.24±2.90	3155.83±300.49	177.50±5.77[a]	144.41±21.13
Septicemia serum (n=6)	55.42±2.92[b]	47.16±2.05	2874.17±125.87	154.58±4.93[b]	118.12±9.62
P	0.05	0.51	0.20	0.03	0.15
Reference ranges (Puls 1994, Radostits et al. 2006, Kaneko et al. 2008)	92-102	28-40	4647-4871	125-173	52-76

Differences in letters within the same column are statistically significant ($p \leq 0.05$).

Table 2. Plasma and serum micro (trace) essential element concentrations of the groups (mg L-1) (mean±SE)

	Fe	Cu	Se	Zn
Control plasma (n=6)	3.23±0.36	0.80±0.07	0.16±0.02[a]	1.22±0.30
Control serum (n=6)	4.69±0.59	0.78±0.06	0.10±0.02[a]	0.72±0.09
Septicemia plasma (n=6)	4.26±0.56	0.67±0.07	0.11±0.02[ab]	0.64±0.05
Septicemia serum (n=6)	5.72±2.35	0.67±0.11	0.08±0.01[b]	0.59±0.13
P	0.58	0.50	0.02	0.07
Reference ranges (Puls 1994, Radostits et al. 2006, Kaneko et al. 2008)	1.66-2.22	0.58-1.6	0.08-0.5	0.8-1.5

Differences in letters within the same column are statistically significant ($p \leq 0.05$).

The findings revealed statistically significant differences in Ca, K, and Se concentrations between the groups ($p \leq 0.05$) in the study. Ca concentrations were found to be significantly lower in septicemia groups (septicemia plasma and septicemia serum) than in the control plasma group. The septicemia serum group's K concentrations were also found to be significantly lower than the other groups. Furthermore, when the septicemia groups were compared, it was found that the plasma K concentration (septicemia plasma) increased, which was statistically significant, more than the serum K concentration (septicemia serum). The concentration of selenium (Se) was found to be significantly higher in the healthy groups (control plasma and control serum) than in the septicemia serum group.

Serum Ca, Cu, Se, and Zn concentrations were within normal limits, Na concentrations were low, and Mg, K, Fe, and P concentrations were high when the data from the control group were compared to the reference ranges.

DISCUSSION

Animals require around 30 of the 103 elements in the periodic table to grow, develop, and survive. Of these, 16 are considered essential trace elements (Se, Zn, Cu, Fe, Mo, Mn, etc.). Because trace elements are difficult to detect in biological tissues and require careful measurements, this categorization was created first. Despite the fact that the development of new instruments has made measurements easier, the term "trace elements" is still used (Kaneko et al. 2008). AAS and ICP-MS were used in this study to determine the concentrations of macro and micro (trace) essential elements in sheep plasma and serum samples, which are more sensitive and reliable approaches than the measuring methods frequently used in clinics.

The link between the elements and the risk of infection and nutritional deficits is complicated because of the metabolic changes in this process (Saner et al. 2000).
Although the results for Na concentration in sheep serum samples are lower than the reference values (Underwood 1999, Radostits et al. 2006), Na deficiency is not a serious condition and Fan et al. (2020) is similar to their study findings.
P serum concentration were above the reference range in the all groups of current study while found to be low compared to other studies (843-1910 mg L-1) (Underwood 1999, Radostits et al. 2006, Fan et al. 2020).
In the previous two decades, Zn, Cu, Se, and other key trace elements have been discovered to have a critical role in modifying the immune response and changing the risk of infection (Saner et al. 2000). The concentrations of elements, such as Cu, Se, and Zn, in sheep tissues can be affected by differences in soil (in terms of plants used in diet) and water concentrations in the region where the sheep are raised, the ration contents, genetic factors, climate conditions, pregnancy, and antagonistic interactions of elements with each other (Erdoğan et al. 2003).
Animals require Se as a trace element. The normal functioning of bone and endocrine metabolism, immunological, endocrine, and reproductive processes all require a sufficient concentration of Se (Huo et al. 2020). Se deficiency in sheep is linked to a variety of clinical conditions, including reproductive problems, muscle degeneration, and cell membrane damage, in addition to its toxic effects. As a result, clinical Se status monitoring is critical. The results are evaluated differently depending on the species, age, race, analysis method, and sample type (Pamukçu et al. 2001). Various ranges of values have been reported in studies. Naghadeh et al. (2015) determined plasma Se concentrations to be 0.01-0.29 mg L-1, Humann-Ziehank et al. (2016) reported that that plasma Se concentrations vary between 1.24 and 21.6 mg L-1 in their study. In their study, Yeltekin et al. (2018) discovered very low values in the control group (0.028 mg L-1) that were associated with regional differences and nutrition. It has been reported that Se can be toxic in this context, and caution should be exercised when using it as a supplement in non-deficient areas (Vázquez-Armijo et al. 2011). While Se concentrations were within normal limits in all groups in the current study, it was found to be significantly higher in healthy groups (control plasma and control serum) than in the septicemia serum group. Yeltekin et al. (2018) found in their study that Se concentrations in sheep with contagious ecthyma were lower than in healthy sheep (0.013-0.028 mg L-1). Huo et al. (2020) discovered that lambs with clinically aberrant gait, weakness, anemia, polypnea, and arrhythmia had significantly lower plasma Se concentrations than healthy sheep.
Despite the lack of a reference study on variations in element concentrations in septicemic sheep, the link between diseases and elements in several animal species has attracted interest. However, the roles and interactions of elements with bacterial diseases have yet to be fully understood (Beisel 1976, Middleton et al. 2004).
Essential trace elements such as iron, Zn, and Cu have been linked to infection and sepsis in living organisms (Coşkun et al 2020). In 25 newborn calves with sepsis, Coşkun et al. (2020) found a large increase in K and Cu concentrations and a significant decrease in Na and Ca concentrations. The same study noted that K, Cu, Na, and Ca elements may play a crucial role in functional impairments caused by sepsis. Similar findings were achieved in the current investigation, and Ca, Se, and K concentrations were shown to be considerably lower in the septicemia groups.
In an E. coli-induced intraperitoneal sepsis model, Zn, Cu, and Fe concentrations were found to increase in rats (Konukoğlu et al. 2001). Again, serum Cu concentrations increased significantly after 12, 24, and 72 hours of endotoxin injection in hamsters, although serum Zn concentrations decreased after 4-48 hours (Etzel et al. 1982).
Serum Fe, Zn, and Cu concentrations were found to be lower in infants with sepsis syndrome than in the control group in another investigation, and it was suggested that Zn concentrations, in particular, could be a prognostic indicator of sepsis in infancy. According to the findings of this investigation, the plasma and serum Zn, Fe, and Cu concentrations of sheep with septicemia exhibited no significant changes.

CONCLUSION

In conclusion, significant differences in Ca, K, and Se concentrations were detected in healthy and septicemic sheep in the current study. More comprehensive research is needed to improve diagnosis and treatment, get new biomarkers, and explain the mechanisms involved in light of the information about the state and changes of elements in septic patients. The present study will contribute to the current literature by linking macro and micro (trace) necessary elements with different diseases and determining their amounts using more sensitive and up-to-date analysis methods such as AAS and ICP-MS.

Conflict of Interest: No potential conflict of interest was reported by the authors.

Authorship Contributions: The authors contributed equally to this work

Ethical Approval: The study was conducted under permission (Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee 30/03/2022 tarih No: 2022/03-03).

KAYNAKLAR

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RESEARCH ARTICLE

Dexpanthenol Inhibits Inflammation and Apoptosis in LPS-Induced Acute Lung Injury by Reducing Increased VCAM-1 and Caspase-3 Expressions in Rats

Nasıf Fatih KARAKUYU[1*], Özlem ÖZMEN[2]

[1]*Suleyman Demirel University, Faculty of Medicine, Department of Pharmacology, 32200, Isparta, Turkey*
[2]*Mehmet Akif Ersoy University, Faculty of Veterinary, Department of Pathology, 15030, Burdur, Turkey*

ABSTRACT

This study aims to investigate the effects of Dexpanthenol (Dex), a stable alcoholic analogue of D-pantothenic acid which has anti-oxidant, antiapoptotic, and antiinflammatory properties, on lipopolysaccharide (LPS)-induced lung damage via caspase-3 (cas-3) and vascular cell adhesion molecule-1 (VCAM-1) levels. According to the experimental plan of study, thirty-two Wistar Albino rats were distributed randomly into four groups as control, LPS (5 mg/kg, intraperitoneally (i.p), single dose), LPS (30 minutes before last Dex treatment) + Dex (500 mg/kg, i.p, for 3 days) and Dex. After six hours of LPS application, lung tissues of the rats were taken for histopathological, immunohistochemical and biochemical examinations. According to results of the study, LPS caused hyperemia, neutrophil leukocyte chemotaxis and thickened septal tissue on lung. Inducing inflammation by increasing VCAM-1 expression and triggered apoptosis by increasing cas-3 expression in lung tissue. In addition, LPS decreased total antioxidant status levels, which is a marker of anti-oxidant capacity, and increased oxidative stress index and total oxidant status values, which are indicators of oxidative stress. Dex has shown its effect by reversing all these alterations and normalizing the values. These results suggest that Dex can be used as a preservative to reduce LPS-induced acute toxicity in the lung.

Keywords: Cas-3, Dexpanthenol, Inflammation, Lung, VCAM-1

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Dekspantenol, Sıçanlarda LPS'nin Neden Olduğu Akut Akciğer Hasarında Artan VCAM-1 ve Kaspaz-3 Ekspresyonlarını Azaltarak İnflamasyonu ve Apoptozu İnhibe Eder

ÖZ

Bu çalışma, D-pantotenik asidin antioksidan, antiapoptotik ve antiinflamatuar özelliklere sahip stabil bir alkolik analoğu olan Dekspantenol'ün (Dex), lipopolisakkarit (LPS) kaynaklı akciğer hasarı üzerindeki etkilerini vasküler hücre adezyon molekülü-1 (VCAM-1) ve kaspaz-3 (cas-3) seviyeleri üzerinden incelemeyi amaçlamaktadır. Çalışmanın deneysel planına göre otuz iki adet Wistar Albino sıçan rastgele dört farklı gruba ayrıldı: Kontrol, LPS (5 mg/kg, intraperitoneal (ip), tek doz), LPS (son Dex uygulamasından 30 dakika önce) + Dex (500 mg/kg, ip, 3 gün boyunca) ve Dex. LPS uygulamasından 6 saat sonra sıçanların akciğer dokuları histopatolojik, immünohistokimyasal ve biyokimyasal incelemeler için alındı. Çalışmanın sonuçlarına göre LPS, akciğerde hiperemi, nötrofil lökosit kemotaksisi ve kalınlaşmış septal dokuya neden oldu. Akciğer dokusunda VCAM-1 düzeylerini artırarak inflamasyonu indükledi ve cas-3 düzeylerini artırarak apoptozu tetikledi. Ayrıca LPS, antioksidan kapasitenin bir belirteci olan total antioksidan status düzeylerini düşürürken, oksidatif stresin göstergeleri olan total oksidant status ve oksidatif stres indeks değerlerini artırdı. Dex, tüm bu değişiklikleri tersine çevirerek ve değerleri normalleştirerek etkisini gösterdi. Bu sonuçlar, Dex'in LPS kaynaklı akut akciğer hasarında toksisiteyi azaltmak için bir koruyucu olarak kullanılabileceğini düşündürdü.

Anahtar Kelimeler: Akciğer, Cas-3, Dekspantenol, İnflamasyon, VCAM-1

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ORCID ID; *NFK: 0000-0002-2249-4668, ÖÖ: 0000-0002-1835-1082*

*Corresponding author e-mail: fatihkarakuyu03@gmail.com

INTRODUCTION

Acute lung injury (ALI) is one of the main causes of mortality, especially in intensive care patients (Bellani et al. 2016). It is seen clinically due to blood transfusions and it can be experimentally induced by acid aspiration, pulmonary ischemia-reperfusion, and sepsis (Matute-Bello et al. 2011).

There is a complex inflammatory process in the pathogenesis of ALI, and recent studies have focused on basement membrane destruction in the development of ALI. Endothelial cells, intrapulmonary leukocytes and their inflammatory products cause ALI by interacting with lung parenchyma cells. (Ashbaugh et al. 2005, Matute-Bello et al. 2008, Butt et al. 2016).

Lipopolysaccharide (LPS), a glycopeptide taking part in the external membrane of gram-negative bacteria, induces inflammation in rat models by acting Toll-like receptor-4 on monocytes, macrophages and other cells (Raetz and Whitfield 2002). This receptor signaling triggers many intracellular post receptor mechanisms and causes apoptosis with an increase of caspase-3 (cas-3) expression and inflammation with an enhancement of vascular cell adhesion molecule-1 (VCAM-1) expression (Wang et al. 2019). It also causes oxidative stress by triggering the formation of reactive oxygen species (Park et al. 2015).

Dexpanthenol (Dex) is a stable alcoholic analogue of D-pantothenic acid (Vitamin B5), which is used especially in wounds, irritations and wrinkles, is easily accessible due to its cheapness, and is frequently found in local pharmacies (Heise et al. 2012, Stettler et al. 2017). In previous studies; it has been proven to be effective against oxidative stress on heart tissue (Kose et al. 2020), against nephrotoxicity (Pınar et al. 2020), hair strengthening (Shin et al. 2021), and prevention of hepatotoxicity (Ucar et al. 2018).

In this study, it was aimed to prove the preservative effect of Dex in the case of ALI in rats.

MATERIAL AND METHODS

Ethical Approval

All experiments were performed in accordance with the ARRIVE (Animal Research: Reporting in Vivo Experiments) guidelines in 2.0, and study approved by the Local Ethical Committee on Animal Research of Suleyman Demirel University (No:2022-02/29).

Study animals and design of experiment

A total of 32 adult female, Wistar Albino rats weighing 310–360 g were placed in a temperature controlled room (about 22°C) with specific humidity (60%[±5]) conditions and 12 hour dark/12 hour light cycle was maintained. Each rat was fed a standard commercial diet (Korkuteli Yem, Antalya, Türkiye).

Experimental design of this study was shown in Figure 1.

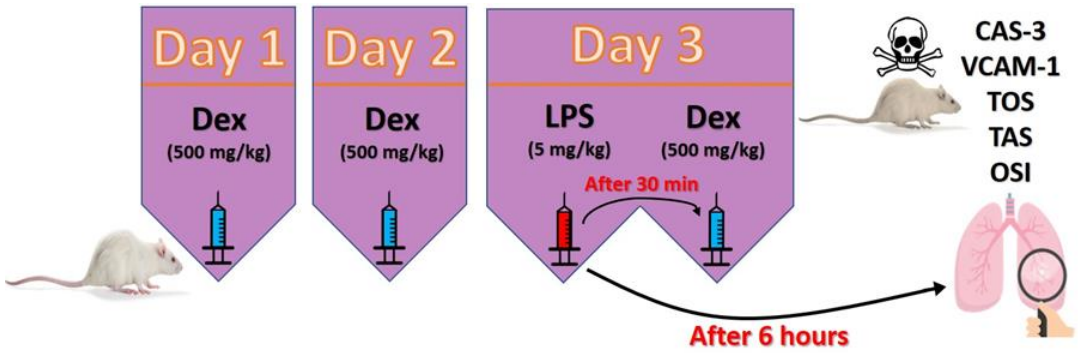

Figure 1: Experimental design of this study

The rats were divided into the following four groups eight rats each. Groups as;

1-Control Group (n = 8); 1 ml of isotonic saline solution given to the rats, intraperitoneally (i.p.) once a day for three days from the left inguinal area and one dose of 1 ml isotonic saline solution i.p. from the right inguinal region of the rats on the third day, 30 minutes before the last saline application.

2- LPS Group (n = 8); 1 ml of saline given to the rats by i.p. injections, once a day for three days from the left inguinal region and a single dose of 5 mg/kg, 0.5-1 ml LPS (048K4126, Sigma Aldrich, USA) i.p. was applied from the right inguinal area of the rats on the third day, 30 minutes before the last saline application.

3- LPS + Dex Group (n = 8); 1 ml, 500 mg/kg Dex (Bepanthene, Bayer Türk Kimya, Türkiye) given to the rats by i.p. once a day for three days from the left inguinal region and a single dose of 5 mg/kg, 0.5-1 ml LPS i.p. was applied from the right inguinal area of the rats on third day 30 minutes before the last Dex application.

4- Dex Group (n = 8); 1 ml, 500 mg/kg Dex given to the rats by i.p. once a day for three days from the left inguinal region and one dose of 1 ml saline solution i.p. from the right inguinal area of the rats on third day 30 minutes before the last Dex application.
Six hours after LPS application, 85-105 mg/kg Ketamine (Keta-Control, Doğa İlaç, Türkiye) and 9-11 mg/kg Xylazine (Rompun, Bayer, Germany) were administered to all rats. Blood was taken from the vena cava inferior for euthanasia from the rats who underwent abdominal incision following anesthesia. One-half of the lung specimens were placed in liquid nitrogen and stored at -25 °C until biochemical analysis. The remaining lung specimens were fixed with 10% neutral buffered formalin for immunohistochemical and histopathological analysis.

Histopathological examination
Lung tissues of rats were taken and fixed in 10% neutral buffered formalin. After fixation, tissues were regularly processed by a fully automated tissue processing equipment (Leica ASP300S; Leica Microsystem, Nussloch, Germany) and embedded with paraffin. Then, 5μm sections were taken from paraffin blocks with a Leica RM 2155 RT microtome (Leica Microsystem, Nussloch, Germany). After 1 day of drying, the slides were passed through xylol and alcohol series. Then stained with Hematoxylin–Eosin (HE) and analyzed through a light microscope.
Lung damage was evaluated in 10 different randomly selected fields under 20x objective for each rat. For injury scores, hyperemia, edema, increase in septal tissue thickness and necrosis were evaluated between 0-4. Accordingly, 0, no damage; 1, damage 1-25% of the field; 2, damage on 26-50% of the field; 3, damage 51-75% of the field; and 4 were assessed as damage above 76%. The score of each animal was found by dividing the total score by the number of fields examined and rounding up this number. Statistical analysis was performed on these data and the difference between the groups was found.

Immunohistochemical examination
Further, two series of sections taken from all blocks drawn on poly-L-lysine coated slides and were stained immunohistochemically for cas-3 (Anti-cas-3 Antibody (E8): sc7272, 1/100 dilution), and VCAM-1 (VCAM-1 (M/K-2):sc-18864, 1/100 dilution) (Santa cruz,Texas, USA) expressions by streptavidin biotin method according to manufacturer's directive. Antibody Diluent (ab64211) (Abcam Cambridge, UK) was used for dilution of the primary antibodies. For antigen retrieval, sections were boiled twice in citrate buffer solution (pH 6.00) using 700 MW irradiation. Sections were incubated with primary antibodies for 12 hours and immunohistochemical analysis was performed. Streptavidin-biotin immunoenzymatic antigen detection system [EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436), (Abcam, Cambridge, UK)] was used for detection. All evaluations were performed on blind specimens by, pathologists from different centers.
Sections were examined one by one for each antibody for immunohistochemical analysis. Ten different areas in each segment were scanned for evaluation under 40X objective magnification.
All slides were analyzed for immunopositivity, and a semiquantitative analysis was carried out. Samples were analyzed by examining five different sections for each sample and each section was then scored from 0 to 3 according to the intensity of staining (0, absence of staining; 1, slight; 2, medium; and 3, marked staining). Rats included in this study were evaluated randomly in a blinded fashion by the researcher without knowing which rat was included in which group. For morphometric examinations of sections, an Olympus CX41 light microscope was used. Morphometric evaluations were made by using the Database Manual CellSens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

Biochemical Analysis
Oxidative stress in lung tissue was analyzed by the following steps: Lung tissues were diluted with 5x (w/v) phosphate buffered saline (10 mM pH 7,4) and homogenized using Janke&Kunkel IKA Ultra-Turrax T25 (Germany) tissue homogenizator. After the homogenization process, the samples were centrifuged at +4°C at 2000 rpm for 20 minutes (Nüve NF 1200 R, Turkey). From the supernatants, tissue total antioxidant status (TAS) and total oxidant status (TOS) levels were measured on Beckman Coulter AU 5800 biochemistry autoanalyzer (USA), and also oxidative stress index (OSI) levels of the samples were calculated (Erel 2004a).
TAS levels were measured using an automated colorimetric measurement method developed by Erel (Erel 2004b). The tissue TAS levels were expressed as μmol trolox equiv/l.
TOS levels of lung tissue were measured using an automated colorimetric method and the results were expressed as μmol H2O2 equiv/l (Erel 2005). OSI was calculated using the formula: OSI = TOS/TAS.

Statistical Analysis
The immunohistochemical findings of the groups were compared between the groups for statistical analysis. For this comparison, One-way ANOVA, Duncan, LSD tests (posthoc tests) were used with SPSS-22.00 package program. Significance level was accepted as $P < 0.05$.

RESULTS

Histopathological Findings
At the histopathological examination, marked hyperemia and increased septal tissue thickness in LPS group was observed. Emphysema was also a

common finding in lungs in LPS group. Dex treatment decreased pathological findings. Normal tissue structure was observed in control and Dex groups. Histopathological appearances are shown in Figure 2.

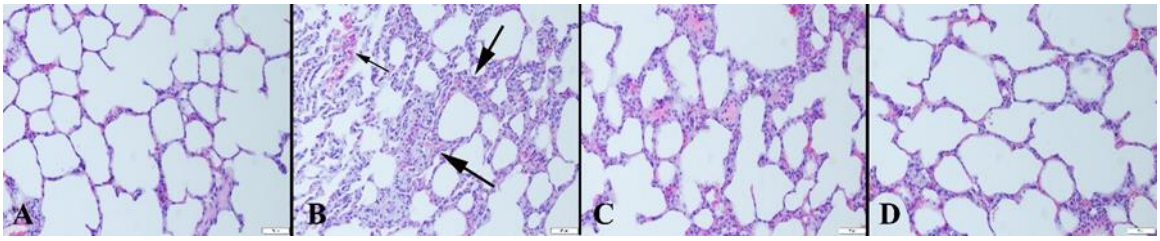

Figure 2: Histopathological view of groups. (A) Normal tissue structure in control group, (B) neutrophil leukocyte chemotaxis and hyperemia (thin arrow) and thickened septal tissue (thick arrows) in LPS group, (C) Decreased hyperemia and septal tissue thickness in LPS+Dex group, (D) Normal lung tissue histology in Dex group, HE, scale bars=0.05mm.

Immunohistochemical Findings

Immunohistochemical examination revealed significantly increased expressions for both cas-3 and VCAM-1 in LPS group (1,50±0,53, 1,12±0,64; respectively) compared to control (0,12±0,35; for both markers) ($p<0.001$; for both markers). Dex treatment (LPS+Dex group) decreased immunohistochemical expressions of both markers (0,37±0,51, 0,25±0,46; respectively) compared to LPS group (Figures 3-4) ($p<0.001$; for both markers). No or very slight expressions noticed in control group and Dex group (0,12±0,35, 0,25±0,46; respectively). Statistical analysis results shown in Figure 5.

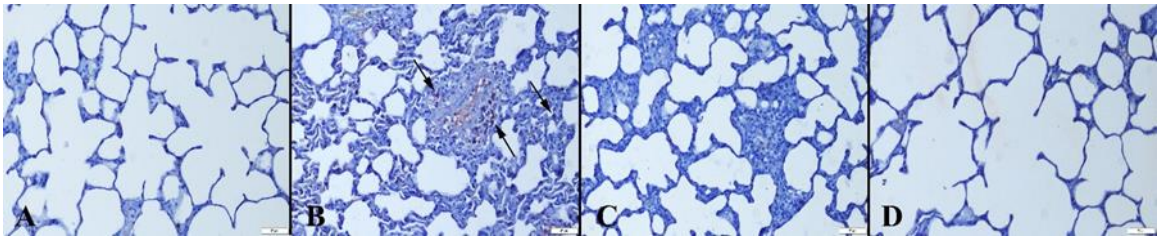

Figure 3: Cas-3 immunohistochemistry findings between the groups. (A) Negative expression in control group, (B) increased expressions (black arrows) in LPS group, (C) decreased expression in LPS+Dex group, (D) no expression in Dex group, Streptavidin-biotin peroxidase method, scale bars=0.05mm.

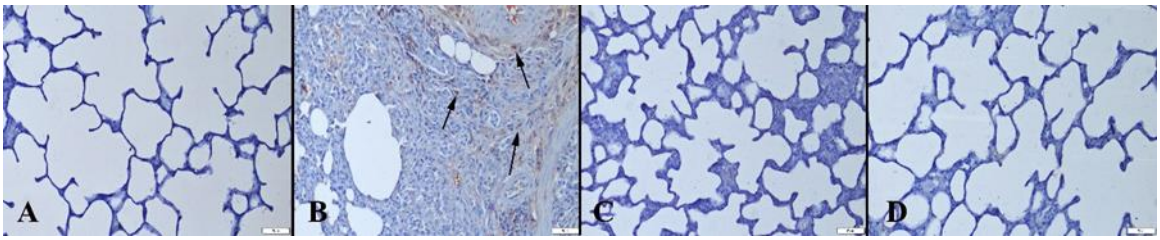

Figure 4: VCAM-1 immunohistochemistry results among the groups. (A) Negative immunoreaction in control group, (B) marked expressions (black arrows) in LPS group, (C) decreased immunoexpression in LPS+Dex group, (D) no expression in Dex group, Streptavidin-biotin peroxidase technique, scale bars=0.05mm.

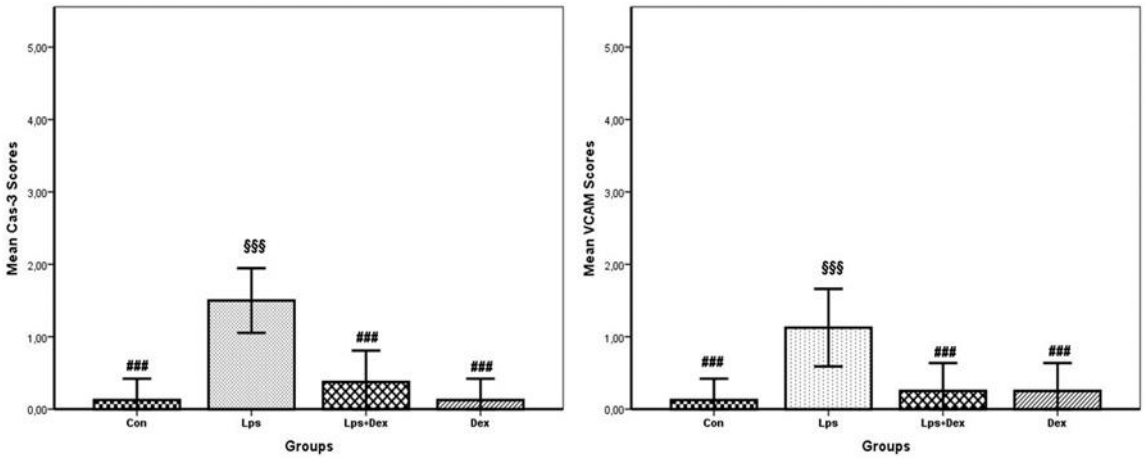

Figure 5: Statistical analysis of immunohistochemical scores. The dissimilarity between the means of groups carrying different signs between the groups are statistically significant, P<0.001. Data standard deviation (SD). Differences between groups and results of immunohistochemical scores are assessed by One way ANOVA test (post hoc Duncan test).

Biochemical Results

As shown in Table 1, the levels of TOS and OSI were significantly higher compared with control group (p=0.023 and p=0.002; respectively) and the grades of TAS were significantly lower in LPS group compared with control group (p=0,043). In the LPS+Dex group, TOS and OSI grades were lower while the TAS grades were higher than LPS group but not significant (p=0,214 for TOS; p=0,358 for OSI; p=0,508 for TAS). On the other hand, TOS and OSI grades were higher in the LPS+Dex group compared to the control group, and TAS grades were lower, but not significant (p=0,264 for TOS; p=0,105 for OSI; p=0,159 for TAS). In the Dex group, TAS grades were significantly higher compared to the LPS group (p=0,024), TOS and OSI grades were significantly lower compared to LPS group (p≤0.001 for both) and LPS+Dex group (p=0.017 and p=0.005; respectively).

Table 1. Oxidative stress markers of lung tissues in rats

	TOS (mmol H2O2Equivalents/L)		**TAS (mmol TroloxEquivalents/L)**		**OSI (TOS/[TAS*10])**	
GROUPS	**Mean ± SD**	***p* value**	**Mean ± SD**	***p* value**	**Mean ± SD**	***p* value**
CONTROL	53,11 ± 9,48		1,14 ± 0,15		4,76 ± 1,32	
LPS	62,78 ± 2,66[a]	**a:**p=0.023	0,99 ± 0,11[a]	**a:**p=0.043	6,36 ± 0,70[a]	**a:**p=0.002
LPS+Dex	57,68 ± 8,08		1,04 ± 0,16		5,56 ± 0,59	
Dex	47,50 ± 9,76[b, c]	**b:**p≤0.001 **c:**p=0.017	1,16 ± 0,12[b]	**b:**p=0.024	4,12 ± 1,02[b, c]	**b:**p≤0.001 **c:**p=0.005

Values are presented as means±SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA (posthoc LSD). 'a' represents comparison with control group, 'b' represents comparison with LPS group, 'c' represents comparison with LPS+Dex group LPS – Lipopolysaccharide; Dex – Dexpanthenol; SD – Standard Deviation.

DISCUSSION

Lungs are one of the vital organs that are at the top of the list of organs necessary for life. Lungs are frequently exposed to infectious factors due to their close relationship with the external environment or their intense blood supply. Neutrophil leukocyte infiltrates cause increase in inflammatory cytokines and protein leakage in acute pulmonary inflammation, are common findings in ALI. In the acute phase of ALI, neutrophils are the most abundant inflammatory cells at the site of injury and this situation is vital to host defense. However, excessive activation of neutrophils triggers tissue damage by releasing various inflammatory mediators. Overall, in treatment studies, reduced neutrophil counts were associated with a favorable prognosis. Because cytokines not only cause recruitment and activation of neutrophils

at the site of inflammation, but also cause severe inflammatory damage. Neutrophil infiltration and cytokine release particularly cause endothelial and epithelial cell damage (Li-Mei et al. 2016). In this study, intraperitoneal LPS application caused a significant inflammatory reaction in the lungs and Dex treatment improved both histopathological and immunohistochemical findings.

Sepsis-related lung injury is frequently observed in the community (Varisco 2011). In this case, the lung can be heavily affected due to the high vascularization of systemic inflammation. Oxidative and pro-inflammatory cytokines circulating in the blood bind to their surface receptors in every tissue they encounter, triggering some intracellular signaling mechanisms. As a result, a cumulative damage picture may occur as some cytokines synthesized in that cell affect other nearby cells (Si-Cong et al. 2021). Neutrophil leukocytes are the first defense cells to come to the damaged area to tissue repair. In order to invade the damaged tissue, these leukocytes must adhere to some adhesion molecules while circulating in the vessel, approach the vessel endothelium and be extravasated by taking advantage of the increase in permeability. As Qureshi et al. mentioned in their study, VCAM-1, which increases in a TNF-α-dependent manner and interacts with neutrophils, is one of these adhesion molecules (Qureshi et al. 2003). There are many studies, such as the studies of Vogel et al., Alapati et al., on reducing the expression of VCAM-1 in inflammatory or pathologic conditions (Alapati et al. 2015, Vogel et al. 2017).

Detection of these neutrophil leucocytes in the damage area with various analysis methods shows that the event is acute. The scene of neutrophilic leukocytosis detected in the LPS group in this study, also supports this view. The fact that Dex causes a decrease in cell density also creates the impression that the intraperitoneally administered drug can be used in acute events. In addition, as demonstrated immunohistochemically, it can be said that the reduction of VCAM-1 expressions, which was increased in the LPS group, by Dex is effective in reducing neutrophilic leukocytosis.

The anti-inflammatory properties of Dex were proven on nephrotoxicity in the Pınar et al research and on cardiotoxicity in the Kose et al research (Kose et al. 2020, Pınar et al. 2022). Cytokine-mediated increase in permeability in vascular structures in the area of inflammation and, accordingly, edematous scene and hyperemia in that tissue can be observed. These developing forms of damage cause septal thickening and dyspnea in the thin and expandable lung tissue. The hyperemia and septal thickening detected in the injury group of this study were reduced by Dex, indicating that it prevented the progression of the damage in the lung tissue.

Although apoptosis is generally described as programmed cell death, some external factors can lead healthy cells to apoptosis (Naim et al. 2005). Caspases are enzymes that play an important role in apoptosis. These enzymes, which are primarily synthesized as inactive proteins, are activated in various ways. A lot of cellular and morphological transformations that consist during cell death, develop as a result of a number of processes in which these enzymes play a role (Berger.et al., 2006). In the study of Miao et al., LPS was shown to cause embryonic damage due to apoptosis via cas-3 (Miao and Cui 2022).

As it is known, inflammation also contributes to the formation of oxidant molecules circulating in the blood and oxidative stress that occurs in the tissue. It is known that these two important damage mechanisms trigger apoptosis resulting in tissue cell death. Activation/inhibition of the aforementioned intracellular pathways mediate cell to undergo apoptosis. The increased cas-3 levels detected in the lung tissues of the injury group in this study, indicate that apoptosis due to this LPS-induced systemic inflammatory condition also occurs in the lung. Dex, which is normally used as an epithelial reparative, has a reducing effect on cas-3 levels, which can be considered as an important finding.

The significant increment in OSI and TOS levels in the injury group indicates that oxidative stress is stimulated in the lung tissue. In addition to this, the significant decrease in TAS is interpreted as the tissue uses the endogenous antioxidant system in the fight against oxidant substances and therefore shows a decrease. The fact that there was no significant change in the Dex applied group compared to the control, indicates that the drug is not harmful to the lung tissue. Moreover, the fact that the amount of antioxidant enzymes in the treatment groups are higher than in the damage group may indicate that the drug reduces oxidative stress secondary to the suppression of inflammatory conditions, or it can be interpreted that the drug administration time-dose requires a change, since the experimental model is an acute model. For this situation, the ideal use of the drug should be determined by studying other models that include different durations and use different doses.

As it was demonstrated by Li-Mei et al. (2016), Dex is improving histological structure of lungs by arranging the changes in tumor necrosis factor alpha, interleukin-6, malondialdehyde, superoxide dismutase, and glutathione. In this study, we obtained results that supports the findings of Li-Mei et al. showed that Dex is providing a protective effect through oxidative stress markers, cas-3 and VCAM-1 levels and improve the histological structure similar to the previous study (Li-Mei et al. 2016). Moreover, the innovative aspects of the study are that Dex, which is used in another indication and is easy to obtain, regresses the serious inflammatory response in the lung, by reducing the levels of VCAM-1, an adhesion molecule, and by reducing the apoptotic process that causes cell death, by reducing the levels of caspase-3,

where various apoptotic pathways intersect in the cell. Thanks to this feature, it is predicted that it can be used in many pathological processes using the same mechanisms and can regress the damage scene.

CONCLUSION

As a result, with Dex application, inflammation and apoptosis were prevented by reducing VCAM-1 and cas-3 expressions in lung injury secondary to systemic inflammation. It is obvious that the intracellular pathways of this active substance should be investigated in animal models in which different duration and dose applications are made to solve the mechanism of action. Unraveling these mechanisms may shed light on many new areas for the indications for use of this drug.
The results of this study showed that Dex may be a potential drug choice for the treatment of the LPS induced lung damage. Further studies are needed on this subject.

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RESEARCH ARTICLE

Determination of Morphological Characteristics of Tumbler Pigeons Reared in Kırıkkale Province

Uğur Coşkun ÖZÇELİK [1], Evren ERDEM[2], Bora ÖZARSLAN[2]*

[1]Ministry of Agriculture and Forestry, Kırıkkale Directorate of Provincial Agriculture and Forestry, 71000, Kırıkkale, Turkey
[2] Department of Animal Breeding and Husbandry, Faculty of Veterinary Medicine, Kırıkkale University, 71450 Kırıkkale, Turkey

ABSTRACT

The aim of this research was to determine the morphological characteristics of Kırıkkale tumbler pigeons. For this purpose, morphological characteristics were determined in 80 pigeons from 7 different breeders in Kırıkkale province. The body weight (P<0.05), body length (P<0.001), wing length (P<0.05), head width (P<0.01), and beak depth (P<0.001) were significantly influenced by sex. Male pigeons had higher values than female pigeons for these traits. Age group affected body weight, chest width, and chest depth. Although age group II was higher than age group I in terms of body weight, the age group I was higher than age group II in terms of chest width and chest depth (P<0.05). As a result of the study, it was determined that most of Kırıkkale tumbler pigeons had brown-eyed (89.53 %) and small muff (78.48 %), and there was a high rate of individuals with gray plumage color (35.16 %) without a crest (45.35 %). Body weight, body length, wing length, thoracic perimeter, and head width values of Kırıkkale tumbler pigeons were lower than the study for Squadron flyer but higher than the Alabadem and Muradiye Dönek pigeons. Kırıkkale tumbler pigeons had similar values to Ankara tumbler pigeons in terms of morphological characteristics. It can be suggested that the genetic relationship level between Ankara pigeons and Kırıkkale pigeons should be clarified by genetic studies.

Keywords: Breeding, Morphological caharacteristics, Tumbler pigeon, Kırıkkale

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Kırıkkale İlinde Yetiştirilen Taklacı Güvercinlerde Morfolojik Özelliklerin Belirlenmesi

ÖZ

Bu araştırmanın amacı Kırıkkale taklacı güvercinlerinin morfolojik özelliklerini belirlemektir. Bu amaçla Kırıkkale ilinde 7 farklı yetiştiriciden 80 güvercinde morfolojik özellikler belirlenmiştir. Canlı ağırlık (P<0.05), vücut uzunluğu (P < 0.001), kanat uzunluğu (P<0.05), baş genişliği (P<0.01) ve gaga derinliği (P<0.001) cinsiyetten önemli ölçüde etkilenmiştir. Bu özelliklerde erkek güvercinler dişi güvercinlerden daha yüksek değerlere sahip olmuştur. Yaş grubu canlı ağırlık, göğüs genişliği ve göğüs derinliğini etkilemiştir. Canlı ağırlık bakımından yaş grubu II, yaşlı grubu I'den daha yüksek olmasına rağmen, göğüs genişliği ve göğüs derinliği bakımından yaş grubu I, yaş grubu II'den daha yüksek olmuştur (P<0.05). Bu araştırmanın sonucu olarak Kırıkkale taklacı güvercinlerinin çoğunun kahverengi gözlü (% 89.53) ve yıldız paçalı olduğu (% 78.48), gri dona sahip (% 35.16) ve tepesiz (% 45.35) bireylerin oranının yüksek olduğu belirlenmiştir. Kırıkkale taklacı güvercinlerinin vücut ağırlığı, vücut uzunluğu, kanat uzunluğu, göğüs çevresi ve baş genişliği değerleri filo güvercinleri için yapılan çalışmaya göre daha düşük, Alabadem ve Muradiye Dönek güvercinlerine göre yüksek olmuştur. Kırıkkale taklacı güvercinleri morfolojik özellikler yönünden Ankara taklacı güvercinlerine benzer değerlere sahiptir. Ankara güvercinleri ile Kırıkkale güvercinleri arasındaki genetik yakınlık düzeyi, genetik çalışmalarla netleştirilmesi önerilebilir.

Anahtar Kelimeler: Morfolojik özellikler, Taklacı güvercin, Kırıkkale, Yetiştiricilik.

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ORCID ID; *UCÖ: 0000-0003-2797-4983, EE: 0000-0001-5674-2538, BÖ: 0000-0001-8245-6383*
*Corresponding author e-mail: boraozarslan@kku.edu.tr

INTRODUCTION

It has been reported that the rock pigeon (Columba livia) was domesticated 3000-5000 years ago and there are more than 350 pigeon breeds in the world today (Stringham et al. 2012, Vickrey et al. 2018). There were many indigenous animal genetic resources with high disease resistance and adaptability in Turkey. One of these animal genetic resources is pigeon breeds/genotypes. The breeds of pigeons in Turkey are classified into nine main groups as diver (dalıcı), tumbler (taklacı), roller (makaracı), spinner (dolap dönücü), fleet flyer (filo uçucusu), high flyer (yüksek uçucu), racing/homer (postacı), ornamental/show (form/süs), and singer (ötücü) (Yılmaz et al. 2013). Pigeon breeding is carried out in small flocks in Turkey. For some breeders, the priority is to care for and see the performance of their birds, and they conduct their bird breeding as financial support. For some other breeders, it is just for hobby purposes and they see their birds as psychological support (Israili and Iqbal, 2017). In addition, small markets for breeder sales are established on certain days of the week, specific to the region, where the experiences of the breeders are shared. Tumbler pigeon genotypes are grown in almost every region of Turkey. Artificial selection by breeders in the desired traits (cranio-facial structures, skeletal differences, plumage color, vocalizations, flight behaviors) revealed high variation in pigeon genotypes in terms of morphological characteristics (Helms and Brugmann, 2007, Vickrey et al. 2018). Artifical selection is conducted according to the flight performance and aerial-display abilities of the tumbler pigeons. The number of studies, which was conducted on tumbler pigeon genotypes bred in Turkey is so scarce (Atasoy et al. 2013). Therefore, the aim of this research is to determine the morphological characteristics of Kırıkkale tumbler pigeons.

MATERIAL and METHODS

Birds

The material of this research consisted of 80 tumbler pigeons (42 male and 38 female) in the hands of 7 breeders in Kırıkkale province. Kırıkkale province is located at 39°50'30" N 33°31'22" E (Anonymous-1, 2022). No changes were applied to the care and management conditions of the pigeons. The age groups of the pigeons were determined according to the private enterprise records. The age groups of pigeons were classified into two groups: 12 - 24 months of age pigeons were as age group I, and 36 months of age and above pigeons were as age group II. The determination of morphological characteristics in pigeons was carried out between March 2018 and January 2019.

Morphological characteristics

Each pigeon was individually examined for morphological characteristics (plumage color, head type, eye color, head crest, presence or absence head, and body marks, wing and tail marks, and the presence or absence of muffs). Wing and tail feather numbers were determined. Wing feathers were enumerated as primary, axillary, and secondary (p-a-s), respectively. Plumage color was determined in consultation with local breeders. Likewise, the classification of eye color, crest type of the pigeons were evaluated as a result of common declarations of the breeders (Erdem et al. 2021, Özbaşer et al. 2021). Feathered feet classification in pigeons adapted from Domyan et al. (2016), and breeders' local nomenclature was given in parentheses.

Morphometric characteristics

Body weight, body length, wing span, wing length, tail length, thoracic perimeter, chest width, chest depth, head length, head width, beak length, beak depth, and tarsus diameter were determined individually for Kırıkkale tumbler pigeons. The body weights of the pigeons were taken with a precision digital scale sensitive to 0.01 g. A metal ruler was used to determine body length, a measuring tape was used to determine trunk length, wing span, wing length, thoracic perimeter, and tail length. A digital caliper was used to determine the head length and head width, beak length and depth, chest width and depth, and tarsus diameter (Figure 1) (Atasoy et al. 2013, Erdem et al. 2021).

Statistical analysis

Data were tested for normality of distribution using the Box Plot, and the outlier data was removed from the data set. The data set was analyzed by The Generalized Linear Model (GLM). Significance of the difference between means was determined by Tukey's test. The statistical significance of the difference between the two groups' means was verified by the Student-T test (age groups and sexes). Statistical analyses were performed using SPSS. Data were presented as means ± standard error ($S\bar{x}$). A value of $P<0.05$ was considered statistically significant (Dawson and Trapp, 2004).

RESULTS

Morphologıcal Characterıstıcs

As a result of breeding selection for different purposes in indigenous pigeon genotypes, various plumage colors and patterns, vocalizations, and flight displays have emerged. Three eye colors were determined in this genotype as brown ('kahverengi' in Turkish) (89.53 %) (Figure 2a), black ('siyah' in Turkish) (8.14 %) (Figure 2b) and grayish-blue ('mısır or çakır' in Turkish) (2.33 %) (Figure 2c) (Table 1). There was no crest ('takkasız' in Turkish) in almost

half of the pigeons (45.35 %) of this genotype. It was determined that 10.47 % of the examined pigeons crested ('takkalı' in Turkish) (Figure 3a), and 41.86 % of pigeons tufted ('perçemli' in Turkish) (Figure 3b). In addition, it was determined that these two feather structures were found together in 2.32 % of them (crested and tufted) ('takka-perçemli' in Turkish) (Figure 3c) (Table 2). Six plumage colors (gray/boz, smoky/dumanlı, azure/gök, yellow/sarı, white/beyaz or süt beyaz, black/arap) were determined in this pigeon genotype. Gray (plumage color and wing tips were fawn-colored, and the tail feathers was gray or brown, which was called 'boz') (35.16 %) (Figure 4a), smoky (plumage color was ash or smoky-colored, which was called 'dumanlı') (20.88 %) (Figure 4b), azure (plumage color was white or azure-colored, the wings are light gray, the tail is gray, and the wingtips are dusky, which was called 'gök') (19.78%) (Figure 4c), yellow (plumage color is off-white with gray stripes along the wing, which was called 'yellow'), (14.29 %) (Figure 4d), white (plumage color was white, which was called 'beyaz or süt beyaz') (6.59 %)(Figure 4e), black (plumage color was black, which was called 'arap') (3.30 %) (Figure 4f) were classified as the plumage colors (Table 1). Head and body marks were classified into six groups as whiskered or beak-feathered ('bıyıklı' in Turkish) (15.05 %) [(the presence of a few curled hairs at the junction of the beak and head. It can be unilateral or bilateral. The uni-lateral one was called as single-whiskered ('tek bıyıklı' in Turkish), the bi-lateral one was called as double-whiskered (' çift bıyıklı' in Turkish)] (Figure 5a), speckled ('benekli' in Turkish) (12.90 %) (It is the presence of small round-like areas on a part of the body or on the wing of a different color from the plumage color) (Figure 5b), white tail or shoved ('ak kuyruk or sokuşturmalı' in Turkish) (11.83 %) [(One or more of the tail feathers are white in pigeons with a dark plumage color - the white tail is named according to the number of white tail feathers, such as single shoved, three shoved and five shoved ('tek sokuşturmalı, üç sokuşturmalı or beş sokuşturmalı' in Turkish)] (Figure 5c), grizzled ('kırçıllı' in Turkish) [(It is the heterogeneous distribution of white feathers in dark plumage colored pigeons (4.30%)] (Figure 5d) and barred (The colored line extending along the wing, different from the wing color, was called as 'kalemli' in Turkish) (2.15 %) (Figure 5e) (Table 1). It was determined that there were three types of feathered-feet in Kırıkkale tumbler pigeons: small-muff (yıldız paça) (78.48 %) (Figure 6a), grouse (silik paça) (11.40%) (Figure 6b) and large-muff (bol paça) (10.12%) (Figure 6c). When the rates were evaluated, it was determined that small muff had the highest rate in this genotype. In the present study, Kırıkkale tumbler pigeons divided into four groups 10-1-11 (45.35%), 10-1-12 (36.05%), 10-1-10 (17.44%), 10-1-13 (1.16 %) according to the number of primary-axial-secondary wing feathers. Also, this genotype was divided into three groups 12 (83.72 %), 13 (11.63 %), 14 (4.65 %), according to the number of tail feathers (Table 2).

Morphometric Characteristics

The body weight was significantly influenced by age group and sex. The body weight of male pigeons was significantly higher than those of female pigeons ($P < 0.05$). Also, age group II was heavier than age group I ($P < 0.05$). Chest width and chest depth were found to be significantly different between age groups. Age group I was higher than the age group II in terms of chest width and chest depth ($P < 0.05$). Sex significantly affected body length, wing length, head width, and beak depth in Kırıkkale tumbler pigeons. Body length ($P < 0.001$), wing length ($P < 0.05$), head width($P < 0.01$) and beak depth ($P < 0.001$) of male pigeons were higher than those of female pigeons (Table 3).

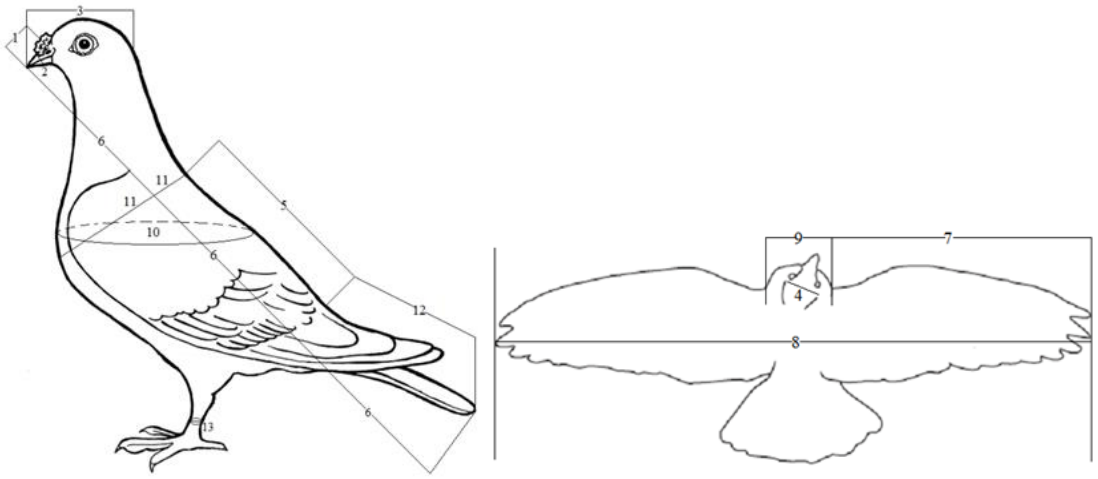


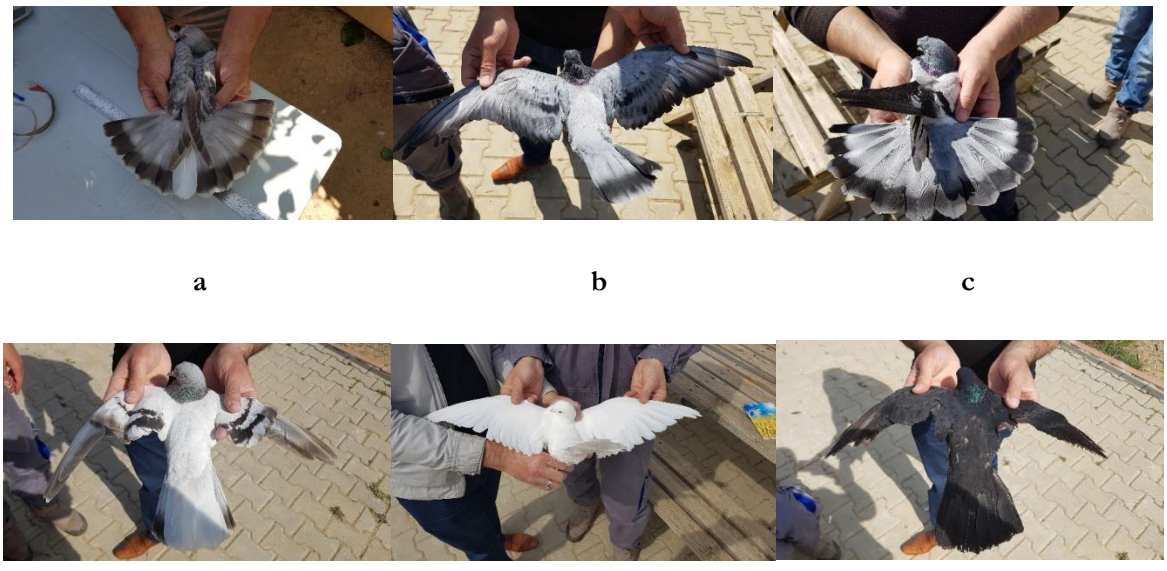
Figure 1: Morphometric body measurement regions (Erdem et al. 2021).
(1 - Beak length; 2 - Beak depth; 3 - Head length; 4 - Head width; 5 - Trunk length; 6 - Body length; 7 - Wing length; 8 - Wing span; 9 - Chest width; 10 - Thoracic perimeter; 11 - Chest depth; 12 - Tail length; 13 - Tarsus diameter)

a **b** **c**

Figure 2: Eye colors of the Kırıkkale tumbler pigeons a. Brown b. Black c. Grayish-blue

a **b** **c**

Figure 3: Head-feather type of the Kırıkkale tumbler pigeons a. Crested b. Tufted c. Crested and tufted



a **b** **c**

d **e** **f**

Figure 4: Plumage colors of the Kırıkkale tumbler pigeons a. Gray b. Smoky c. Azure d. Yellow e. White f. Black

a b c

d e

Figure 5: Head and body marks of the Kırıkkale tumbler pigeons a. Whiskered b. Speckled c. White-tail d. Grizzled e. Barred

a b c

Figure 6. Fetahered feet types of the Kırıkkale tumbler pigeons a. Small muff b. Grouse c. Large muff

Table 1. Eye color, plumage color, head and body mark in Kırıkkale Tumbler pigeons (%).

Morphological characteristics	Ratio (%)
Eye color	
Brown (Kahverengi)	89.53
Black (Siyah)	8.14
Grayish-blue (Mısır)	2.33
Plumage Color	
Gray (boz)	35.16
Smoky (dumanlı)	20.88
Azure (gök)	19.78
Yellow (sarı)	14.29
White (beyaz/süt beyaz)	6.59
Black (arap)	3.30
Head and body mark	
Unmarked	53.77
Whiskered (bıyıklı)	15.05
Speckled (benekli)	12.90
White tail (ak kuyruk)	11.83
Grizzled (kırçıllı)	4.30
Barred (kalemli)	2.15

Table 2. Table 2. Head-feather type, feathered-feet type, number of wing and tail feathers in Kırıkkale Tumbler pigeons (%).

Morphological characteristics	Ratio (%)
Head-feather type	
Non-crested (Düz)	45.35
Tufted (Perçemli)	41.86
Crested (Takkalı)	10.47
Crested and tufted (Takka perçemli)	2.32
Feathered-feet type	
Small muff (Yıldız paça)	78.48
Grouse (Silik paça)	11.40
Large muff (Bol paça)	10.12
Number of wing feather	
10−1−11	45.35
10−1−12	36.05
10−1−10	17.44
10−1−13	1.16
Number of tail feather	
12	83.72
13	11.63
14	4.65

Table 3. Morphometric characteristics of Kırıkkale tumbler pigeons (X ± S$\bar{x}$)

Age group	Sex	n	Body weight (g)	Body length (cm)	Wing Span (cm)	Wing length (cm)	Tail length (cm)	Thoracic perimeter (cm)	Chest width (mm)	Chest depth (mm)	Head length (mm)	Head width (mm)	Beak length (mm)	Beak depth (mm)	Tarsus diameter (mm)
I	Male	16	321.04±5.82	34.14±0.25	68.44±0.75	30.72±0.34	14.06±0.16	20.29±0.17	50.72±0.68	58.11±1.01	47.96±0.39	19.62±0.29	15.40±0.25	5.12±0.09	4.81±0.01
	Female	24	309.93±4.75	33.83±0.21	67.10±0.61	31.24±0.28	13.55±0.13	20.15±0.13	51.26±0.56	59.52±0.83	48.38±0.32	18.55±0.24	15.80±0.20	4.99±0.08	4.70±0.08
II	Male	26	338.18±4.56	35.03±0.12	67.78±0.59	30.01±0.27	13.99±0.13	20.69±0.13	50.74±0.53	57.55±0.79	48.66±0.31	18.99±0.23	15.75±0.20	5.42±0.07	4.88±0.08
	Female	14	323.96±6.22	33.57±0.27	67.66±0.81	30.56±0.37	13.93±0.17	20.22±0.18	48.54±0.73	55.83±1.08	48.16±0.42	18.25±0.31	16.16±0.27	4.82±0.09	4.67±0.10
TOTAL															
Age group	I	40	315.48±3.75	33.99±0.16	67.77±0.48	30.36±0.22	13.80±0.11	20.22±0.11	50.99±0.44	58.81±0.65	48.17±0.25	19.09±0.19	15.60±0.16	5.05±0.06	4.76±0.06
	II	40	331.07±3.86	34.30±0.17	67.72±0.50	30.90±0.23	13.96±0.11	20.45±0.11	49.64±0.45	56.69±0.67	48.41±0.26	18.62±0.19	15.95±0.16	5.12±0.06	4.77±0.06
Sex	Male	42	329.61±3.69	34.59±0.12	68.11±0.48	30.98±0.22	14.02±0.10	20.49±0.11	50.73±0.43	57.83±0.64	48.31±0.25	19.31±0.19	15.58±0.16	5.27±0.06	4.84±0.06
	Female	38	316.94±3.91	33.70±0.17	67.38±0.51	30.29±0.23	13.74±0.11	20.18±0.11	49.90±0.46	57.68±0.68	48.27±0.26	18.31±0.20	15.98±0.16	4.90±0.06	4.68±0.06
Grand mean		80	323.28±2.69	34.14±0.12	67.74±0.34	30.63±0.16	13.88±0.08	20.34±0.08	50.31±0.32	57.75±0.47	48.29±0.18	18.85±0.14	15.78±0.12	5.09±0.04	4.76±0.04
P															
Age group			*	—	—	—	—	—	*	*	—	—	—	—	—
Sex			*	***	—	*	—	—	—	—	—	**	—	***	—

DISCUSSION

Morphological Characteristics

Pigeon genotypes, one of our indigeonous genetic resources, are important in terms of their unique flight display characteristics, and adaptability to the region where they are raised. For this reason, it is necessary to define the morphological characteristics of indigenous genotypes (i.e. eye color, crest structure, plumage color, muff type, body morphometric traits) and compare them with other indigenous pigeon genotypes (Atasoy et al., 2013; Erdem et al., 2021). In the present study, brown (kahverengi) (89.53 %), black (siyah) (8.14 %) and grayish-blue (çakır) (2.33 %) eye colors were determined in Kırıkkale tumbler pigeons. In the studies conducted in other indigenous breeds, it was stated that the Ankara tumbler pigeons had grayish-blue (çakır) (51.76 %), dark grayish-blue (nar/koyu çakır) (25 %), gray (gri) (18.59 %), dark brown (üzüm/koyu kahverengi) (7.04 %) light brown (açık kahverengi) eye colors, and the Squadron flyer pigeons had dark brown (kehribar/kahverengi) (26.62 %), yellow (23.02 %), dark grayish-blue (nar/koyu çakır) (18.71%), grayish-blue (çakır) (15.83%), gray (10.06 %) and crimson/dark red (5.76 %) eye colors. In another study conducted on Bursa Oynarı pigeons, it was reported that the majority of the pigeons had dusty rose (gül kurusu) eye color (67.44 %). In the same study, it was reported that pigeons also had white (23.26 %) (beyaz) and dark dusty rose (koyu gül kurusu) colored eyes (9.3 %). When the researches were examined, the eye color of grayish blue was defined as the 'çakır' in Kırıkkale tumblers, Ankara tumblers and Squadron flyer pigeons, and 'gülkurusu' in Bursa Oynarı pigeons and Mulakat pigeons (Atasoy et al. 2013, Özbaşer et al. 2016, Balcı et al. 2018, Özbaşer et al. 2020). Atasoy et al. (2013) emphasized that pigeons with grayish-blue (çakır) eye color were preferred in the selection of breeding animals in the tumbler pigeons in Ankara. Crest structure in pigeons was defined as the growth of head or neck feathers towards the top of the head (Shapiro et al. 2013). In the studies carried out in Turkey, the crest structure in pigeon genotypes were determined in Ankara tumblers (8.54 %), Edremit Kelebek roller (46 %), and Alabadem pigeons (100 %). The absence of crest in Ankara tumbler pigeons was found to be 78.89%, while it was 43.35% in Kırıkkale tumbler pigeons. These rates are quite high for both genotypes. These findings show that there was a high rate of absence of a crest in Kırıkkale tumblers, similar to Ankara tumblers (Atasoy et al. 2013, Erdem et al. 2018, Erdem et al. 2021). The plumage colors of Kırıkkale tumbler pigeon were divided into six groups as gray (boz) (35.16 %), smoky (dumanlı) (20.88 %), azure (gök) (19.78 %), yellow (sarı) (14.29 %), white (beyaz) (6.59 %), and black (arap) (3.30 %). Similar to this finding, the plumage color of the tumbler pigeons reared in Ankara was divided into six groups gray (boz - 47.77 %), blue (gök - 17.56 %), smoky (dumanlı - 16.07 %), siyah (black - 9.05%), white (beyaz - 7.04 %) and shiny (yanardöner - 2.51 %) (Atasoy et al. 2013). The fact that gray plumage color ratios in these two genotypes were higher than other plumage color ratios shows that breeders give importance to plumage color as well as flight characteristics while applying selection. In Turkish pigeon breeds/genotypes, one or a few feathers on the wing/tail were white, it was called shoved (sokuşturmalı). This mark was defined according to the number of white primaries on the wing or the tail such as single-shoved (tek-sokuşturmalı), double-shoved (çift-sokuşturmalı), or five-shoved (beş-sokuşturmalı). If this mark was found as a wing mark in Ankara tumblers and squadron flyer pigeons, it was defined as grizzle color-wing (kır-kanat) (6.03 %) and shoved-wing (sokuşturmalı/arans) (29.03 %), respectively. In the present study, it was determined that one or more of the tail primaries in pigeons are white, which is called the white-tail (ak-kuyruk/sokuşturmalı) (11.83 %). This situation is called white tail (ak-kuyruk) or mirror tail (ayna-kuyruk) in Ankara tumbler pigeons (15.58 %) and silver-tail (gümüş-kuyruk) in squadron flyer pigeons (19.35 %) (Atasoy et al. 2013, Özbaşer et al. 2016). In addition, the white-wing and white-tail marks with red plumage color in the Cakal pigeon genotype can be interpreted as a breed character (Özbaşer et al. 2020). Another common point morphologically between Ankara tumblers and Kırıkkale tumblers is that some pigeons have lines on the wing that are different from the wing color. This wing mark is called barred (kalemli) in Kırıkkale tumbler pigeons and aleph (elifli/kuşaklı) in Ankara tumbler pigeons (Atasoy et al. 2013). These marks were defined as 'strips' in the Oriental pigeon genotype reared in the Marmara region (Özbaşer et al. 2020).

The feathered-feet was defined as the abnormal growth and distribution of the feathers extending towards the foot (Baptista et al. 2009, Kabir, 2015). Although foot feathering in poultry species is a desirable trait in ornamental birds, it is found in many indigenous pigeon genotypes bred for flight display performance purposes (Ankara tumblers, Edremit kelebek rollers, Mulakat and Oriental pigeons) (Atasoy et al. 2013; Erdem et al. 2018, Özbaşer et al. 2020). Bortoluzzi et al. (2020) defined the absence of feathers on the feet (non feather- legged) as 'scale'. Also, he stated that some feather-legged birds had short and tight foot feathers, and some had long foot feathers similar to wing feathers. On the other hand, the foot feathering in pigeons was divided into four groups as scale, grouse, small muff, and large muff (Domyan et al. 2016, Boer et al. 2019). In Kırıkkale tumbler pigeons, foot feathering was divided into three groups as small muff (yıldız paça) (78.48 %), grouse (silik paça) (11.40 %), and large muff (bol

paça) (10.12 %). This situation was classified into four groups as grouse (normal paça), small muff (kılıç paça), medium muff (sote paça), and large muff (bol paça or Kayseri paça) in Ankara tumbler pigeons, and it was emphasized that all pigeons had muffs (paçalı) (100 %). The situation of having trotters in all Kırıkkale tumbling pigeons was similar to Ankara tumbling pigeons. The feather-legged ratio in the tumbler pigeons bred in Kırıkkale province (all pigeons have feathered-feet) (100 %) was similar to the tumbler pigeons bred in Ankara province (100 %). In this genotype, the number of the wing primary-axial-secondary feathers (p-a-s) were divided into four groups as 10 - 1 - 11 (45.35 %), 10 - 1 - 12 (36.05 %),10-1-10 (17.44 %), (10-1-13) (1.16 %). Wing primaries were determined as 9-1-13 (50.84%), 9-1-12 (22.91 %), 9-1-11 (12.85 %), 9-1-14 (10.05 %) and 9-1-10 (3.35 %) in Ankara tumbler pigeons. The number of wing primaries was reported as 8-1-9 (87%), 9-1-9 (13%) in the Mülakat pigeons, 11-1-11(87%), 10-1-11 (13%) in the Oriental pigeons, and 9-1-10 (87%), 8-1-10 (13%) in the Çakal pigeons. Three groups were determined according to the number of tail primaries (12 - 83.72 %, 13 - 11.63 %, 14 - 4.65) in Kırıkkale tumbler pigeons. Similarly in the previous research, Ankara tumblers were divided into three groups in terms of the number of tail primaries (12- 85.43 %, 13 - 9.04 %, 14 - 5.53 %) (Atasoy et al. 2013, Özbaşer et al. 2020).

Morphometric Characteristics

Body weight, wing length, thoracic perimeter, and head width of Kırıkkale tumbler pigeons, which was obtained in this study (323.28g, 30.63 cm, 20.34 cm, and 18.85 mm) were lower than the study for Squadron flyer pigeons (428.85 g, 31.34 cm, 22.11 cm, and 21.35 mm), but higher than the Alabadem (231.17 g, 31.56 cm, 19.78 cm, and 18.67 mm) and Muradiye dönek pigeons (319.74 g, 29.30 cm, 19.34 cm, 18.20 mm) (Erdem et al., 2021 Özbaşer et al., 2016; Özbaşer et al., 2021). In the present study, it was determined that Kırıkkale tumbler pigeons (323.28 g, 34.14 cm, 67.74 cm, 30.63 cm, 20.34 cm, and 4.76 mm) had similar values to Ankara tumbler pigeons (321.62 g, 34.95 cm, 68.82 cm, 31.55 cm, 19.70 cm, and 4.01 mm) in terms of body weight, body length, wing span, wing length, thoracic perimeter, and tarsus diameter (Atasoy et al., 2013). Body weight, body length, wing length, head width and beak depth were significantly affected by sex. However wingspan, tail length, thoracic perimeter, chest width and depth, head length, beak length and tarsus diameter were not affected by sex. Body weight ($P < 0.05$), body length ($P < 0.001$), wing length ($P < 0.05$), head width ($P < 0.01$) and beak depth ($P < 0.001$) of male pigeons were signifcantly higher than that of female pigeons. These results were consistent with the other studies emphasizing that the effect of sex difference is statistically significant in terms of body weight, body length, wing length, head width, and beak depth in Ankara tumbler pigeons and Muradiye Dönek pigeons (Atasoy et al. 2013, Özbaşer et al. 2021). Body weight, chest width, and chest depth were significantly affected by age group. However body, length, wing span, wing length, tail length, throracic perimeter, head length, head width, beak length, beak depth, tarsus diameter were not affected by age group. Although age group II was higher than age group I in terms of body weight ($P < 0.05$), age group I was higher than age group II in terms of chest width ($P < 0.05$), and chest depth ($P < 0.05$). This might be due to the different management conditions applied by the breeders in different enterprises. Body development could be affected by the aerial-display and racing performance in pigeons. Mercieca et al. (2017) reported that speed and racing performance in pigeons changed positively or negatively depending on external (diet, geographical and environmental factors) and internal factors (health, innate homing ability, and body condition).

CONCLUSION

It is obvious that the nomenclature of head-feather type, eye color, plumage color, body mark and feathered-feet type are disparities in pigeons according to the region. These definitions varies according to the regions, and it is created by the breeders in that region. The high rate of absence of crest, high rate of gray plumage color, and fethered-feet of all the pigeons in Kırıkkale tumbers were completely overlap with the morphological findings in Ankara Tumblers. The high ratio of gray plumage color in both genotypes (Ankara tumbler and Kırıkkale tumbler) shows that breeders give importance to plumage color as well as aerial-display characteristics in artificial selection. In addition to these findings, when Kırıkkale tumbler pigeons and Ankara tumbler pigeons were compared in respect to body structure, it was determined that these two pigeons had very close mean values in terms of body weight, body length, wing span, wing length, thoracic perimeter and tarsus diameter. For these reasons, the degree of similarity between Ankara tumblers and Kırıkkale tumblers should be clarified by genetic studies. The number of morphological studies carried out on tumbler pigeon genotypes in Turkey should be increased. The morphological and molecular characteristics of indigenous pigeon genotypes should be determined throughout the country.

Conflict of Interest: Authors declared there is no conflict of interest.

Author Controbution: Each author declared same controbution.

Ethical approval: This study does not need ethical approval of HADYEK.

Data, attachment and other knowledge of this study is reviewed with ethical concerns.

Statement: This study is summarized from the same name master thesis.

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RESEARCH ARTICLE

Structural Features of Sheep Farms in Elazığ Province

İbrahim ŞEKER[1], Selim KUL[1], Abdurrahman KÖSEMAN[2]*

[1] Firat University, Faculty of Veterinary Medicine, Zootechny Department, Elazığ, 23000, Turkey
[2] Malatya Turgut Ozal University, Akcadag Vocational School, Plants and Animal Production Department, 44600, Malatya, Turkey

ABSTRACT

This study was carried out to determine the structural characteristics of sheep farms in Elazığ province. For this purpose, 167 farms were included in the research and visited, evaluated on-site, and a face-to-face survey was conducted with their owners. According the study, 56.3% of the breeders owned their own land, 60.0% owned 10.1-50 decares of land, 86.2% of the barns were detached, 10.8% were adjacent to the house, 88.0% were closed type, 42.5% were on the north-south long axis, 60.0% were brick-briquettes walled, 66.9% were soil ground, 62.9% had adequate ventilation shafts, 84.5% had sufficient windows. In addition, 94.5% of the farms use troughs as sheep irrigation equipment, 55.8% prefer wooden mangers, 76.6% of the farms had two barn doors, 86.2% had 1 m² or less walking area per sheep. But there was no feed mill/mixer in 88.5% and no shade/porch in 47.2%. As a result, it is expected that the sheep breeding in the province will reach the desired level by considering the identified problems and proposed solutions through this study by both the breeders and the relevant institutions and organizations. It is recommended to increase incentives, support and interest-free loans in order to eliminate structural problems in businesses.

Keywords: Breeders, Facility, Sheep, Sheep Farms, Structural Features

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Elazığ İlindeki Koyunculuk İşletmelerinin Yapısal Özellikleri

ÖZ

Bu çalışma Elazığ ilinde bulunan koyunculuk işletmelerinin yapısal özelliklerini belirlemek amacıyla yapılmıştır. Bu amaçla 167 çiftlik araştırmaya dâhil edilerek ziyaret edilmiş, yerinde değerlendirilmiş ve sahipleri ile yüz yüze anket çalışması yapılmıştır. Araştırmada işletmelerin %56,3'ünün kendi arazisine, %60,0'ının ise %10,1-50 dekar araziye sahip olduğu belirlendi. Çalışmada ağılların %86,2'sinin müstakil, %10,8'inin eve bitişik, %88,0'inin kapalı tip, %42,5'inin kuzey-güney uzun aksında, %60,0'ının tuğla-briket duvarlı, %66,9'unun toprak zeminli olarak yapıldığı; %62,9'unda yeterli havalandırma bacası ve %84,5'inde yeterli pencere bulunduğu belirlendi. Ayrıca çiftliklerin %94,5'inde koyun sulama ekipmanı olarak yalak kullanıldığı, %55,8'inde ahşap yemlik tercih edildiği, çiftliklerin %76,6'sında iki ağıl kapısı, %86,2'sinde koyun başına 1 m² veya daha az gezinti alanı bulunduğu, %88,5'inde yem hazırlama değirmeni/mikser, %47,2'sinde ise gölgelik/sundurma bulundurulmadığı tespit edildi. Sonuç olarak hem yetiştiriciler hem de ilgili kurum ve kuruluşlar tarafından bu çalışma ile tespit edilen sorunlar ve çözüm önerileri dikkate alınarak İlde koyun yetiştiriciliğinin uygun düzeye getirilmesi beklenmektedir. İşletmelerde yapısal sorunların giderilmesi için teşvik, destek ve faizsiz kredilerin artırılması önerilmektedir.

Anahtar kelimeler: Ağıl, İşletme, Koyun, Yapısal Özellik, Yetiştirici

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ORCID ID; *İŞ: 0000-0002-3114-6411, SK: 0000-0003-3032-8050, AK: 0000-0001-6491-9962.*

*Corresponding author e-mail: abdurrahman.koseman@ozal.edu.tr

GİRİŞ

Koyunculuk, birçok ülkenin ekonomisi ve tarım sektörü içerisinde önemli bir paya ve potansiyele sahiptir. İnsanların sağlıklı ve dengeli beslenmeleri için gerekli olan özellikle hayvansal kaynaklı protein ve diğer fizyolojik ihtiyaçların temini açısından çok önemli bir rolü vardır (Güneş ve Akın 2017).

Türkiye'de koyunculuk sektörünün en önemli sorunlarının başında düşük maliyete sahip uygun barınakların azlığı gelmektedir. Ekonomik ve doğru bir yetiştiricilikte en düşük maliyetle en yüksek verimi elde etmek temel amaçtır. Bu maliyetlerin içerisinde barınak yapımı ve amortismanı önemli bir yer tutmaktadır. Barınaklar, koyunların yaşam alanları olup, onları olumsuz dış etkenlerden korumaktadır (Şeker ve ark. 2020).

Koyun yetiştiriciliğinde barınak içi ve barınak dışı çevresel şartlar koyunların refahına uygun olmalı, koyun üzerinde stres oluşturmayacak ve koyunların rahat edebileceği nitelikleri taşımalıdır (Şahin 2016). Hayvanın türü, ırkı, yaşı ve verim yönü ile doğrudan ilişkisi olan barınak planlaması; sıcaklık, hava akış hızı, bağıl nem, radyasyon, havanın kimyasal bileşimi, ışık gibi barınak içi ve dışı çevre şartları dikkate alınarak yapılmalıdır. Barınak içi koşulların en önemli unsuru 'barınak iklimi' olarak adlandırılan sıcaklık, havalandırma, aydınlatma ve bağıl nem özellikleridir (Ekmekyapar 1991).

Koyunculuk işletmelerinin yapısal özellikleri de sektördeki gelişmeyi etkilemektedir. Ekonomik ve kârlı bir yetiştiricilik için sağlıklı ve verimli koyunlara ihtiyaç vardır. Uygun koşullara sahip olan barınaklar, birçok çevresel özelliğin ideal seviyelerde tuttuğu için koyunların beslenme, sağlık, gelişim ve verimlerini en üst düzeye yükseltir (Şeker ve ark. 2020).

Koyun, ekonomik amaçla yetiştiriciliği yapılan diğer türlerle kıyaslandığında daha kanaatkâr, olumsuz çevre şartlarına karşı daha dayanaklı, maliyeti ve kalitesi düşük besin maddelerinden besin ihtiyaçlarını karşılayabilmesi ve daha düşük maliyetli işletmelerde yetiştirilebilmesi bakımından tercih edilmektedir. Koyun yetiştiriciliği, bölgedeki iklim koşulları dikkate alınarak, düşük maliyetle ve koyunların tüm ihtiyaçları göz önüne alınarak planlanmış ağıllarda yapıldığı takdirde verimliliği yüksek, ekonomik bir faaliyet olarak yürütülebilir. Bundan dolayı özellikle işletmeler açısından barınakların özenli ve gereksinimlere göre inşa edilmesi kritik öneme sahiptir (Güneş ve Akın 2017).

Koyunculuğun daha verimli ve karlı bir düzeye çıkarılabilmesi için işletmelerdeki mevcut durumun belirlenmesi, öncelikli problemlerin tespit edilmesi oldukça önemlidir. Bu amaçla ilgili alanda yapılacak bilimsel nitelikli tüm çalışmalar yararlı olmaktadır. Bu araştırma, Elazığ İli koyunculuk işletmelerinde yapısal durumun tespit edilmesi amacıyla yürütülmüştür.

MATERYAL ve METOT

Bu araştırma, Elazığ İli Damızlık Koyun Keçi Yetiştiriciler Birliği'ne üye olan koyunculuk işletmeleri sahipleriyle gönüllülük esasına göre yüz yüze yapılan anketler ve işletmelere yapılan ziyaretler sırasındaki gözlem, değerlendirme ve ölçümlerle gerçekleştirilmiştir.

Araştırmada, öncelikle Elazığ ilinin en fazla koyun sayısına ve işletmesine sahip ilçeleri Elazığ İl Tarım ve Orman Müdürlüğü'nden edilen veriler yardımıyla belirlenmiştir. Bu kapsamda Merkez, Kovancılar, Karakoçan, Palu, Sivrice, Baskil, Keban ilçeleri öne çıkmıştır. Bu ilçelerde araştırmaya dâhil edilecek işletmelerin seçiminde öncelikle Elazığ Damızlık Koyun Keçi Yetiştiriciler Birliği'ne üyeliği bulunması koşulu aranmış olup, bu koşulu sağlayan işletmeler arasından tesadüfi örnekleme metodu ile işletmeler seçilmiştir. Araştırmada kullanılan anketin güvenilirliğini ve geçerliliğini yükseltmek için deneme amaçlı olarak bazı işletmelerde ön çalışmalar yürütülmüş, bu çalışmalara göre araştırmadaki anket sorularına son şekilleri verilmiştir. Bu araştırmadaki anket soruları daha önceki benzer araştırmalardan (Altınçekiç 2014, Ceyhan ve ark. 2015, Tüfekci 2020) yararlanarak, araştırma ekibi tarafından oluşturulmuştur.

Çalışma kapsamında belirlenen işletme sahipleriyle ön görüşmeler yapılmış, anket uygulaması için daha önceden seçilerek belirlenmiş, gönüllülük esasına dayalı yüz yüze görüşmeyi kabul edenlerin işletmeleri bir takvime bağlı olarak araştırma ekibi tarafından ziyaret edilerek anket uygulanmış, ayrıca ziyaret edilen işletmelerde araştırma kapsamında gözlemler yapılmış ve gerekli metrik ölçümler gerçekleştirilmiştir. Yapılan görüşmelerden elde edilen veriler kayıt altına alınmıştır.

Bu araştırmayla benzerlik gösteren ve anket uygulaması içeren birçok çalışmada gerekli örnek büyüklüğünün saptanmasında, popülasyonun %3 (Yamane 2010) ila %10'unu (Sümbüloğlu ve Sümbüloğlu 2000, Cochran 1997) temsil edecek büyüklükte olacak şekilde hesaplanmasının yeterli olacağı kaydedilmiştir. Bu araştırma için gerekli örnek büyüklüğü %10 örnekleme hatası ve %95 güvenilirlik sınırları içerisinde olacak şekilde aşağıdaki formül yardımıyla 92 olarak belirlenmiştir (Çiçek ve Erkan 1996).

$$n=\frac{N.t^2.p.q}{d^2.(N-1)+t^2.p.q}$$

n: Örnek
N: Populasyon büyüklüğü (1669)
t: %95 güven aralığındaki t değeri (1.96)
p: 0.5 (%50 görülme sıklığı)
q: 0.5 (%50 görülmeme sıklığı)
d: Örnekleme hatası (0.10)

Formüle göre belirlenmiş olan örnek büyüklüğü bu araştırma için en küçük örnek büyüklüğü olarak hedeflenmiştir. Bilimsel araştırmalarda ele alınan ve üzerinde çalışılan örnek ne kadar büyük olursa ilgili popülasyonu temsil edilebilme gücü de o oranda artmaktadır. Bu çerçevede mevcut araştırma sonuçlarını daha güvenli kılmak amacıyla Birliğe ait aktif üye sayısı olan 1669 işletmeden oluşan popülasyonun en az %10'unun örneğe dâhil edilmesi uygun bulunmuştur. Bu kapsamda örnek büyüklüğü 167 işletme olarak belirlenmiştir.

Bu çalışmanın yürütülmesi için gerekli olan etik onay, Fırat Üniversitesi Girişimsel Olmayan Araştırmalar Etik Kurul'undan alınmıştır (29.4.2020 tarih ve 2020/07-24 sayılı izin).

İstatistiki analizlerde, yetiştiricilerin arazi sahiplik durumları, ağılların topografik ve mimari özellikleri, ağılların yapı malzemeleri ve koyun başına kullanım alanları ile işletmelerin koyun ihtiyaçlarına yönelik donatım ve ekipman varlığı konularına yönelik sorulara ankete katılan yetiştiricilerin verdikleri cevaplar analiz edilmiştir. Elde edilen veriler kullanılarak ilgili sorulardaki parametrelere ait seçeneklerin sayısal ve % frekansları hesaplanmıştır. Bu amaçla SPSS programından yararlanılmıştır (SPSS 2015).

BULGULAR

Araştırmada, işletmelerin %56.3'ünün kendi arazi varlıklarına sahip olduğu, %31.0'inin kiralık olan arazide faaliyet yaptığı, %60.0'ının 10.1-50 dekar, %29.3'ünün ise 10 dekar ve daha az arazi varlığına sahip olduğu belirlenmiştir. Ağılların %84.3'ünün köyde, %14.5'inin köyde ve yaylada bulunduğu, %86.2'sinin müstakil ve %10.8'inin eve bitişik olduğu, %88.0'inin kapalı olduğu, %87.4'ünün "I" şeklinde tasarlandığı, %42.5'inin kuzey-güney uzun eksenli yapıldığı saptanmıştır. Ağılların %77.6'sının eninin 10.0 m ve daha az, %59.6'sının boyunun 21-30 m, %56.0'sının yüksekliğinin 3.1 m ve daha az olduğu tespit edilmiştir (Tablo 1).

Yetiştiricilerin arazi sahiplik durumları ile ağılların topografik ve mimari özelliklerine ait bulgular Tablo 1'de verilmiştir.

Tablo 1. Elazığ'da yetiştiricilerin arazi sahiplik durumları ile ağılların topografik ve mimari özellikleri
Table 1. Land ownership status of breeders and topographic and architectural characteristics of barns in Elazığ

Anket sorusu		Frekans	
		n	%
Arazi sahiplik durumu			
Kendi malı		89	56.3
Kiralık olarak kullanıyor		49	31.0
Bir kısmı kendi malı- bir kısmı kiralık		20	12.7
	Toplam	158	100
Arazi varlığı			
10 da ve daha az		44	29.3
10.1-50 da		90	60.0
50.1-100 da		13	8.7
101.1 da ve üstü		3	2.0
	Toplam	150	100
Ağılın yeri			
Köyde		140	84.3
Köyde ve yaylada		24	14.5
Merada		2	1.2
	Toplam	166	100
Ağılın konumu			
Bağımsız		144	86.2
Eve bitişik		18	10.8
Evin altında		5	3.0
	Toplam	167	100
Ağılın tipi			
Açık		9	5.4
Kapalı		147	88.0
Yarı açık		11	6.6
	Toplam	167	100
Ağılın şekli			
U		7	4.2
L		14	8.4
I		146	87.4
	Toplam	167	100
Ağılın uzun eksen yönü			
Doğu-batı		36	21.6
Kuzey-güney		71	42.5
Güney-batı		60	35.9
	Toplam	167	100
Ağılın genişliği			
10 m ve daha az		128	77.6
11 m ve daha fazla		37	22.4
	Toplam	165	100
Ağılın uzunluğu			
20 m ve daha az		23	13.9
21-30 m		99	59.6
30.1 m ve daha fazla		44	26.5
	Toplam	166	100
Ağılın yüksekliği			
2 m den az		5	3.0
2 - 3 m		68	41.0
3.1 m ve daha fazla		93	56.0
	Toplam	166	100

Ağılların, %60.0'ının duvarının tuğla-briketten ve %40.0'ının kerpiçten yapıldığı, %66.9'unun zemininin toprak, %48.5'inin çatı örtü malzemesinin oluklu sac olduğu ve %73.0'ünün beşik çatı şeklinde inşa edildiği belirlenmiştir. Araştırmada, işletmelerin %86.2'sinin koyunlar için 1 m² ve daha az gezinti alanına, %40.7'sinin 2.25 m² ve daha az kuzusuz koyun başına alana, %40.1'inin ise 2.26-3.80 m² kuzusuz koyun başına alana sahip olduğu belirlenmiştir (Tablo 2).

Ağılların yapısal malzemelerine ve koyun başına kullanım alanlarına ait bulgular Tablo 2'de verilmiştir.

Tablo 2. Elazığ'da ağılların koyun başına kullanım alanları ve yapısal malzemeleri
Table 2. Usage areas per animal and structural materials of barns in Elazig

		Frekans	
		n	**%**
Ağılın duvar malzemesi			
Tuğla - Briket		90	60.0
Kerpiç		60	40.0
	Toplam	150	100
Ağılın zemin malzemesi			
Toprak		111	66.9
Beton		55	33.1
	Toplam	166	100
Ağılın çatı malzemesi			
Kiremit		52	31.1
Eternit		2	1.2
Oluklu sac		81	48.5
Naylon		20	12.0
Diğer		12	7.2
	Toplam	167	100
Ağılın çatı şekli			
Beşik çatı		122	73.0
Sundurma		35	21.0
Diğer		10	6.0
	Toplam	167	100
Ağılın dinlenme yerinde koyun başına ayrılan alan			
1 m² ve daha az		144	86.2
1 m² den daha fazla		23	13.8
	Toplam	167	100
Ağılın kuzusuz koyun başına gezinti avlusu büyüklüğü			
2.25 m² ve daha az		68	40.7
2.26-3.80 m²		67	40.1
3.81 m² ve daha fazla		12	7.2
Avlusuz		20	12.0
	Toplam	167	100

Yapılan çalışmada, işletmelerin %76.6'sında iki adet ağıl kapısı bulunduğu, %62.9'unda havalandırma bacasının yapıldığı, %84.5'inin yeterli pencere varlığın olduğu saptanmıştır. Araştırmada, işletmelerin %94.5'inde koyun sulama ekipmanı olarak yalak kullanıldığı, %55.8'inde ahşap yemlik tercih edildiği, metal yemlik tercih edenlerin oranının %40.4 olduğu, %88.5'inde yem hazırlama değirmeni/karıştırıcı bulunmadığı ve %47.2'sinde ise gölgelik/sundurma olmadığı tespit edilmiştir (Tablo 3).

İşletmelerin koyun ihtiyaçlarına yönelik donatım ve ekipman varlığına ait bulgular Tablo 3'de verilmiştir.

Tablo 3. Elazığ'da işletmelerin koyun ihtiyaçlarına yönelik donatım varlığı
Table 3. Equipment for the animal needs of enterprises in Elazığ

		Frekans	
		n	%
Ağılın kapı sayısı			
1		10	6.0
2		128	76.6
3 ve daha fazla		29	17.4
	Toplam	167	100
Ağılda baca varlığı			
Var		105	62.9
Yok		62	37.1
	Toplam	167	100
Ağılda pencere varlığı			
Yetersiz		26	15.5
Yeterli		141	84.5
Toplam		167	100
Suluk varlığı			
Yalak		155	94.5
Otomatik-şamandıralı suluk		9	5.5
	Toplam	164	100
Yemlik varlığı			
Metal yemlik		65	40.4
Ahşap yemlik		86	53.4
Plastik yemlik		4	2.5
Beton-taş yemlik		6	3.7
	Toplam	161	100
Yem hazırlama değirmen-karıştırıcı varlığı			
Var		19	11.5
Yok		146	88.5
Toplam		165	100
Gölgelik/sundurma varlığı			
Var		86	52.8
Yok		77	47.2
	Toplam	163	100

TARTIŞMA

Araştırmada, işletmelerin yarısından fazlasının (%56.3) kendi arazisine sahip olduğu, bir kısmının ise (%31.0) kiralanmış arazilerde faaliyet yaptığı belirlenmiştir (Tablo 1). Yapılan çalışmalarda, kendi arazisinde koyunculuk yapan işletmelerin oranı Karaman'da %95.36 (Koca 2014), Küçük Menderes Havzası'nda %76.5 (Aydıner 2018), Malatya'da ise %56.7 belirlenmiştir (Köseman ve ark. 2022). Bir kısmı kendi malı bir kısmı kiralık olan arazide faaliyet yapan işletmelerin oranı (%12,5) ise Malatya'da %32.4 (Köseman ve ark. 2022) ve Kastamonu'da %68.8'dir (Tüfekci ve Olfaz 2015). İzmir'deki işletmelerin %35.2'sinin arazisi bulunmamaktadır (Kandemir ve ark. 2015). Elazığ'da kendi arazisinde faaliyet yapanların oranı İzmir'deki yetiştiricilerden daha yüksek, Karaman ve Küçük Menderes Havzasında saptanandan daha düşük, Malatya'da belirlenenle benzerdir. Kiralık arazilerde faaliyet yapanların oranı ise İzmir'dekinden düşüktür. Sürdürülebilir ve kârlı üretim yapılabilmesi için işletmelerin kendi arazilerinde faaliyet yapmaları olumlu, tamamı kiralanan arazilerde yetiştiricilik yapılması ise bir kısmı kiralanmış arazide faaliyet sürdürmeye göre daha dezavantajlı olarak değerlendirilmektedir.

Araştırmada, işletmelerin %60.0'ının 10.1-50 dekar (da), %29.3'ünün ise 10 da ve daha az arazi varlığına sahip olduğu belirlenmiştir (Tablo 1). Malatya'daki koyunculuk işletmelerinin %57.3'ü 10.1-50 da, %35.4'ü ise 10 da ve daha az arazi varlığına sahiptir (Köseman ve ark. 2022). İzmir yöresindeki küçükbaş işletmelerinin % 52,4'ünde arazi varlığı 100 da ve

bunun altındadır (Kandemir ve ark. 2015). Karaman'da koyunculuk işletmelerinde arazi büyüklüğü ortalama 122.18 da'dır (Koca 2014). Bulgular ışığında, Elazığ'daki koyunculuk işletmelerinin arazi varlığının Malatya ilindekilere yakın olduğu, buna karşın İzmir ve Karaman illerindekilerden daha küçük olduğu, arazi büyüklüklerinin bölge ve iller arasında değişkenlik gösterdiği görülmektedir. Doğru ve ekonomik bir koyunculuk faaliyeti için arazi büyüklükleri koyun sayısına uygun ve yeterli olmalıdır (1 da arazi / 7-10 baş koyun). Elazığ'daki işletmelerin sahip oldukları arazi varlığının mevcut koyunların ihtiyaçlarını karşılayacak büyüklükte olduğu söylenebilir.

Araştırma bulgularına göre ağılların %84.3'ü sadece köyde, %14.5'i ise hem köyde hem de yaylada bulunurken (Tablo 1), Diyarbakır'da ağılların %84.2'si, Şanlıurfa, Gaziantep ve Adıyaman illerinde tamamı (%100) köyde bulunmaktadır. Bununla birlikte Diyarbakır'da ağılların %14.5'inin köy ile birlikte yaylada bulunduğu bildirilmiştir (Dellal ve ark. 2002). Bu araştırmada sadece köyde ve hem köyde hem de yaylada ağılı bulunan yetiştiricilerin oranı Diyarbakır'dakilerle oldukça yakındır. Sadece köyde ağılı bulunan yetiştiricilerin oranı Şanlıurfa, Gaziantep ve Adıyaman'daki yetiştiricilerden ise daha düşüktür. Elazığ ilinde toplam alanının çoğunu platolar oluşturmaktadır. Daha düşüklü rakımlı olan yerleşkelerde havaların ısınması ve sıcak mevsimlerde yayla koşullarının koyunlar için daha elverişli olması nedeniyle yetiştiriciler koyunlarıyla birlikte yaylalara göç etmektedirler.

Araştırma bulgularına göre ağılların, %86.2'sinin müstakil, %10.8'inin ise eve bitişik olduğu saptanmıştır (Tablo 1). Niğde ilindeki ağılların %69.8'i müstakil (Ceyhan ve ark. 2015), Siirt ilindekilerin ise % 56.8'i eve bitişiktir (Bakır ve ark. 2017). Elazığ'da saptanan müstakil ağılların oranı Niğde ilindekilerden daha yüksek, buna karşın eve bitişik olanların oranı ise Siirt'tekilerden daha düşüktür. Ağılların müstakil inşa edilmeleri özellikle biyogüvenlik bakımından olumlu ve gerekli bir uygulamadır.

Bu çalışmada, Elazığ'da ağılların %87.4'ünün "I" şeklinde inşa edildiği (Tablo 1), bu oranın Malatya'daki işletmelerde saptanandan (%60.9) (Köseman ve ark. 2022) daha yüksek olduğu tespit edilmiştir. Türkiye'de ağılların açık yönünün güneye, güney-doğuya veya doğuya bakması, kuzey tarafının ise kapalı olması gerekmektedir. Bu sayede ağılların ve koyunların güneşin ısıtıcı ve kurutucu etkisinden yararlanmaları mümkün olmaktadır.

Bu araştırmada, ağılların %42.5 kuzey-güney, %21.6'sının doğu-batı uzun eksenli yapıldığı belirlenmiştir (Tablo 1). Barınakların, Kahramanmaraş'ta %51.0'inin kuzey-güney (Paksoy ve ark. 2006), Tokat ilinde ise %52.0'sinin doğu-batı (Karaman ve ark. 2012) uzun eksenli olduğu bildirilmiştir. Elazığ'da kuzey-güney uzun eksenli inşa edilen ağılların oranı Kahramanmaraş'ta tespit edilenden, doğu-batı uzun eksenli yapılanların oranı ise Tokat'taki ağılların oranından daha düşüktür. Yanlış konumlandırılan barınaklar kışın solar radyasyondan, yazın ise serinletici rüzgârlarda yararlanamamaktadır. Elazığ'da rüzgâr hızlarının düşük ve birbirine yakın değerlerde her yönden estiği görülür. Elazığ'da yıl içinde hâkim rüzgâr yönü kuzeybatıdır. Bunu sırasıyla batı ve doğu yönleri izlemektedir. Rüzgâr yönleri genel hava dolaşımına uygun olup, topografyaya göre kanalize olmaktadır. Bu nedenle Elazığ'da ağılların kuzey-güney uzun eksenli yapılmasının doğru olduğu değerlendirilmektedir.

Çalışmada, kapalı tipte inşa edilen ağılların oranı %88.0, açık tip ağılların oranı %5.4, yarı açık ağılların oranı ise %6.6 olarak belirlenmiştir (Tablo 1). Kapalı tip ağılların oranı, Kahramanmaraş'taki işletmelerde (%81.0) (Paksoy ve ark. 2006), Siirt'teki işletmelerde (%95.8) (Bakır ve ark. 2017) ve Yozgat'taki işletmelerde (%95.0) (Tüfekci 2020) tespit edilenden daha düşüktür. Elazığ'da saptanan açık tip ağılların oranı, Aydın ve Keskin (2018) tarafından Muğla'da belirlenenden (% 36.0), yarı açık ağılların oranı ise Elmaz ve ark. (2014) tarafından Burdur'da tespit edilenden (%84.4) daha düşüktür. Barınaklar, koyunları dış etkenlerden koruyan yapılar olup iklimsel zorunluluk yoksa kapalı tip barınaklar yerine açık veya yarı açık tipteki barınakların tercih edilmeleri gerekmektedir. Elazığ'da kapalı tipte yapılan ağılların daha fazla tercih edilmiş olması bir olumsuzluk olarak değerlendirilmektedir.

Araştırmada ağılların en fazla 10 m ve daha az genişlik (%77.6), , 21-30 m uzunluk (%59.6), 3.1 m ve daha az yükseklikte (%56.0) inşa edildiği tespit edilmiştir (Tablo 1). Tokat'taki işletmelerde en yüksek orandaki ağıl ölçüleri 6-6.9 m genişlik (%35.4), 10.1-15 m uzunluk (%32.9) ve 2.1-2.5 m yüksekliktir (%41.8) (Karaman ve ark. 2012). Niğde'deki işletmelerde ortalama ağıl ölçüleri 9.67 m genişlik, 21.73 m uzunluk ve 3.51 m yüksekliktir (Ceyhan ve ark. 2015). Kahramanmaraş'taki işletmelerde ise ortalama ağıl ölçüleri 5.3 m genişlik, 13.7 m uzunluk ve 2.20 m yüksekliktir (Paksoy ve ark. 2006). Bu araştırmada belirlendiği şekliyle, Elazığ'daki ağıllar, genişlikleri bakımından Niğde'deki işletmelerle benzer ve yakın ölçülere sahip olup, Tokat ve Kahramanmaraş'taki ağıllardan ise daha geniştir. Ayrıca, Tokat ve Kahramanmaraş'taki ağıllardan daha uzun, Niğde'deki işletmelerin ortalama uzunluklarına yakındır. Ağıl yükseklikleri ise Niğde'deki ağılların ortalama yüksekliklerinden daha düşük, Tokat ve Kahramanmaraş'taki ağılların yükseklikleri ile benzerdir.

Koyunculuk işletmelerinde ağıl genişliğinin en fazla 12 metre olması, duvar yüksekliğinin ise 200 başlık ağıllar için 3.0-3.5 metre, 500 başlık ağıllar için 3.5-4.0 metre olması gerekmektedir. Ağıl uzunlukları barındırılacak koyun sayısına göre değişmektedir. Bu araştırmada belirlenen ağıl genişlikleri ve uzunlukları normal ölçülerde olup, ağıl yükseklikleri ise düşüktür.

Bu araştırmada, ağıl duvarlarının %60.0 oranında tuğla ve briketten, %40.0 oranında ise kerpiçten yapıldığı belirlenmiştir (Tablo 2). Kahramanmaraş'taki ağılların %50.0'sinde tuğla ve briket, %43.0'ünde ise taş kullanılmıştır (Paksoy ve ark. 2006). Bursa bölgesinde ağılların %83.0'ünün duvarları tuğla ve briket (Onuk 2015), Siirt'teki ağılların %61.8'inin taş (Bakır ve ark. 2017), Konya'daki ağılların ise %60.0'ının briket, %15.0'inin taş ve %15.0'inin kerpiç (Oğuz ve ark. 2019) malzemeden yapıldığı tespit edilmiştir. Elazığ'da, Kahramanmaraş ve Siirt'teki işletmelerin aksine kâgir taşıyıcı yapı malzeme olarak ağıllarda taş kullanılmamaktadır. Bulgulara göre, kerpiç malzeme Konya, Siirt, Bursa ve Kahramanmaraş'taki işletmelere göre yüksek oranda kullanılmaktadır. Ağıl duvarlarında tuğla ve briket kullanımı bakımından ise Elazığ'daki işletmeler, Kahramanmaraş, Bursa ve Konya'daki işletmelerle benzerlik göstermektedir. Duvarlarda kullanılan kâgir taşıyıcı yapı malzemeleri iklim, kültür, maliyet, temin edilebilirlik gibi çok sayıda faktöre göre belirlenmektedir. Kullanılan malzemenin dezenfeksiyona elverişli olması da gerekmektedir. Elazığ'daki işletmelerde ağıl tabanlarında %66.9 oranında toprak malzeme kullanılmaktadır (Tablo 2). Siirt'teki ağılların %90.8'inin (Bakır ve ark. 2017), Burdur'daki ağılların %89.1'inin (Elmaz ve ark. 2014), Yozgat'taki ağılların ise %95.0'inin (Tüfekci 2020) zemininde toprak kullanıldığı saptanmıştır. Bu araştırma bulgularına göre ağıl zeminlerinde toprak malzemenin kullanım oranı Siirt, Burdur ve Yozgat'taki işletmelerden daha düşüktür. Bu durum olumlu görülmekle birlikte zeminde toprak kullananların oranı yine de oldukça yüksek düzeydedir. Toprak, temizlik ve dezenfeksiyon için elverişli olmayan bir malzemedir. Bu nedenle ağıl zeminlerinde toprak kullanılması uygun görülmemektedir.

Bu araştırmada, ağılların %48.5'inde oluklu sac kullanıldığı ve çatıların %73.0'ünün beşik çatı olarak inşa edildiği tespit edilmiştir (Tablo 2). Bursa'da yapılan araştırmada ağılların %44.0' ünde oluklu sac kullanıldığı ve çatıların %70.0 oranında beşik çatı olarak inşa edildiği belirlenmiştir (Onuk 2015). Kahramanmaraş'taki ağılların çatısında %30.0 oluklu çinko kullanıldığı ve ağılların %30.0'unun beton damla kapatıldığı kaydedilmiştir (Paksoy ve ark. 2006). Siirt'te ise ağılların %45.4'ün toprak damla örtüldüğü belirlenmiştir (Bakır ve ark. 2017). Yapılan bu çalışmada, en fazla beşik tip çatı yapılması ve çatı malzemesi olarak en çok sac kullanılmasında Bursa'daki işletmelerle benzerlik ve oransal bir yakınlık vardır. Ele alınan her iki özellik yönünden gerek Kahramanmaraş gerekse Siirt illerindeki işletmelerle ise farklılıklar tespit edilmiştir.

Çatı, ağılların en önemli unsurlarındandır. Çatının şekli, ağılın bulunduğu bölgenin iklim tipi, yıllık yağış çeşidi ve yağış miktarıyla ilişkilidir. Kullanılan çatı örtü malzemesinin çeşidi ise ağıl içi sıcaklık ve nem miktarlarını belirlemede büyük role sahiptir. Bu çalışmada ağıllarda en fazla tespit edilen beşik tipi çatı, Elazığ'ın sahip olduğu iklime uygun bir çatı tipidir. İl genelinde ağıl çatılarında en fazla oluklu sac kullanılması ise olumsuz bir durumdur. Sac, yazın sıcağın yüksek oranda ağıl içine girmesine, kışınsa ağıl içinde yüksek nem oluşmasına ve altlığın ıslanmasına neden olur.

Araştırmada, ağılların %86.2'sinin dinlenme yerinde koyun başına 1 m² ve daha az alan olduğu belirlenmiştir. İşletmelerin bir kısmı 2.25 m² ve daha az (%40.7), bir kısmı ise 2.26-3.80 m² (%40.1) kuzusuz koyun başına gezinti avlusuna sahiptirler (Tablo 2). Bolu'daki işletmelerde koyunlar için ayrılan dinlenme yeri, ağılların %31'inde 0.8 m2'nin altında ve %40.5'inde 1 m² üstündedir. Bu ildeki ağılların %19.0'unda gezinti avlusu büyüklüğünün yeterli olduğu belirlenmiştir (Şişman ve ark. 2009). Tokat ilinde yapılan araştırmada ise ağılların %54.7'sinde gezinti alanı bulunmamaktadır (Karaman ve ark. 2012). Bu çalışmada tespit edilen koyun başına 1 m² ve daha az alana sahip işletmelerin oranı Bolu'daki işletmelerde belirlenenden daha yüksektir. Diğer bir deyişle dinlenme yerindeki koyun başına alan bakımından Bolu'daki işletmelerden daha yetersizdir. Yapılan araştırmada kuzusuz koyun başına tespit edilen gezinti alanı ise kısmen Bolu'daki ve Tokat'taki işletmelerden daha elverişlidir. Ağıllarda koç başına 1.5-2.0 m², koyun ve kuzusu için 1.25-1.5 m², toklu başına 0.8-1.0 m² taban alanı gereklidir. Kuzusuz koyun başına gezinti alanı ise dinlenme alanının iki katı kadardır. Bu çalışmada tespit edilen koyun başına dinlenme alanı düşük, kuzusuz koyun başına gezinti alanı ise normal değerlere yakındır. Elazığ'daki işletmelerde sürü sıkışıklığına neden olan dinlenme alanlarının elverişli biçimde genişletilmesi gerekmektedir.

Bu çalışmada, ağılların %76.6'sında iki adet kapı bulunduğu belirlenmiştir (Tablo 3). Fonksiyonel ve modern bir işletmecilikte giriş ve çıkışlarda sıkışıklık olmaması için koyun sayısı fazla olan ağıllarda en az iki adet kapı bulunması gerekmektedir. Ayrıca büyük kapasiteli işletmelerde ağılların kısa kenarlarında traktör, makine ve ekipmanların girip çıkacağı büyüklükte karşılıklı kapılar da olmalıdır. Araştırmada tespit edilen kapı sayıları bakımından işletmelerin elverişli olduğu düşünülmektedir.

Araştırmada, ağılların %62.9'unda uygun havalandırma bacasının bulunduğu, %84.5'inde de yeteri kadar pencere varlığının olduğu saptanmıştır (Tablo 3). Havalandırma bacası, Niğde'deki ağılların %95.8'inde bulunmakta (Ceyhan ve ark. 2015), Bursa'daki ağılların ise hiç birinde bulunmamaktadır (Altınçekiç 2014). Kahramanmaraş işletmelerinin %96.0'sında ise pencere alanının yeterli olmadığı bildirilmiştir (Paksoy ve ark. 2006).

Bu araştırma bulguları ışığında Elazığ ilindeki ağılların havalandırma bacası varlığı bakımından yetersiz, pencere varlığı bakımından ise kısıtlı olduğu görülmektedir. Bununla birlikte yeterli havalandırma bacası bulunan ağılların oranı Bursa'daki işletmelerden, yeterli pencere bulunan ağılların oranı ise Kahramanmaraş'taki işletmelerden daha yüksektir. Biyogüvenlik ve refah koşullarına uygun olmayan bu eksikliğin düzeltilmesi gerekmektedir.

Araştırmada, koyunları sulamak için %94.5 oranında yalak kullanıldığı tespit edilmiştir (Tablo 3). Bursa'daki işletmelerde de %100 (Altınçekiç 2014), Siirt'teki işletmelerde %48.3 (Bakır ve ark. 2017) yalak kullanılmaktadır. Elazığ'daki işletmelerde ağıllarda sulama amaçlı yalak kullanım oranı, Bursa'daki işletmelerde tespit edilen orandan düşük, Siirt'teki işletmelerde belirlenen orandan ise daha yüksek saptanmıştır. Yalaklar otomatik suluklara göre koyunların istedikleri zaman suya erişimine daha az imkân vermektedir. Bu nedenle otomatik suluklar yaygınlaştırılmalıdır.

Bu çalışmada, işletmelerde %55.8 oranında ahşap yemlik, %40.4 oranında metal yemlik kullanılmaktadır (Tablo 3). Ahşap yemliklerin kullanım oranı Tokat'taki işletmelerde %93.7 (Karaman ve ark. 2012), Niğde'deki işletmelerde ise %91.7'dir (Ceyhan ve ark. 2015). Ahşap yemlik kullanım oranı Tokat ve Niğde'deki işletmelerde saptanandan daha düşüktür. Ahşap yemlikler, ıslanıp çürümeye son derece elverişli malzemelerdir. Bunlar yıkama ve dezenfeksiyon uygulamaları açısından da elverişli değillerdir.

Elazığ'daki işletmelerin %88.5'inde yem hazırlama değirmeni/karıştırıcı, %47.2'sinde ise gölgelik/sundurma bulunmamaktadır (Tablo 3). İşletmelerin yem hazırlama değirmeni/karıştırıcı ve sundurma/gölgeliklerin sahibi olmamaları önemli bir eksikliktir.

SONUÇ

Ekonomik ve sosyal bakımdan önemli bir faaliyet kolu olan koyunculuğun etkin ve yeterli sürdürülmesi için yapısal imkânların yerinde ve doğru kullanılması gerekmektedir. Yapılan bu araştırmada, Elazığ'daki koyunculuk işletmelerinin yapısal özellikleri, hem Türkiye'deki farklı bölge ve illerdeki örnekleriyle hem de mevcut durum bakımından kendi içerisinde değerlendirilmiş, bilimsel ve teknik olarak analiz edilmiştir. Elazığ'daki işletmelerin, araştırma kapsamında ele alınan yapısal ölçütler bakımından genel olarak standartlara uygun olduğu belirlenmiştir. Bununla birlikte işletmelerin verimlilik ve sürdürülebilirlik bakımından daha iyi bir duruma getirilmesi için aşağıda sıralanan önerilerin daha fazla göz önüne alınması gerektiği kanaatine ulaşılmıştır.

- Yetersiz sayıda olan havalandırma bacası ve kısıtlı sayıdaki pencere varlığı artırılmalıdır.
- Normalden daha düşük olan ağıl yükseklikleri ile dinlenme yerinde koyun başına alanlar artırılmalıdır.
- Çatılarda sac yerine kiremit ya da izolasyonlu malzemelerin kullanılması teşvik edilmelidir.
- Ağıl zeminlerinde toprak ve duvarlarında kerpiç malzeme yerine, temizlik ve dezenfeksiyona elverişli malzemelerin kullanılması sağlanmalıdır.
- Koyunlar için sulak olarak kullanılan yalaklar yerine otomatik suluklar daha fazla yaygınlaştırılmalıdır.
- Yazlak ve kışlak ağılların olduğu yerlerde koyunlar için mutlaka gölgelik ve sundurmalar yapılmalıdır.
- Yem ve rasyon hazırlamak için değirmen/karıştırıcı alımı teşvik edilmelidir.
- Verilecek faizsiz kredilerle koyunculuk amaçlı kullanmak şartı ile arazi satın alımı sağlanmalı ve desteklenmelidir.
- Koşullar bakımından elverişsiz olanların yerine yeni ve uygun ağılların yapılması sağlanmalıdır.
- Ağıl yapacak yetiştiricilere ağıl projesi sağlanmalı, elverişli yerlerde yarı açık, açık veya çadır-sera tipi ağıllar yaygınlaştırılmalıdır.
- İşletmelerdeki yapısal sorunların giderilmesi için mali teşvik, destek ve faizsiz krediler verilmelidir.

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RESEARCH ARTICLE

Evaluation of Platelet Count and Platelet Indices in Cats and Dogs Diagnosed with Lymphoma

E. Merve ALAN[1] Özge ERDOĞAN BAMAÇ[2] Lora KOENHEMSİ[1]

[1]Department of Internal Medicine, Istanbul University –Cerrahpasa, Istanbul, Avcılar, Turkey
[2]Department of Pathology, Istanbul University – Cerrahpasa, Istanbul, Avcılar, Turkey

ABSTRACT

In this study, it was aimed to evaluate the platelet count and platelet indices in cats and dogs diagnosed with lymphoma. Lymphoma, a malignant tumor that is very common in cats and dogs, is more common in middle-aged and older cats and dogs. For diagnostic purposes, complete blood count, serum biochemistry profile, urine analysis and some biomarkers as well as ultrasonography, radiography and advanced imaging techniques are used. Non-regenerative anemia, thrombocytopenia and leukocyte variability can be encountered in the complete blood count of cats and dogs with lymphoma. In recent years, the reflections of platelet indices on different diseases have been the subject of new studies in the fields of human medicine and veterinary medicine. In this direction, 13 cats with lymphoma, 20 healthy cats, 33 dogs with lymphoma and 20 healthy dogs were included in the study, which was conducted to examine PLT (Thrombocyte), MPV (Mean platelet volume-fl), PDW (Platelet distribution width) and PCT (Thrombocytocrit-%) of cats and dogs with lymphoma. The lymphoma diagnosis of the patients was confirmed by cytological and histopathological examinations. According to the statistical evaluations obtained from the hemogram data, it was determined that the RBC (Red blood cell-m/mm3), HGB (Hemoglobin-g/dL), HCT (Hematocrit-%) and MCHC (Mean platelet volume-fl) values were significantly lower ($P \leq 0.01$) and the MPV value was higher ($P < 0.05$) in sick cats compared to healthy cats. It was determined that RBC, HGB, HCT values were lower ($P < 0.05$), WBC ($P \leq 0.01$) and PDW ($P < 0.05$) values were higher in sick dogs compared to healthy dogs. In this study conducted on cats and dogs with lymphoma, it is thought that changes in some of the platelet indices may be significant for treatment management and prognosis. It has been concluded that further studies on the subject will contribute to the field of veterinary medicine.

Keywords: Lymphoma, platelet, platelet indices, thrombocytopenia

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Lenfoma Teşhisi Konulan Kedi ve Köpeklerde Trombosit Sayısı ve Trombosit İndekslerinin Değerlendirilmesi

ÖZ

Bu araştırmada, lenfoma teşhisi konulan kedi ve köpeklerdeki trombosit sayısı ve trombosit indekslerinin değerlendirilmesi amaçlanmıştır. Kedi ve köpeklerde çok fazla rastlanılan malign bir tümör olan lenfoma, daha sık olarak orta yaş ve üzerindeki kedi ve köpeklerde görülmektedir. Diyagnostik amaçla, tam kan sayımı, serum biyokimya profili, idrar analizi ve bazı biyobelirteçlerin yanında ultrasonografi, radyografi ve ileri görüntüleme tekniklerinden faydalanılmaktadır. Lenfomalı kedi ve köpeklerin tam kan sayımında non-rejeneratif anemi, trombositopeni ve lökosit değişkenlikleriyle karşılaşılabilmektedir. Son yıllarda beşeri hekimlik ve veteriner hekimliği alanlarında, trombosit indekslerinin farklı hastalıklar üzerindeki yansımaları yeni çalışmalara konu olmuştur. Bu doğrultuda, lenfomalı kedi ve köpeklerde PLT(Trombosit), MPV (Ortalama trombosit hacmi-fl), PDW (Trombosit dağılım genişliği) ve PCT (Trombositokrit-%)'in incelenmesi amacıyla yürütülen çalışmaya 13 lenfomalı kedi, 20 sağlıklı kedi, 33 lenfomalı köpek ve 20 sağlıklı köpek dahil edilmiştir. Hastaların lenfoma teşhisi, sitolojik ve histopatolojik incelemelerle doğrulanmıştır. Hemogram verilerinden elde edilen istatistiki değerlendirmelere göre, hasta kedilerde RBC (Eritrosit-m/mm3), HGB (Hemoglobin-g/dL), HCT (Hematokrit-%) ve MCHC (Ortalama Eritrosit Hemoglobin Konsantrasyonu) değerlerinin sağlıklı kedilere göre anlamlı düzeyde düşük ($P \leq 0.01$), MPV (Ortalama trombosit hacmi-fl), değerinin ise yüksek ($P < 0.05$) olduğu tespit edilmiştir. Hasta köpeklerde RBC, HGB, HCT değerlerinin sağlıklı köpeklere göre düşük ($P < 0.05$), WBC ($P \leq 0.01$) ve PDW ($P < 0.05$) değerlerinin ise yüksek olduğu belirlenmiştir. Lenfomalı kedi ve köpekler üzerinde yapılan bu çalışmada, trombosit indekslerinin bir kısmında meydana gelen değişimlerin tedavi yönetimi ve prognoz için anlamlı olabileceği düşünülmektedir. Konuyla ilgili daha fazla çalışmanın yapılmasının veteriner hekimlik alanına katkıda bulunacağı kanısına varılmıştır.

Anahtar Kelimeler: Lenfoma, platelet, platelet indeksleri, trombositopeni

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ORCID ID; *EMA: 0000-0002-4422-2241, ÖEB: 0000-0002-0352-4841, LK: 0000-0002-4979-170X*

*Corresponding author e-mail: dvm.mervealan@gmail.com

GİRİŞ

Lenfoma, kedi ve köpeklerde sık görülen malign bir tümördür. Kanin lenfoma, yıllık olarak 100.000 köpekten 20-100 arası köpekte görülmekle birlikte, en çok teşhis edilen hematopoietik neoplazilerin başında yer almaktadır. Kesin nedeni tam olarak bilinmemekle birlikte, çevresel faktörlerin ve genetik yatkınlığın hastalığın köpeklerdeki oluşumunda önemli bir rol oynadığı düşünülmektedir (Zandevliet, 2016). Tüm ırklarda görülebilmesine rağmen, orta ve büyük ırk köpeklerin lenfomaya daha yatkın olduğu belirtilmiştir (Teske ve ark., 1994a). Hastalık için belirgin bir cinsiyet eğilimi yoktur fakat dişi köpeklerde riskin daha az olduğu bilinmektedir (Villamil ve ark., 2009).

Günümüze kadar yayınlanan çalışmalar, lenfomalı kedilerin yaklaşık %70'inin kedi lösemi virüsü (FeLV) ile enfekte olduğunu göstermiştir. Aynı zamanda kedi immun yetmezlik virüsü (FIV)'nün de, kedilerde lenfoma gelişme riskini önemli ölçüde artırdığı bilinmektedir. FeLV ile enfekte kedilerde lenfoma görülme yaşının ortalama 3, enfekte olmayan kedilerdeki lenfoma görülme yaşının ise 7 ile 8 arasında olduğu bilinmektedir (Couto, 2000).

Lenfomalı kedi ve köpeklerde hastalığın evresi ve yerleşim yerindeki farklılıklar sebebiyle klinik bulgular oldukça değişkenlik göstermektedir. Hastaların anamnezinde sıklıkla iştahsızlık, halsizlik, kusma, ishal, kilo kaybı, solunum güçlüğü, görme bozukluğu veya denge kaybı gibi hastalığa spesifik olmayan semptomlar bildirilmektedir. Klinik muayene sırasında hepatomegali, splenomegali ve ağrısız, serbestçe hareket edebilen büyümüş lenf yumruları tespit edilebilmektedir. Lenf yumrularındaki büyümeler tek taraflı veya multipl olabilmektedir (Couto, 2000; Zandevliet, 2016).

Diyagnostik anlamda radyografik ve ultrasonografik incelemeler ve bilgisayarlı tomografi gibi ileri görüntüleme tekniklerine başvurulmaktadır (Blackwood ve ark., 1997; Nyman ve ark., 2005; Ballegeer ve ark., 2013). Tam kan sayımı, serum biyokimya profili, idrar analizi ve bazı biyobelirteçlerin incelenmesi gibi tahlillerin yanı sıra tedaviye başlamak amacıyla kesin teşhis, ancak sitolojik ve histopatolojik incelemeler sonucunda konulabilmektedir (Teske, 1994b; Couto, 2000; Bryan, 2016; Zandevliet, 2016).

Lenfomalı kedi ve köpeklerin tam kan sayımında sıklıkla non-rejeneratif anemi, trombositopeni ve lökosit değişkenlikleriyle karşılaşılmaktadır (Grindem ve ark., 1994; Ettinger, 2003; Gavazza ve ark., 2008). İnsanlarda yapılan çalışmalar, tümörlerle alakalı hematolojik değişimlerin bazı tümör tiplerinde daha sık gerçekleştiğini göstermektedir. Bunlar arasında lenfoma da yer almaktadır. Son yıllarda beşeri hekimlikte hematolojik parametreler arasında özellikle trombosit ve indeksleri daha fazla sayıda araştırmaya konu olmuştur. Beşeri alanda yapılan bu çalışmalar, trombositlerin çeşitli hastalıklarda, organ yetmezliklerinde ve tümör olgularında etkin rol oynadığını göstermiştir (Uçmak ve ark., 2021).

Trombosit indeksleri arasında yer alan ortalama trombosit hacmi, trombosit dağılım genişliği ve trombositokrit parametrelerinin ölçümü, günümüz veteriner hekimliği alanında kullanılan hematolojik cihazlar tarafından rahatlıkla yapılabilmektedir. Son yıllarda veteriner hekimliği alanında birçok çalışmaya dahil olan bu parametrelerin klinik önemleri daha belirgin hale gelmiştir (Alan ve ark., 2022).

Lenfomalı hayvanların hematolojik yönden incelenmesi, özellikle prognozun belirlenebilmesi ve tedavi metodlarının uygun bir şekilde oluşturulması açısından önem taşımaktadır. Lenfomalı kedi ve köpeklerde trombosit ve indekslerinin araştırmaya açık ve faydalı bir konu olabileceği öngörülmüştür (Graff ve ark., 2014; Phillips ve ark., 2022). Bu amaçla planlanan çalışmada, lenfomalı kedi ve köpeklerdeki trombosit sayıları ve trombosit indeksleri, sağlıklı kedi ve köpekler ile karşılaştırmalı olarak değerlendirilmiştir.

GEREÇ VE YÖNTEM

İstanbul Üniversitesi-Cerrahpaşa Veteriner Fakültesi İç Hastalıkları Anabilim Dalı'na bir veya birden çok bölgede ağrısız lenfadenopati şikayeti ile getirilmiş ve yapılan tetkikler sonucunda lenfoma teşhisi konulmuş çeşitli ırk, cinsiyet ve yaştaki kedi ve köpekler çalışmanın hasta grubunu oluşturmuştur. Herhangi bir şikayeti olmaksızın genel sağlık kontrolü amacıyla getirilen kedi ve köpekler vücut sıcaklığı, nabız, solunum sayısı, mukoz membranların ve deri turgorunun kontrolü olacak şekilde genel muayeneye tabi tutulmuştur. Genel muayene ve hemogram bulguları normal olanlar çalışmanın sağlıklı gurubunu oluşturmuştur. Bu hayvanlar arasında hemogram bulgularında eksiklik bulunanlar ile aynı anda başka bir hastalığa daha sahip olanlar ve daha önceden tedavi görmüş olanlar çalışma kapsamına alınmamıştır. Buna ek olarak, Cavalier King Charles, Cairn Terrier, Norfolk Terrier ve Greyhound ırkı köpekler kongenital makrotrombositopeniye sahip olduklarından dolayı çalışmaya dahil edilmemiştir. Bu doğrultuda, 13 lenfomalı kedi, 20 sağlıklı kedi, 33 lenfomalı köpek ve 20 sağlıklı köpek çalışma kapsamına alınmıştır.

Çalışma kapsamında değerlendirilen kedi ve köpeklerin anamnez bilgisi, klinik muayene bulguları ve tam kan sayımı sonuçları, polikliniğimizin elektronik tıbbi kayıt veri tabanı üzerinden bulunarak not edilmiştir.

Veri tarama sisteminden edinilen bilgiye göre, çalışma kapsamına dahil edilen hayvanların kanı jugular, cephalik veya lateral saphenous venaların herhangi bir tanesinden alınarak, K3-EDTA (Tri-potasyum etilendiamintetraasetik asit) içeren tüplere konulmuştur. Kan örnekleri Idexx ProCyte Dx model hemogram cihazıyla incelenmiştir. Hemogram verilerinden; PLT (Trombosit), MPV (Ortalama Trombosit Hacmi-fl), PDW (Trombosit Dağılım Genişliği), PCT (Trombositokrit-%), WBC (Lökosit-m/mm3), RBC (Eritrosit-m/mm3), MCV (Ortalama Eritrosit Hacmi-fl), MCH (Ortalama Eritrosit Hemoglobini-pg), MCHC (Ortalama Eritrosit

Hemoglobin Konsantrasyonu), RDW (Eritrosit Dağılım Genişliği), HCT (Hematokrit-%) ve HGB (Hemoglobin-g/dL) bulguları değerlendirmeye alınmıştır. Lenfoma şüpheli hastaların büyümüş lenf yumrusundan ince iğne aspirasyon yöntemi ile alınan örnekler sitolojik incelemeye, lenf yumrularından alınan biyopsi örnekleri ise histopatolojik incelemeye gönderilmiştir. Alınan örneklerin incelemesi fakültemizin patoloji anabilim dalında gerçekleşmiştir. Lenf yumrusu aspiratlarından elde edilen tüm sitolojik smearlar May-Grünwald Giemsa ile boyanmıştır. Histopatolojik muayene için gönderilen tümör dokuları % 10'luk tamponlu formalinde fikse edildikten sonra parafine gömülmüş ve rotary mikrotomda 4 m kalınlığında kesitler alınarak ışık mikroskobunda incelenmek üzere Hematoksilen Eozin ile boyanmıştır.

Bu çalışma için gerekli olan etik kurul izni İstanbul Üniversitesi-Cerrahpaşa Rektörlüğü Veteriner Fakültesi Birim Etik Kurulu Başkanlığı'ndan alınmıştır (25.05.2022 tarih ve 2022/21 nolu karar).

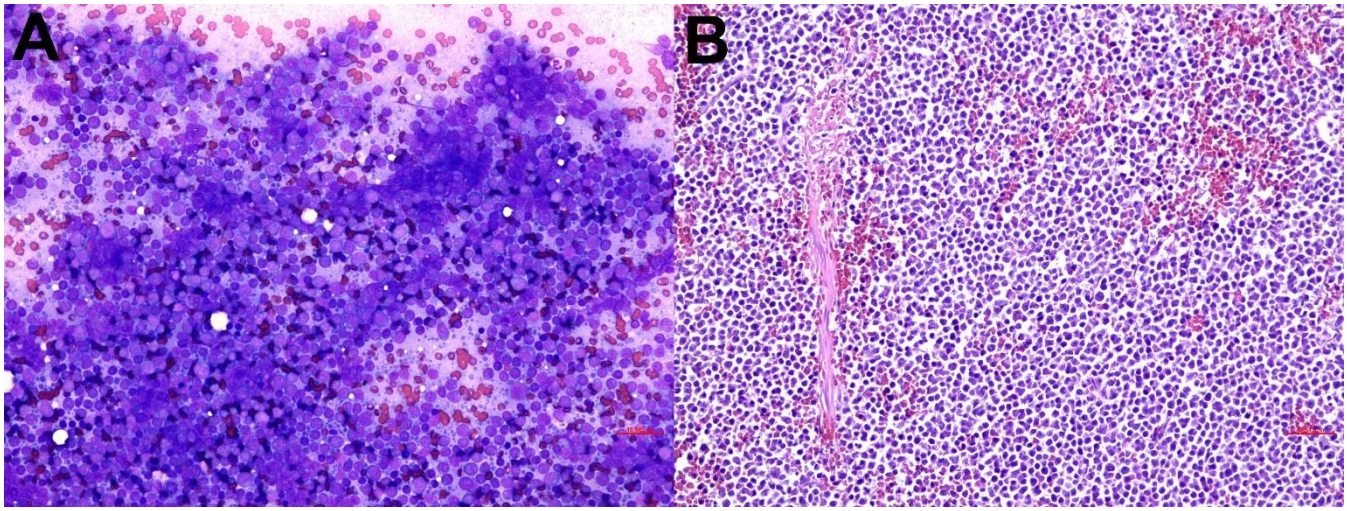
Verilerin dağılımı Shapiro-Wilk testi ile kontrol edilmiştir. Verilerde normal dağılım gözlendiği için parametrik bir test olan Univariate ANOVA testi kullanılmıştır. Verilerin gösteriminde "aritmetik ortalama ± standart hata" kullanılmıştır. Veriler, "IBM SPSS Statistics 25" paket programı ile analiz edilmiştir. Sonuçlar için anlamlılık seviyesi $P<0.05$ olarak kabul edilmiştir.

BULGULAR

Lenf nodu aspiratlarının sitolojik incelemesi sonucu 8 kedi ve 21 köpeğe, lenf nodundan alınan biyopsilerin histopatolojik incelemesi sonucu ise 5 kedi ve 12 köpeğe lenfoma tanısı konulmuştur (Resim 1). Neoplazik lenfositler, literatürde yer aldığı şekilde; hücre ve çekirdek boyutuna, kromatin paternine, çekirdekçiklerin ve mitotik figürlerin varlığına göre değerlendirilmiştir (Moulton JE, 1990).

Her gruptaki kedi ve köpeklerin ırk, yaş ve cinsiyet bilgileri kaydedilmiştir. Lenfomalı kedilerden 8'inin dişi (%61,54), 5'inin erkek (%38,46); lenfomalı köpeklerden 11'inin dişi (%33,33), 22'sinin erkek (%66,66) olduğu belirlenmiştir. Hasta kedilerden 12'sinin tekir (%92,31), 1'inin Scottish Fold ırkı (%7,69); hasta köpeklerden 13'ünün melez (%41,93), 8'inin Golden Retriever (%25,80), 3'ünün American Cocker Spaniel (%9,67), 3'ünün Rottweiler (%9,67), 1'inin Cavalier King Charles Spaniel (%3,22), 1'inin Boxer (%3,22), 1'inin Pekingese (%3,22) ve 1' nin Terrier (%3,22) ırkı olduğu belirlenmiştir. Hasta kedilerin yaş ortalaması 6,28±1,26; hasta köpeklerin yaş ortalaması ise 8,65±0,68 olarak tespit edilmiştir.

Elde edilen istatistiki değerlendirmelere göre, hasta kedilerde RBC, HGB, HCT ve MCHC (Tablo 1 ve 2) değerlerinin sağlıklı kedilere göre anlamlı düzeyde düşük ($P\leq0.01$), MPV (Tablo 3) değerinin ise yüksek ($P<0.05$) olduğu tespit edilmiştir. Hasta köpeklerde RBC, HGB, HCT (Tablo 5) değerlerinin sağlıklı köpeklere göre düşük ($P<0.05$), WBC ($P\leq0.01$) ve PDW ($P<0.05$) değerlerinin ise yüksek olduğu belirlenmiştir (Tablo 6 ve 7).



Resim 1: Histopatolojik muayenede lenfomanın mikroskobik görüntüsü.
Image 1: Microscopic image of lymphoma on histopathological examination.

A) Köpek. Lenf yumrusu. Lenfoma. Belirgin anizositozis, anizokaryozis gösteren pleomorfik, çok çekirdekçikli lenfositik seriden hücreler ve zeminde bol miktarda lenfoglandüler cisimcikler. May-Grünwald Giemsa boyama.
B) Köpek. Dalak. Diffuz immunoblastik lenfoma. Hematoksilen- Eozin boyama.

A) Dog. Lymph node. Lymphoma. Significant anisocytosis, cells from the pleomorphic, multinucleated lymphocytic series showing anisokaryosis, and abundant lymphoglandular bodies in the background. Painting by May-Grünwald Giemsa.
B) Dog. Spleen. Diffuse immunoblastic lymphoma. Hematoxylin-Eosin staining.

Tablo 1. Kedilerde lenfoma durumu ve cinsiyetin RBC, HCT ve HGB üzerine etkileri
Table 1. The effect of lymphoma status and sex on RBC, HCT and HGB in cats

Faktörler	RBC		HCT		HGB	
	Ort	SH	Ort	SH	Ort	SH
Lenfoma durumu (L)						
Lenfoma (L)	7,10	0,65	30,72	2,38	9,91	0,77
Sağlıklı (S)	9,62	0,49	39,23	1,78	13,37	0,58
Cinsiyet (C)						
Dişi (D)	8,73	0,63	37,32	2,31	12,25	0,75
Erkek (E)	8,00	0,51	32,62	1,87	11,03	0,61
L x C etkileşimi						
Lenfoma-Dişi	7,77	1,08	35,15	3,96	11,27	1,28
Lenfoma-Erkek	6,44	0,72	26,29	2,64	8,54	0,86
Sağlıklı-Dişi	9,68	0,65	39,50	2,39	13,22	0,77
Sağlıklı-Erkek	9,56	0,72	38,96	2,64	13,52	0,86
P değeri						
Lenfoma	0,00**		0,01**		0,00**	
Cinsiyet	0,38		0,12		0,22	
L x C	0,46		0,17		0,13	

RBC (Eritrosit-m/mm3), HCT (Hematokrit-%), HGB (Hemoglobin-g/dL)
(*P<0.05, **P≤0.01)
Veriler Ort (Aritmetik ortalama) ve SH (Standart hata) olarak verilmiştir.

Tablo 2. Kedilerde lenfoma durumu ve cinsiyetin MCV, MCH, MCHC ve WBC üzerine etkileri
Table 2. The effect of lymphoma status and sex on MCV, MCH, MCHC and WBC in cats

Faktörler	MCV		MCH		MCHC		WBC	
	Ort	SH	Ort	SH	Ort	SH	Ort	SH
Lenfoma durumu (L)								
Lenfoma (L)	45,52	2,11	14,73	0,71	32,38	0,54	12,67	3,31
Sağlıklı (S)	41,04	1,58	13,99	0,53	34,15	0,40	10,58	2,48
Cinsiyet (C)								
Dişi (D)	43,26	2,05	14,13	0,69	32,77	0,52	11,26	3,22
Erkek (E)	43,30	1,66	14,59	0,55	33,77	0,42	12,00	2,60
L x C etkileşimi								
Lenfoma-Dişi	45,52	3,52	14,52	1,17	31,92	0,89	9,84	5,51
Lenfoma-Erkek	45,51	2,35	14,94	0,78	32,84	0,60	15,51	3,67
Sağlıklı-Dişi	41,00	2,12	13,74	0,71	33,61	0,54	12,68	3,32
Sağlıklı-Erkek	41,09	2,35	14,24	0,78	34,69	0,60	8,49	3,67
P değeri								
Lenfoma	0,10		0,41		0,01**		0,62	
Cinsiyet	0,99		0,60		0,147		0,86	
L x C	0,98		0,96		0,91		0,24	

MCV (Ortalama Eritrosit Hacmi-fl), MCH (Ortalama Eritrosit Hemoglobini-pg), MCHC (Ortalama Eritrosit Hemoglobin Konsantrasyonu), WBC (Lökosit-m/mm3)
(*P<0.05, **P≤0.01)
Veriler Ort (Aritmetik ortalama) ve SH (Standart hata) olarak verilmiştir.

Tablo 3. Kedilerde lenfoma durumu ve cinsiyetin PLT, MPV ve PCT üzerine etkileri
Table 3. The effect of lymphoma status and sex on PLT, MPV and PCT in cats

Faktörler	PLT		MPV		PCT	
	Ort	SH	Ort	SH	Ort	SH
Lenfoma durumu (L)						
Lenfoma (L)	282,64	38,07	18,05	0,58	0,49	0,06
Sağlıklı (S)	306,40	28,47	16,29	0,44	0,49	0,04
Cinsiyet (C)						
Dişi (D)	319,93	36,99	16,55	0,57	0,52	0,05
Erkek (E)	269,11	29,86	17,79	0,46	0,46	0,04
L x C etkileşimi						
Lenfoma-Dişi	294,50	63,35	17,07	0,97	0,49	0,09
Lenfoma-Erkek	270,78	42,23	19,02	0,65	0,48	0,06
Sağlıklı-Dişi	345,36	38,20	16,02	0,59	0,54	0,06
Sağlıklı-Erkek	267,44	42,23	16,57	0,65	0,45	0,06
P değeri						
Lenfoma	0,62		0,02*		0,91	
Cinsiyet	0,29		0,10		0,45	
L x C	0,57		0,35		0,56	

PLT (Trombosit), MPV (Ortalama Trombosit Hacmi-fl), PCT (Trombositokrit-%)
(*P<0.05, **P≤0.01)
Veriler Ort (Aritmetik ortalama) ve SH (Standart hata) olarak verilmiştir.

Tablo 4. Kedilerde yaş durumu ve cinsiyetin lenfoma üzerine etkileri
Table 4. Effects of age and sex on lymphoma in cats

Faktörler	YAŞ	
	Ort	SH
Lenfoma durumu (L)		
Lenfoma (L)	6,28	1,26
Sağlıklı (S)	4,39	0,85
Cinsiyet (C)		
Dişi (D)	5,39	1,23
Erkek (E)	5,28	0,89
L x C etkileşimi		
Lenfoma-Dişi	6,33	2,18
Lenfoma-Erkek	6,22	1,26
Sağlıklı-Dişi	4,45	1,14
Sağlıklı-Erkek	4,33	1,26
P değeri		
Lenfoma	0,22	
Cinsiyet	0,94	
L x C	0,99	

(*P<0.05, **P≤0.01)
Veriler Ort (Aritmetik ortalama) ve SH (Standart hata) olarak verilmiştir.

Tablo 5. Köpeklerde lenfoma durumu ve cinsiyetin RBC, HCT ve HGB üzerine etkileri
Table 5. The effect of lymphoma status and sex on RBC, HCT and HGB in dogs

Faktörler	RBC		HCT		HGB	
	Ort	SH	Ort	SH	Ort	SH
Lenfoma durumu (L)						
Lenfoma (L)	4,83	0,22	30,82	1,45	10,91	0,52
Sağlıklı (S)	6,90	0,27	45,20	1,79	16,10	0,65
Cinsiyet (C)						
Dişi (D)	5,99	0,25	39,22	1,64	13,87	0,59
Erkek (E)	5,74	0,25	36,80	1,62	13,13	0,58
L x C etkileşimi						
Lenfoma-Dişi	4,84	0,36	31,75	2,37	11,14	0,85
Lenfoma-Erkek	4,83	0,25	29,89	1,68	10,68	0,60
Sağlıklı-Dişi	7,14	0,34	46,70	2,27	16,61	0,82
Sağlıklı-Erkek	6,66	0,42	43,71	2,78	15,59	1,00
P değeri						
Lenfoma	0,00**		0,00**		0,00**	
Cinsiyet	0,49		0,30		0,38	
L x C	0,50		0,81		0,74	

RBC (Eritrosit-m/mm3), HCT (Hematokrit-%), HGB (Hemoglobin-g/dL)
(*P<0.05, **P≤0.01)
Veriler Ort (Aritmetik ortalama) ve SH (Standart hata) olarak verilmiştir.

Tablo 6. Köpeklerde lenfoma durumu ve cinsiyetin MCV, MCH, MCHC ve WBC üzerine etkileri
Table 6. The effect of lymphoma status and sex on MCV, MCH, MCHC and WBC in dogs

Faktörler	MCV		MCH		MCHC		WBC	
	Ort	SH	Ort	SH	Ort	SH	Ort	SH
Lenfoma durumu (L)								
Lenfoma (L)	63,89	0,76	22,49	0,28	36,62	0,82	27,49	2,90
Sağlıklı (S)	65,56	0,93	23,33	0,34	35,62	1,02	9,48	3,58
Cinsiyet (C)								
Dişi (D)	65,53	0,85	23,00	0,31	36,49	0,93	18,14	3,27
Erkek (E)	63,92	0,85	22,80	0,31	35,75	0,92	18,83	3,24
L x C etkileşimi								
Lenfoma-Dişi	65,56	1,23	22,75	0,45	37,44	1,34	26,97	4,73
Lenfoma-Erkek	62,22	0,87	22,24	0,32	35,79	0,95	28,01	3,34
Sağlıklı-Dişi	65,49	1,18	23,25	0,43	35,55	1,29	9,31	4,53
Sağlıklı-Erkek	65,62	1,45	23,41	0,53	35,70	1,58	9,64	5,55
P değeri								
Lenfoma	0,17		0,06		0,45		0,00**	
Cinsiyet	0,19		0,69		0,57		0,88	
L x C	0,15		0,44		0,50		0,94	

MCV (Ortalama Eritrosit Hacmi-fl), MCH (Ortalama Eritrosit Hemoglobini-pg), MCHC (Ortalama Eritrosit Hemoglobin Konsantrasyonu), WBC (Lökosit-m/mm3)
(*P<0.05, **P≤0.01)
Veriler Ort (Aritmetik ortalama) ve SH (Standart hata) olarak verilmiştir.

Tablo 7. Köpeklerde lenfoma durumu ve cinsiyetin PLT, MPV, PDW ve PCT üzerine etkileri
Table 7. The effect of lymphoma status and sex on PLT, MPV, PDW and PCT in dogs

Faktörler	PLT		MPV		PDW		PCT	
	Ort	SH	Ort	SH	Ort	SH	Ort	SH
Lenfoma durumu (L)								
Lenfoma (L)	230,68	25,38	12,03	0,52	12,46	0,52	0,26	0,03
Sağlıklı (S)	292,27	31,38	11,40	0,62	10,86	0,54	0,33	0,03
Cinsiyet (C)								
Dişi (D)	280,67	28,69	12,24	0,58	12,08	0,56	0,32	0,03
Erkek (E)	242,28	28,38	11,19	0,57	11,23	0,49	0,27	0,03
L x C etkileşimi								
Lenfoma-Dişi	252,18	41,45	12,92	0,86	13,63	0,88	0,30	0,04
Lenfoma-Erkek	209,18	29,31	11,13	0,60	11,29	0,55	0,23	0,03
Sağlıklı-Dişi	309,17	39,69	11,57	0,78	10,54	0,70	0,35	0,04
Sağlıklı-Erkek	275,37	48,61	11,24	0,96	11,17	0,82	0,31	0,05
P değeri								
Lenfoma	0,13		0,44		0,04*		0,13	
Cinsiyet	0,35		0,20		0,26		0,21	
L x C	0,91		0,37		0,05		0,74	

PLT (Trombosit), MPV (Ortalama Trombosit Hacmi-fl), PDW (Trombosit Dağılım Genişliği), PCT (Trombositokrit-%)
(*P<0.05, **P≤0.01)
Veriler Ort (Aritmetik ortalama) ve SH (Standart hata) olarak verilmiştir.

Tablo 8. Köpeklerde yaş durumu ve cinsiyetin lenfoma üzerine etkileri
Table 8. Effects of age and sex on lymphoma in dogs

Faktörler	YAŞ	
	Ort	SH
Lenfoma durumu (L)		
Lenfoma (L)	8,65	0,68
Sağlıklı (S)	7,48	0,84
Cinsiyet (C)		
Dişi (D)	8,04	0,77
Erkek (E)	8,08	0,76
L x C etkileşimi		
Lenfoma-Dişi	9,00	1,11
Lenfoma-Erkek	8,29	0,78
Sağlıklı-Dişi	7,08	1,06
Sağlıklı-Erkek	7,87	1,30
P değeri		
Lenfoma	0,28	
Cinsiyet	0,97	
L x C	0,49	

(*P<0.05, **P≤0.01)
Veriler Ort (Aritmetik ortalama) ve SH (Standart hata) olarak verilmiştir.

TARTIŞMA VE SONUÇ

Hayvanlarda ve insanlarda tümör oluşumuna bağlı şekillenen çeşitli hematolojik değişimler bildirilmiştir (Grindem ve ark., 1994; Graff ve ark., 2014). Lenfomalı insanlar üzerinde yapılan çalışmalarda trombositopeni ve indekslerinin kullanımı üzerinde hala bir görüş birliğine varılmadığından konuyla alakalı araştırmalara devam edildiği fark edilmiştir. Hayvanlar üzerinde yapılan araştırmalarda ise trombositopeni en sık görülen hematolojik bulgu olmasına rağmen trombosit indeksleri ile alakalı köpekler üzerine yayınlanmış az sayıda makale bulunmaktadır. Lenfomalı kedilerde ise trombosit ve indeksleriyle ilgili yapılmış herhangi bir çalışma olmadığı belirlenmiştir. Yapılmış olan bu çalışma ile lenfomalı kedi ve köpeklerdeki trombosit indekslerindeki değişimler incelenmiştir.

Özellikle 3 yaşın altındaki genç kedilerde lenfoma gelişiminin FIV/FeLV enfeksiyonu ile yüksek oranda ilişkili olduğu bilinmektedir (Couto, 2000). Bu çalışmadaki 13 lenfomalı kediye yapılan FIV/FeLV testleri negatif sonuçlanmıştır. Çalışmaya dahil edilen hasta kedilerin yaş ortalamalarının (6,28±1,26) yüksek olması sebebiyle hastalığın; yaş, çevresel faktörler ve genetik yatkınlık sonucu oluştuğu kanısına varılmıştır.

Günümüze kadar yapılan çalışmalar, lenfoma insidansının özellikle orta ve büyük ırk köpeklerde daha yüksek olduğunu göstermiştir (Teske ve ark., 1994a). Önceki çalışmalara paralel olarak bu çalışmadaki köpeklerin büyük oranda orta ve büyük ırk olduğu belirlenmiştir. Dorn ve ark., (1967) yaptıkları çalışmada, lenfoma insidansının 1 yaşından küçük köpeklerde 100.000 köpek başına 1.5 olduğunu tespit ederken, 10 yaşından büyük köpeklerde bu oranın 100.000'de 84'e yükseldiğini bildirmişlerdir. Teske (1994), lenfomaların ağırlıklı olarak orta yaştaki (6 ila 8 yaş arası) köpekleri etkilediğini belirtmiştir. Cinsiyetin (kısırlaştırma dahil), kanin lenfoma prevalansı veya insidansı üzerindeki etkileri çoğu araştırmacı tarafından önemsiz bulunmuştur (Greenlee ve ark., 1990; Edwards ve ark., 2003). Bu çalışmada daha önce bilidirilen yaş ortalamasına benzer şekilde lenfomalı köpeklerin yaş ortalaması 8,65±0,68 olarak belirlenmiştir. Aynı zamanda çalışmanın istatistik verilerine göre, erkek ve dişilerde hastalık durumunun grup etkisi bakımından farklılık göstermediği belirlenmiştir.

Lenfomalı kedi ve köpeklerin tam kan sayımında, non-rejeneratif anemi ve lökositozis sıklıkla karşılaşılan anormalitelerdir (Ettinger, 2003; Gavazza ve ark., 2008). Bu çalışmada, hasta kedi ve köpeklerin RBC, HCT, HGB değerlerinin, sağlıklı kedi ve köpeklere göre anlamlı düzeyde ($P\leq0.01$) düşük olduğu saptanmıştır. Bu doğrultuda çalışmaya dahil edilen lenfomalı kedi ve köpeklerin non-rejeneatif anemisi olduğu tespit edilmiştir. Lenfomalı kedilerin MCHC değeri, sağlıklı kedilere göre anlamlı düzeyde ($P<0.05$) düşük bulunmuş ve hipokromik anemi varlığını doğrulamıştır. Ayrıca lenfomalı köpeklerin WBC değerinin, sağlıklı köpeklerinkine göre anlamlı düzeyde ($P<0.05$) yüksek olduğu tespit edilmiştir. Bu bulgular, Phillips ve ark., (2022) hematolojik tümörleri olan köpekler üzerinde yaptıkları çalışmayla benzerlik göstermektedir. 2021 yılında meme tümörü olan köpekler üzerine yayınlanan başka bir çalışmada ise hasta köpeklerin HCT, HGB ve MCH değerlerinin sağlıklılara daha oranla düşük olduğu bildirilmiştir (Uçmak ve ark. 2021).

Grindem ve ark., (1994) neoplazili köpekler üzerinde yaptıkları bir çalışmada, lenfomalı 57 köpeğin %36'sında trombositopeni saptamışlardır. Graff ve ark. 2014 yılında, 107 lenfomalı köpek üzerinde yaptıkları çalışmada da, lenfoma sınıfı 5 olan köpeklerin trombositopenik olduğunu belirlemişlerdir. Bununla birlikte, mevcut çalışmada hasta kedilerin 2'sinde, hasta köpeklerin ise 13'ünde trombositopeni tespit edilmiştir. Yapılan çalışmada az sayıdaki hastada trombositopeni tespit edilmiş olmasının sebebinin lenfoma sınıfıyla ilgili olabileceği düşünülmüştür.

Phillips ve ark. (2022) hematolojik tümörleri olan köpeklerde yaptıkları çalışmada PDW değeri yüksek olarak saptamış ve bu çalışmaya benzer olarak çalışmamızın sonucunda, PDW değeri lenfomalı köpeklerde de anlamlı olarak yüksek bulunmuştur ($P<0.05$). PDW, trombositlerdeki hacim değişkenliğini ve anizositozu ifade eden bir değerdir. Trombosit heterojenitesi, trombosit aktivasyonunun değerlendirilmesinde önemlidir. Fakültemizdeki mevcut hemogram cihazının kedilerdeki PDW değerini ölçemiyor olması çalışma için kısıtlayıcı bir faktör olmuştur. Bununla birlikte çalışmaya dahil edilen lenfomalı kedilerde, trombosit indekslerinden MPV'nin anlamlı olarak artış gösterdiği belirlenmiştir ($P\leq0.01$). MPV, trombosit aktivasyonunu gösteren parametrelerden biridir. Herhangi bir sorunda kemik iliğinden genç trombositlerin fazlaca üretilmesi ve genç trombosit sayısının yaşlı trombositlerden fazla olmasıyla MPV değerinde artış şekillenmektedir (Bayleyegn ve ark., 2021). MPV ve PDW değerlerinin endotoksemi durumlarında normalden yüksek ölçüldüğü daha önceki yapılan çalışmalarda da saptanmıştır. Neoplastik hastalıklarda genellikle kronik inflamatuvar yanıtın şekillendiği bilinmektedir. Bu doğrultuda, çalışmamızın sonucunda kedi ve köpeklerde belirlediğimiz trombosit indekslerindeki değişimlerin kronik inflamatuvar yanıtla alakalı olabileceği düşünülmüştür. Buna ek olarak, bu değerlerdeki değişimlerin kemik iliğindeki rejenerasyonla da ilgili olabileceği de unutulmamalıdır (Phillips ve ark., 2022). Lenfomalı kedi ve köpekler üzerinde yapılan bu çalışmada, trombosit indekslerinin bir kısmında meydana gelen değişimlerin tedavi yönetimi ve prognoz için anlamlı olabileceği düşünülmektedir.

Tedavi öncesi ve sonrasi hemogram sonuçlarının ve klinik tablonun karşılaştırılamamış olması ve hastalara lenfoma teşhisi konulduktan sonraki sağkalım sürelerinin belirnememiş olması bu çalışma için kısıtlayıcı bir faktör olmuştur. Trombosit indekslerinin,

kısıtlayıcı faktörlerin giderilerek incelendiği, tedavi öncesi ve sonrası değerlendirmesinin yapılabildiği ileri çalışmaların veteriner hekimliği alanına katkıda bulunacağı düşünülmektedir.

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

Yazarların Katkı Oranı: Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan etmişlerdir.

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RESEARCH ARTICLE

Evaluation of Antioxidant Properties and Total Phenolic and Flavonoid Contents of Honey Bee Hive Products Collected from the Ankara Region

Efe KURTDEDE[1]*, Sedat SEVİN[2]

[1]Ankara University, Veterinary Faculty, Biochemistry Department, 06110, Ankara, Türkiye
[2]Ankara University, Veterinary Faculty, Pharmacology and Toxicology Department, 06110, Ankara, Türkiye

ABSTRACT

In this study, the total phenolic (TP) and total flavonoid (TF) profiles of multifloral honey, bee bread, bee pollen, and drone larvae (apilarnil), which are among the products of bee hives, were determined. In addition, the antioxidant activities of the aforementioned products were determined by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, ferric reducing antioxidant power (FRAP) assay, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay methods. Honey, bee bread, bee pollen, and apilarnil samples collected from 10 honey bee hives in the Ankara region were the material of the study. As a result of the analysis, TP content levels were found to be in the order of bee pollen > bee bread > honey > apilarnil, while TF contents were in the order of bee pollen > bee bread > apilarnil > honey. Considering the results of the DPPH, FRAP, and ABTS assays, the levels of activity were determined in the order of honey > bee pollen > bee bread > apilarnil. The highest antioxidant activity level determined in honey was concluded to be the result of synergistic antioxidant effects of other bioactive complex substances contained in honey. Therefore, we believe that bioactive complex substances that increase the antioxidant activity level of honey should be evaluated in future studies.
Keywords: ABTS assay, Ankara region, DPPH assay, FRAP assay, Total phenol contents, Total flavonoid contents

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Ankara Bölgesinden Toplanan Bal Arısı Kovanı Ürünlerinin Antioksidan Özelliklerinin ve Toplam Fenolik ve Flavonoid İçeriklerinin Değerlendirilmesi

ÖZ

Bu çalışmada, arı ürünleri olan multifloral bal, arı ekmeği, arı poleni ve erkek arı larvası (apilarnil)'in toplam fenolik (TP) ve toplam flavonoid (TF) profilleri ortaya konuldu. Bunun yanı sıra söz konusu örneklerin antioksidan aktiviteleri, 2,2'-azinobis (3-ethylbenzothiazolin)-6-sulfphonate (ABTS) testi ve ferric reducing antioxidant power (FRAP) testi ve 1,1-diphenyl-2-picrylhydrazy (DPPH) testi metotları kullanılarak belirlendi. Çalışmada materyalini Ankara yöresindeki 10 bal arısı kovanından toplanan bal, arı ekmeği, arı poleni ve apilarnil örnekleri oluşturdu. Yapılan analizlerin sonucunda bal arısı ürünlerinin toplam fenolik içerik düzeyine göre sıralaması arı poleni > arı ekmeği > bal > apilarnil olarak bulunurken, bal arısı ürünlerinin toplam flavonoid içeriği düzeyine göre sıralaması ise şu şekildedir: arı poleni > arı ekmeği > apilarnil > bal. Bal arısı kovan ürünlerinin DPPH, FRAP ve ABTS analizine göre sıralaması bal > arı poleni > arı ekmeği > apilarnil olarak belirlendi. Sonuç olarak balın en yüksek antioksidan aktivite düzeyi balın içerdiği diğer biyoaktif kompleks maddelerin sinerjistik antioksidan etkileri olarak değerlendirilmiştir ve bu nedenle balın antioksidan aktivite düzeyini artıran biyoaktif kompleks maddelerin ileriki çalışmalarda değerlendirilmesi gerektiği kanaatindeyiz.
Anahtar Kelimeler: ABTS assay, Ankara yöresi, DPPH assay, FRAP assay, Toplam Fenolik ve Flavonoid içerik.

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ORCID ID; *EK: 0000-0001-8436-3332, SS: 0000-0003-0475-9092*
*Corresponding author e-mail: efekurtdede@gmail.com

INTRODUCTION

The breeding of honey bees has been practiced since ancient times, and it is stated in the scientific literature that honey bee products such as honey, bee pollen, bee bread, and drone larvae (apilarnil) have strong curative properties and are used for various supportive treatments due to their high contents of bioactive molecules (Crane et al. 1990). Scientific studies indicate that honey bee hive products have antioxidant, antibacterial, anti-inflammatory, antitumor, and antiviral effects (Bartkiene et al. 2020, Viuda-Martos et al. 2008). These properties of honey bee hive products are a result of the various phenolic substances and flavonoids, vitamins, and enzymes that these products contain (Dzugan et al. 2018). While complex sugars make up about 80% of honey, which is one of the food items most widely consumed by people around the world, various amino acids, minerals, lipids, sterols, phenolic and flavonoid substances, vitamins, and enzymes are also present in honey and these compounds attract the attention of researchers (Ajibola et al. 2012). Bee pollen, which is a honey bee hive product, contains approximately 4-15% water, 7.5-40% protein, 15-82% sugar, 1.3-7% lipids, and 1-3.5% various vitamins and minerals (Kostic et al. 2015). In addition, similar to honey, bee pollen also contains significant levels of organic acids, phenolic and flavonoid substances, and enzymes (Komosinska-Vassev et al. 2015). Bee bread is reported to contain approximately 21.70-23.33% protein and 57.06-58.89% carbohydrates (Mohammad et al. 2019), as well as various bioactive peptides, minerals and vitamins, organic acids, and phenolic and flavonoid substances, which have been the subject of important studies (Margaoan et al. 2019). Apilarnil, also produced in honey bee hives, contains 9-12% protein, 6-10% carbohydrates, 5-8% lipids (Barnutiu et al. 2013), various vitamins and minerals, phenolic and flavonoid substances, and sex hormones. Generally speaking, the most important compounds responsible for the biological activities of honey bee hive products are thought to be flavonoids and phenolic compounds (Velasquez et al. 2022, Giampieri et al. 2022). The strong antioxidant effects of flavonoids are a result of their free radical scavenging activities (Martinello & Mutinelli 2021, Persuric et al. 2021). For this reason, in the present study, total phenolic (TP), total flavonoid (TF), and total antioxidant levels of samples of these products are examined in the evaluation of the bioactivity levels of honey bee hive products. We believe that it is important to reveal the levels of bioactive substances contained in honey bee hive products and determine the physicochemical structural differences among these products, which vary according to the type of product, region, and year. In this study, the TP and TF profiles of bee hive products including honey, bee bread, bee pollen, and drone larvae (apilarnil) were determined. In addition, the antioxidant activities of these samples were evaluated by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay methods.

MATERIALS AND METHODS

Honey, bee pollen, bee bread, and apilarnil samples collected separately from each of 10 honey bee hives in one apiary in the Ayaş district of the Ankara region in June 2022 constituted the material of the study. The bees breed in the hives from which the samples were obtained were Apis mellifera anatoliaca. Samples (0.5 g each for apilarnil, pollen, honey, and bee bread) were brought to the laboratory in propylene transport bags with ice packs and placed quickly in a refrigerator at 4 ° C until analysis was performed.

Preparation of Extracts

Apilarnil samples of 0.5 g in weight were extracted with 5 mL of distilled water or 70% ethyl alcohol. Samples were homogenized with a tissue homogenizer at medium speed (15000 rpm) for 2 minutes. The extracts were then centrifuged for 20 minutes in a Sigma 3-30KS centrifuge (Sigma, Darmstadt, Germany) at 10000 rpm and 4 °C (refrigeration temperature). The collected supernatants were held at -20 °C until analysis. Samples of pollen, honey, and bee bread of 0.5 g in weight were combined with 5 mL of 80:20 analytical grade methanol/double-distilled water , respectively, and the mixtures were vortexed (Biosan Biovorteks VI, Biosan, Riga, Latvia) for 5 minutes. They were then centrifuged in a cooled centrifuge at 4 °C for 10 minutes. Afterwards, the prepared samples were stored at -20 °C until analysis was performed (Wilcynska et al. 2010).

Determination of Total Phenol Concentrations

TP concentration levels were determined according to the Folin-Ciocalteu method as modified by Beretta et al (2005). For this, after adding 900 µL of distilled water and 5 mL of Folin-Ciocalteu reagent to 100 µL of extract, 4 mL of Na2CO3 (75 g/L) was added to the mixture 4 minutes later. These mixtures were incubated for 2 hours and activity levels were determined at 750 nm with a Shimadzu UV-1201 Uv-Vis spectrophotometer (Shimadzu, Kyoto, Japan). The total amount of phenolics was calculated as mg gallic acid equivalent (GAE) in 100 g of extract (Bertoncelj et al. 2007, Diminis et al. 2010).

Determination of Total Flavonoid Concentrations

Using the method developed by Dewanto et al. (2002), mixtures were evaluated colorimetrically using aluminum chloride. For this, 1.25 mL of distilled water, 75 µL of 5% NaNO3, and 150 µL of 10% AlCl3 were added to 250 µL and this mixture was incubated for 2 hours and spectrophotometrically evaluated at 765 nm.

FRAP assay

For FRAP assays, the method modified by Benzie and Strain (1996) was used. The working principle of this method is based on the reduction of a ferrous 2,4,6-tripyridyl-s-triazine complex to its colored form in the presence of antioxidants. To prepare the FRAP reagent, 40 mM HCL, 2.5 mL of 20 mM FeCl3, and 2.5 mL of 10 mM TPTZ solution in a total volume of 25 mL were mixed. The pH of the mixture was adjusted to 3.6 with 0.3 M sodium acetate. Subsequently, 200 µL of extract was mixed with 1.5 µL of FRAP reagent and incubated for 10 minutes, and the absorbance of the reagent was measured spectrophotometrically at 593 nm.

ABTS assay

For ABTS assays, the method modified by Re et al. (1999) was used. ABTS cation radicals were synthesized by the reaction of 7 mM ABTS solution with 2.45 mM potassium persulfate solution. The mixture was kept in the dark at room temperature for 16 hours. The ABTS+ solution was then diluted with distilled water until an absorbance of 0.7 at 734 nm was reached. The extract was added to this solution immediately after preparing ABTS+ solution aliquots of 2.0 µL to obtain final concentrations between 0 and 100 µg/mL. After 10 minutes, percent inhibition was calculated for each concentration at 734 nm.

DPPH assay

DPPH assays were conducted based on the free radical scavenging effect of DPPH for extracts prepared using an indirect method. DPPH (0.1 mM) was prepared in methanol in a volume of 1 mL and 3 mL of methanolic extract was added. With reference to ascorbic acid, the measurements were modified at 520 nm by spectrophotometer and they were carried out according to the methods of Meda et al. (2005) and Diminis et al. (2010) (Wilczynska et al. 2010). As positive controls, 1.8 mL of DPPH solution and 0.2 mL of ascorbic acid/Trolox solution were used. Antioxidant activity (%) was calculated using the absorption values of the samples against the negative control at 517 nm.

RESULTS AND DISCUSSION

In this study, the values of TP and TF contents, DPPH assay measurements, ABTS assay measurements, and FRAP assay measurement were obtained for the analysis of honey, bee bread, bee pollen, and apilarnil samples. TP levels were measured as 31.1±1.75 mg quercetin equivalent (Qeurcetin (QE))/100 g, 11.37±0.87 mg QE/100 g, 29.36±5.1 mg QE/g, and 47.5±3.62 mg QE/100 g for honey, bee bread, bee pollen, and apilarnil, respectively. TF levels were measured as 2.51±0.42 mg GAE/100 g, 3.52±0.53 mg GAE/g, 5.11±0.72 mg GAE/g, and 14.35±3.2 mg GAE/100 g, respectively. DPPH assay measurements yielded values of 58.61±3.24 SC 50 mg/mL, 0.48±0.02 SC 50 mg/mL, 1.33±0.26 SC 50 mg/mL, and 4.93±0.95 SC 50 mg/mL, respectively. The ABTS assay results were 38.3±2.27 SC 50 mg/mL, 0.31±0.01 SC 50 mg/mL, 24.8±7.47 SC 50 mg/mL, and 35.89±4.06%, respectively. The values of the FRAP assay measurements were determined as 101.97±9.12 µmol Trolox/g, 39.71±1.06 µmol Trolox/100 g, 84.36±28.87 µmol Trolox/100 g, and 0.59±12.73 mmol/100 g, respectively. It is important to consider the TP and TF contents in determining the antioxidant capacity of honey bee hive products. Didaras et al. (2021) reported the TP contents of bee bread samples collected from 18 different regions of Greece as ranging between 2.34±0.22 and 5.27±0.00 mg GAE/g bee bread. Bakour et al. (2017) determined the average TP contents of bee bread samples from Morocco to be 14.88±0.98 mg GAE/g. Malkoç et al. (2019) determined the average total TF contents to be 2.79 mg QE/100 g in measurements that they performed for 11 different Anzer honey samples collected from the Black Sea region of Turkey. Rocchetti et al. (2018) reported TP values of 4.20±0.40-29.60 mg GAE/g for 32 pollen samples from the Marche region of Italy. Socha et al. (2016) found the mean TP level to be 0.47±0.04 mg GAE/g for five samples of multifloral honey collected from the southern regions of Poland. Sawicki et al. (2022) reported average TP levels of 11.77±0.15 mg QE/g for bee pollen samples and 0.07±0.00 mg QE/g for honey samples collected from the Kujawy region of Poland. The same researchers revealed that the TP levels of bee bread were 60% lower than those of other bee hive products. Rzepecka-Stojko et al. (2015) found the average TP level of three pollen samples collected from southern Poland to be 20.22 mg QE/g. Mayda et al. (2020) reported the mean TP level of five honey bee hive product samples collected from five different regions of Turkey as 2.62-4.44 mg QE/g. Saral et al. (2019) analyzed samples collected from the Tekirdağ, Ankara, Hatay, and Artvin regions of Turkey and reported mean TP levels ranging from 28±5 to 58±27 mg GAE/100 g for honey samples and 41±14 to 1258±505 mg GAE/100 g for bee pollen samples. Silici (2019) reported the average TP content of six apilarnil samples collected from the Kayseri region of Turkey as 834.05 mg GAE/100 g. Sidor et al. (2021) obtained average TP levels ranging between 144.8±16.6 and 399.3±14.6 mg GAE/100 g for three apilarnil samples obtained from southeastern Poland.

The levels of flavonoid substances in honey bee hive products have also been revealed in various studies. Didaras et al. (2021) determined that the levels of flavonoid contents of bee bread samples collected from 18 different regions of Greece ranged between 6.49±0.04 and 14.64±0.26 mg QE/g. Bakour et al. (2017) reported the TF contents in bee bread samples collected in Morocco as 1.67±0.12 mg QE /g. Malkoç et al. (2019) found that the average value of TF for 11 different Anzer honey samples collected from the

Black Sea region of Turkey was 2.79 mg QE/100 g. Saral et al. (2019) analyzed honey bee hive products collected from the Tekirdağ, Ankara, Hatay, and Artvin regions and determined average TF levels of 1±1 to 5±2 mg QE/100 g honey for samples and 253±64 to 499±99 mg QE/100 g for bee pollen samples. Sidor et al. (2021) reported TF levels of three apilarnil samples obtained from southeastern Poland ranging between 15.0±4.8 and 57.2±4.1 mg/100 g.

There are various studies in which the antioxidant properties of honey bee hive products were revealed using DPPH assay, ABTS assay, and FRAP assay methods. Didaras et al. (2021) found the IC50 value of the DPPH assay to range between 0.18±0.02 and 1.25±0.04 for bee bread samples collected from 18 different regions of Greece. Bakour et al. (2017) determined the mean IC50 value of the DPPH assay to be 0.05±0.01 mg/mL for bee bread collected in Morocco. Malkoç et al. (2019) reported an average DPPH level of 49.12 mg/mL for measurements performed for 11 different Anzer honey samples in the Black Sea region of Turkey. Rocchetti et al. (2018) determined the DPPH levels of 32 pollen samples collected from the Marche region of Italy to range between 11.9±6.4 and 108.7±6.1. Saral et al. (2019) analyzed honey bee hive products collected from the Tekirdağ, Ankara, Hatay, and Artvin regions and they reported DPPH levels of 30.90±1.3 to 155.70±76.68 SC 50 mg/mL for honey samples and 0.47±0.5 to 0.47±0.51 SC 50 mg/mL for bee pollen samples. Sidor et al. (2021) found the DPPH activity of three apilarnil samples obtained from southeastern Poland to range from 6.3±1.3% to 20.5±0.1%. Previous studies have confirmed that the ABTS assay reflects the scavenging capacity of antioxidants by means of a free radical (i.e., ABTS). Didaras et al. (2021) determined the average IC50 values of the ABTS assay to be 0.38-1.80 for bee bread samples collected from 18 different regions of Greece. Bakour et al. (2017) reported the average ABTS IC50 level to be 0.08±0.05 mg/mL for bee bread samples collected in Morocco. Rocchetti et al. (2018) reported ABTS levels of bee pollen samples collected from the Marche region of Italy within the range of 48.8±14.1 to 224.6±18.6 μmol TE/g DW. Sidor et al. (2021) found that ABTS activities of apilarnil samples collected from southeastern Poland ranged between 32.1±0.5% and 71.3±0.4%. The FRAP assay analysis method is based on the evaluation of a change in the color of a solution as a result of the conversion of ferric (Fe3+) ions in that solution into ferrous (Fe2+) ions by the antioxidants in the environment, and this method has been used in many previous studies. Zuluaga et al. (2015) reported an average value of 46.1±13.0 FRAP μmol Trolox/g for bee bread. Sahin and Kemal (2019) reported average FRAP assay results of 72.38±0.21 μmol FeSO4.7H2O/g for samples of bee pollen. Malkoç et al. (2019) determined an average FRAP level of 110.11 μmol Trolox/100 g for 11 different Anzer honey samples collected from the Black Sea region. Saral et al. (2019) analyzed honey bee hive products collected from the Tekirdağ, Ankara, Hatay, and Artvin regions and found the FRAP assay levels of honey samples to range from 1.37±0.17 to 1.37±0.17 μmol FeSO4.7H2O/g while the values obtained for bee pollen ranged from 8.69±1.64 to 84.89±10.09 μmol FeSO4.7H2O/g. Sidor et al. (2021) found the average FRAP levels of three apilarnil samples obtained from southeastern Poland to be between 0.4±0.1 and 1±0 mmol/100 g. The data obtained from the present study are generally similar to the results of previous studies on this subject. However, in addition to differences in analysis methods used, such as the DPPH assay, ABTS assay, and FRAP assay, variables such as extraction method, botanical species, and geographical conditions may have roles in the levels of phenolic and flavonoid compounds determined in honey bee hive products and may explain the differences detected in the antioxidant properties of these products.

In this study, it was determined that the samples could be ranked in the order of bee pollen > bee bread > honey > apilarnil according to the levels of TP contents in these samples. In the evaluation of the TF levels of the collected samples, an order of bee pollen > bee bread > apilarnil > honey was obtained. In evaluations of the data obtained from DPPH, FRAP, and ABTS assays, the collected samples were ranked as honey > bee pollen > bee bread > apilarnil, respectively. It was determined that the antioxidant capacity of honey was higher than that of the other considered products, which could be attributed to the synergistic effects of bioactive substances contained in honey.

CONCLUSION

This study was undertaken to reveal the TP and TF profiles of honey, bee bread, bee pollen, and apilarnil collected from honey bee hives in the Ankara region of Turkey and to determine the antioxidant activities of these products using ABST, FRAP, and DPPH assays. It is thought that the present findings will serve as a guide for future studies. In addition, this study has provided an up-to-date assessment of the bioactivity levels of honey bee hive products in the Ankara region. We believe that our finding of higher antioxidant capacity for honey samples compared to bee bread, bee pollen, and apilarnil samples can be attributed to the synergistic effects of other bioactive complex substances contained in honey, and this result will be evaluated in further studies using honey.

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

Yazarların Katkı Oranı: 1EK%50, 1SS%50

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan etmişlerdir.

Çalışmamız etik kurul onayı gerektirmemektedir.

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RESEARCH ARTICLE

Effects of Levamisole Application on Immunity System in Anthrax-Vaccinated Cattle

Ertan DOĞAN

Ardahan University, Vocational School of Nihat Delibalta Gole, Department of Laborant and Veterinary Health, 75700, Ardahan, Turkey

ABSTRACT

The present study investigated the effect of levamisole administration on the immune system of anthrax-vaccinated cattle. Totally, 40 healthy cattle were employed in the study. Cattle were divided into 4 even groups. In the control group, saline was subcutaneously injected four times as a placebo, every three days, and one dose was administered on the 10th day. In the second group (vaccine group), anthrax vaccine was administered subcutaneously on the 10th day following the administration of physiological saline three times every three days. The 3rd group (vaccine-levamisole) was subcutaneously administered levamisole at a dose of 2.5 mg/kg three times with three-day intervals and on the following 10th day, anthrax vaccine was administered. The 4th group (levamisole) was subcutaneously administered levamisole at a dose of 2.5 mg/kg three times with three-day intervals and physiological saline was subcutaneously injected on the following 10th day. After vaccination neutrophils, lymphocytes, monocytes count, and the serum IgG amount of the vaccine-levamisole group were found to increase significantly ($p<0.05$) compared to the vaccine-only group. It was concluded that subcutaneously administered levamisole at a dose of 2.5 mg/kg three times with three-day intervals before administrating the anthrax vaccine to cattle, had a stimulating effect on the immune system.
Keywords: Anthrax vaccine, cattle, immune system, levamisole.

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Levamizol Uygulanmasının Şarbon Hastalığına Karşı Aşılanmış Sığırlarda Bağışıklık Sistemi Üzerine Etkileri

ÖZ

Bu araştırmada şarbon hastalığına karşı aşılanan sığırlarda levamizol uygulamasının bağışıklık sistemi üzerine olan etkisi araştırıldı. Araştırmada toplam 40 adet sağlıklı sığır kullanıldı. Sığırlar dört eşit gruba ayrıldı. Kontrol grubuna üçer gün arayla ve bir dozu da 10. gün olmak üzere plasebo olarak serum fizyolojik dört defa deri altına enjekte edildi. İkinci gruba (aşı grubu) üçer gün arayla üç kez serum fizyolojik sonrası 10. gün şarbon aşısı deri altı, üçüncü gruba (aşı-levamizol) üçer gün arayla üç kez levamizol çözeltisinden 2,5 mg/kg dozda deri altı ve sonrası 10. gün şarbon aşısı deri altı, dördüncü gruba (levamizol) ise üçer gün arayla üç defa levamizol 2,5 mg/kg dozda deri altı ve sonrası 10. günde serum fizyolojik deri altına enjekte edildi. Aşılama sonrası aşı-levamizol grubunda nötrofil, lenfosit, monosit ve serum İgG miktarının sadece aşı uygulanan gruba göre anlamlı düzeyde ($p<0,05$) arttığı görüldü. Sığırlara şarbon aşısı uygulanmadan önce üçer gün ara ile üç kez 2,5 mg/kg dozda deri altı verilen levamizolün bağışıklık sistemi üzerine uyarıcı etki gösterdiği sonucuna varıldı.
Anahtar kelimeler: Bağışıklık sistemi, levamizol, sığır, şarbon aşısı.

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ORCID ID; *ED: 0000-0003-0751-0559*
*Corresponding author e-mail: ertandogan@ardahan.edu.tr

INTRODUCTION

Anthrax is an infectious disease caused by Bacillus anthracis (Lewerin et al. 2010, Suchitra et al. 2010, Parlak et al. 2015, Gobeli Brawand et al. 2019). The agent responsible for the disease is a gram-positive, spore-forming bacterium (Lewerin et al. 2010). These spores make bacteria resistant to unfavorable environmental conditions (temperature, dry air, changes in pH, chemicals, etc.). Therefore, they maintain their ability to cause disease by maintaining their vitality in the external environment for a long time (Suchitra et al. 2010, Dettwiler et al. 2018, Gobeli Brawand et al. 2019).

Cattle usually ingest the pathogen into the digestive and respiratory tract. Spores that enter the body in this way are captured by macrophages and transported into the lymph nodes. They are transformed from spore-form to vegetative-form in lymph nodes. Bacteria that enter the vegetative-form proliferate quickly. They also synthesize toxins that are responsible for the pathogenesis of the disease. The bacteria enter the bloodstream is resulted in systemic anthrax which is usually fatal (Hanna et al. 1994, Lewerin et al. 2010).

Levamisole is a widely used anthelmintic against parasites which settle in the lungs, stomach, and intestines of humans and animals. The drug is very effective against nematodes and eliminates the parasite from the place where it is settled by 90% to 100% (Siwicki and Cossarini-Dunier 1990, Sajid et al. 2006). This drug, belongs to the imidazothiazoles group, has been used since 1970. The drug is indicated to have immunomodulatory and immunostimulating properties as well as potential anthelmintic properties (Siwicki and Cossarini-Dunier 1990, Baydan 1995, Sajid et al., 2006, Kızıltepe 2018). The anthelmintic dose of this drug, widely used in veterinary medicine, is 7.5 mg/kg, and its immune system stimulating dose is 2-2.5 mg/kg. It is suggested that the drug should be used 5-6 times a week or 2-3 successive days to boost the immune system (Baydan 1995). It has been reported to regulate immune function when given to mice at a dose of 150 mg or 2.5 mg/kg body weight for one week (Sajid et al. 2006). A single dose of 2.5mg/kg levamisole administration boosts the immune system for 48 hours, and it is recommended to repeat the drug for a more effective stimulation (Pancarcı et al. 2009). When levamisole is injected into cattle in the non-lactating period decreases the incidence of mastitis. The use of levamisole in an intermittent manner is more effective than continuous use for regulating the immune system (Sajid et al. 2006).

The immunomodulating and stimulating effect of levamisole is still not completely unknown (Kızıltepe 2018). However, some research suggests that by stimulating phagocytes, they become sensitive to mitogens and antigens, and increase the number of T cells. It is reported that levamisole does not have direct effects on B-lymphocytes, but indirectly (by affecting T-lymphocytes and phagocytic cells) stimulates the humoral immune response and causes an increase in antibody level (Van Der Maaten et al. 1983, Baydan 1995, Sajid et al. 2006, Pancarcı et al. 2009). Levamisole demonstrates its effect on the cellular immune response through imidazole and sulphide structures. It is noted that it has a hormone-like effect due to the increase in the amount of intracellular cGMP and serum factors responsible for leukocyte activation in lymphocytes exposed to levamisole (Kızıltepe 2018).

As we know, the immune system is a defense that protects living beings from all kinds of harmful factors. This system is mainly examined under two groups cellular and humoral. While macrophages and T-lymphocytes form cellular immunity, B-lymphocytes are responsible for the humoral system. When a foreign substance (such as bacteria, virus, fungus) enters the organism, they are recognized and processed by macrophages and T-lymphocytes. T-lymphocytes stimulate B-lymphocytes secreting lymphokines (Baydan 1995). Stimulated B lymphocytes convert to plasmocytes and stimulus-specific immunoglobulins (Ig antibodies) are synthesized. These immunoglobulins combine with antigens and impair their ability to cause disease. The main antibodies found in cattle are IgA, IgE, IgM and IgG. Of these antibodies, IgG is the most abundant in serum. This molecule is made of two subunits that are called IgG1 and IgG2 (Arda 1994).

Anthrax progresses quickly in cattle and leads to sudden death. The disease is passed on to people and threatens their health. Therefore, it is very important to fight the disease in order to protect human and animal health and prevent economic losses. As such, healthy animals would need to be vaccinated against the disease. In the literature review, no research was found on the effect of levamisole on the immunity of anthrax-vaccinated cattle. The purpose of this study was to determine the effect of levamisole at the immunomodulatory dose on the immune system of cattle vaccinated against anthrax.

MATERIALS and METHODS

This study was approved by the Committee for Ethics of the University of Kafkas (date of decision 27.11.2020 and number 2020-156) and the Ministry of Agriculture and Forestry (letter of 30.10.2020 and number E-3056045).

The animal experimentation part of the research consisted of 40 cattle of different breeds and gender, aged from 10 to 15 months on a cattle farm in the Ardahan region. Cows that had not been vaccinated with the anthrax vaccine and were in good health following the exam were used in the study. The cattle were divided into 4 groups with 10 animals in each group. The groups were determined as control, vaccine, vaccine-levamisole, and levamisole groups

respectively. The cattle used in the study were fed ad libitum with grass and tap water obtained from the same resource in the same environment. 0.2 mg/kg of ivermectin (Vilmectin-Vilsan®, Turkey) was given subcutaneously for the antiparasitic purpose to all animals in the groups. There was one month waiting period after the administration. In the control group, 4 mL of saline (Polifleks-Polifarma®, Turkey) was subcutaneously injected four times as a placebo, every three days, and one dose was administered on the 10th day. In the second group (vaccine group), 1 mL of anthrax vaccine (Ant Etvac-Ministry of Agriculture and Forestry®, Turkey) was administered subcutaneously on the 10th day following the administration of physiological saline three times every three days. The 3rd group (vaccine-levamisole) was subcutaneously administered 10% levamisole HCl (Levatek %10-Teknovet®, Turkey) solution at a dose of 2.5 mg/kg three times at three-day intervals and on the following 10th day, 1ml of the anthrax vaccine was administered. The 4th group (levamisole) was subcutaneously administered 10% levamisole HCl solution at a dose of 2.5 mg/kg three times with three-day intervals and 4 mL of physiological saline was subcutaneously injected on the following 10th day. Before the vaccination and the drug administration (day zero) and on the 7th, 14th, 28th, 35th, and 45th days following vaccination, blood samples from all animals in the groups were collected 2 mL tubes with anticoagulant (EDTA) (BD Vacutainer® K2E 5.4 mg, UK) and 10 mL tubes without anticoagulant (BD Vacutainer® CAT, UK) from vena jugularis. The blood samples were brought to laboratory. The blood samples from the no-anticoagulant tubes were centrifuged at 3000 rpm and 20 minutes. The serum obtained was stored at -20 ^{0}C in Eppendorf (ISOLAB®, Germany) tubes prior to analysis. The number of formula leucocytes in the blood sample collected to contain the anticoagulant was determined using the conventional method (Yaman 2016).

Bovine Immunoglobulin G ELISA kit (Bioassay Technology Laboratory, Cat. No: E0010Bo, China) was used in the determination of total IgG amounts in the serum sample. The results of the analysis were recorded using a 450 nm wavelength reading in an ELISA reader (BioTek ELx800, U.S.A) (Aydin 2015).

IBM SPSS 20.0 software was used for statistical evaluation of research findings. The normal distribution curve was checked using the Shapiro-Wilk test. In comparison, of group means, one-way analysis of variance (ANOVA) and multiple comparisons were performed using Tamhane's T2 test. The results were presented as a mean ($\bar{x}$) and standard deviation (SD). In this survey, $P<0.05$ was found to be statistically significant.

RESULTS

In this study, the number of formula leukocyte in the groups is given in Table 1, and the amount of IgG in Table 2. As shown in Table 1 above, there is no difference in the number of neutrophils, eosinophils, monocytes and lymphocytes in the blood sample collected on day zero among the groups. On the 7th days of drug and vaccine administration, an increase in the number of neutrophils and monocytes in the vaccine group-levamisole is statistically significant ($p<0.05$) and increase of the number of lymphocytes is not important statistically ($p>0.05$) compared to the vaccine group. The number of neutrophils, monocytes and lymphocytes in the levamisole-vaccine group increased dramatically ($p<0.05$) compared to the 14th day vaccine group. It is determined that there was a single difference in the number of lymphocytes on the days 28th, 35th and 45th when the blood sample was taken. The number of lymphocytes in the vaccine-levamisole group is higher than the vaccine group during 28th, 35th and 45th days. The amount of serum IgG in the cattle groups is given in Table 2 above. It has been demonstrated that there is no difference in the amount of serum IgG among groups on day zero. It is determined that the amount of serum IgG in the vaccine group is 98.90 ± 22.99 μg/mL, and the mean of vaccine-levamisole group is 232.50 ± 69.49 μg/mL on the 7th days. It appears that the increase in the amount of antibodies is statistically significant ($P<0.05$). The amount of serum IgG in the vaccine-levamisole group increased substantially ($P<0.05$) compared to the vaccine group on the 14th, 28th, 35th, and 45th. There is no difference between the control and levamisole groups in terms of mean antibody. It was observed that the amount of mean antibodies in the vaccine and vaccine-levamisole groups boosted over time.

Table 1. The number of formula leukocyte in cattle groups (%)
[a,b,c]: Those with different letters in the same row in the range of P<0.05 were found to be statistically significant

Days	Parameters	Control	Vaccine	Vaccine-Levamisole	Levamisole
Day 0	Neutrophils	25.80 ± 2.57	25.80 ± 21.61	26.50 ± 1.43	27.00 ± 1.05
	Eosinophils	6.40 ± 1.57	6.70 ± 1.49	7.60 ± 1.07	7.50 ± 0.84
	Monocytes	2.30 ± 0.94	3.20 ± 1.03	2.60 ± 1.07	3.40 ± 0.96
	Lymphocytes	57.50 ± 1.84	59.60 ± 2.50	58.50 ± 1.71	58.50 ± 2.06
Day 7	Neutrophils	24.90 ± 1.59 [a]	33.80 ± 2.85 [b]	36.60 ± 1.34 [c]	25.70 ± 1.88 [a]
	Eosinophils	5.80 ± 1.68	5.40 ± 1.07	5.50 ± 0.84	6.00 ± 1.33
	Monocytes	2.80 ± 0.91 [a]	3.10 ± 1.10 [a]	5.20 ± 1.03 [b]	4.10 ± 1.44 [a]
	Lymphocytes	57.60 ± 1.71 [a]	67.60 ± 1.83 [b]	70.40 ± 3.20 [b]	58.30 ± 2.31 [a]
Day 14	Neutrophils	25.10 ± 1.52 [a]	26.70 ± 0.94 [a]	32.70 ± 1.94 [b]	25.50 ± 1.08 [a]
	Eosinophils	4.30 ± 0.94	4.80 ± 1.31	4.60 ± 0.96	4.70 ± 0.94
	Monocytes	2.60 ± 1.07 [a]	4.70 ± 0.94 [b]	7.00 ± 1.24 [c]	2.70 ± 0.94 [a]
	Lymphocytes	55.20 ± 1.13 [a]	67.80 ± 3.96 [b]	75.70 ± 1.56 [c]	54.30 ± 1.88 [a]
Day 28	Neutrophils	25.80 ± 2.57	26.20 ± 2.29	26.50 ± 1.43	26.30 ± 1.41
	Eosinophils	6.40 ± 1.57	6.70 ± 1.49	7.60 ± 1.07	7.10 ± 1.37
	Monocytes	2.30 ± 0.94	3.20 ± 1.03	3.20 ± 1.47	2.80 ± 1.03
	Lymphocytes	57.80 ± 1.81 [a]	61.60 ± 2.27 [b]	71.70 ± 1.70 [c]	58.70 ± 2.35 [a]
Day 35	Neutrophils	21.70 ± 3.36	23.90 ± 2.28	23.10 ± 1.91	23.90 ± 2.02
	Eosinophils	5.70 ± 1.15	5.30 ± 1.49	6.30 ± 1.33	6.10 ± 1.44
	Monocytes	2.00 ± 1.33	2.70 ± 1.05	2.80 ± 1.31	2.50 ± 1.35
	Lymphocytes	57.20 ± 2.09 [a]	61.30 ± 2.26 [b]	70.00 ± 2.94 [c]	57.70 ± 2.35 [a]
Day 45	Neutrophils	21.30 ± 2.94	23.10 ± 3.54	22.00 ± 2.21	23.50 ± 2.63
	Eosinophils	5.40 ± 1.07	5.70 ± 1.15	5.60 ± 1.64	5.50 ± 1.84
	Monocytes	2.70 ± 0.94	2.60 ± 0.84	2.00 ± 1.24	2.20 ± 1.31
	Lymphocytes	56.00 ± 3.01 [a]	61.60 ± 1.95 [b]	69.90 ± 0.99 [c]	56.10 ± 2.07 [a]

Table 2. The amount of IgG in serum of the cattle groups (μg/mL)
[a,b,c]: Those with different letters in the same row in the range of P<0.05 were found to be statistically significant

Days	Control	Vaccine	Vaccine-Levamisole	Levamisole
Day 0	79.60 ± 39.38	77.10 ± 12.54	75.40 ± 8.24	81.10 ± 17.52
Day 7	82.40 ± 9.55 [a]	98.90 ± 22.99 [a]	232.50 ± 69.49 [b]	77.90 ± 7.40 [a]
Day 14	69.20 ± 17.99 [a]	141.30 ± 27.77 [b]	235.10 ± 57.64 [c]	65.60 ± 12.73 [a]
Day 28	71.90 ± 30.25 [a]	153.70 ± 38.60 [b]	242.30 ± 36.58 [c]	73.70 ± 28.11 [a]
Day 35	72.30 ± 12.86 [a]	170.00 ± 56.37 [b]	247.50 ± 48.20 [c]	70.00 ± 27.48 [a]
Day 45	74.60 ± 15.43 [a]	174.00 ± 43.38 [b]	251.50 ± 47.84 [c]	72.50 ± 21.24 [a]

DISCUSSION

Levamisole is used to accelerate healing and to protect against some diseases in cattle. It has been suggested that levamisole accelerates healing by increasing serum IgG amount when intramammal (Yarım and Salmanoğlu 2002) and oral used in cattle with subclinical mastitis during 6 days after milking (Ishikawa et al. 1982). It is reported that levamisole adminstration in non-milking cattle decreased incidance of mastitis (Sajid et al. 2006). Levamisole, given intramuscularly at a dose of 2.5 mg/kg starting 5 or 6 weeks before birth and until two weeks before birth, has shown positive effects on reproductive organs by accelerating postpartum uterine involution, providing early follicle wave development (Pancarcı et al. 2009). In addition, it is reported that positive results are obtained with the use of levamisole in the early period of Malignant catarrhal fever in cattle (Van Der Maaten et al. 1983).

For treatment of some diseases such as leprosy, rheumatoid arthritis, systemic lupus erythromatosis, human Immunodeficiency Virus-HIV, colorectal cancers in human is used levamisole due to stimulating effect on the immune system. It is also used to accelerate recovery in malnourished children with disease (Baydan 1995, Yarım and Salmanoğlu 2002, Sajid et al. 2006).

In this study, the usage of levamisole increased the number of neutrophils before administrating anthrax vaccine for cattle. This increase in the number of neutrophils is consistent with the results reported by Mohri et al. (2005) and Bilandžić et al. (2010). In a study, it has been reported that PPR vaccine and levamisole administration to goat did not show any change in neutrophil count (Undiandeye et al. 2014), while in another study, neutrophil count decreased in sheep that received FMD vaccine (Rahman et al. 2002, Abdullah and Başbuğan 2020). In the current study, the anthrax vaccine injected into cattle is a bacterial vaccine. The reason for the increase in the number of neutrophils may be due to both vaccines administered includes bacteria (number of neutrophils increases to bacterial infections) and neutrophils of levamisole sensitivity to antigen. Some studies have shown that levamisole does not affect or reduce neutrophil numbers (Rahman et al. 2002, Undiandeye et al. 2014, Abdullah and Başbuğan 2020). It is believed that the reason for this finding may be that the vaccines used with levamisole are viral and that levamisole is not used in immunostimulating amounts.

In the study, on the 7th and 14th days following the vaccine and drug administration, the number of monocytes in the levamisole-vaccine group increased compared with the vaccine group only. The results of the research are similar to those reported by Stelletta et al. (2004), Mohri et al. (2005), Undiandeye et al. (2014), Das et al. (2016), and Rao et al. (2017). It is thought that the increase in monocytes in the levamisole-vaccine group could be due to the stimulating effect of levamisole on macrophages for antigens. Abdullah and Başbuğan (2020) reported that the FMD vaccine in combination with 5 mg/kg levamisole decreased the number of monocytes. This is thought to be due to the fact that levamisole is not used in immunostimulants and may be due to the viral structure of the vaccine.

It is noticed that the number of lymphocytes in the vaccine-levamisole group insignificantly increased compared to vaccine-only group on the 7th days ($P>0.05$), and significantly increased on the 14th, 28th, 35th, and 45th days ($P<0.05$). It is reported by Rahman et al. (2002), Gürbulak and Kılıçarslan (2004), Mojžišová et al. (2004), Undiandeye et al. (2014), Das et al. (2016), Rao et al. (2017), and Abdullah and Başbuğan (2020) that the administration of levamisole increased the number of lymphocytes. The results obtained form this study are consistent with the results of the research given above. The reason for the increased number of lymphocytes may be due to the stimulant effect of levamisole on T- lymphocytes by imidazole and its sulphur structure. After vaccination, antigen-specific antibodies are synthesized thanks to B lymphocytes that transform into plasmocytes. This increase in the number of lymphocytes during the post-vaccination period is due to the stimulation of the cellular and humoral immune response. It has been reported that the number of lymphocytes decreased 35 days after the 5 mg/kg dose of levamisole injected into sheep with enterotoxemia vaccine (Rashid and Yüksek 2019). Many factors (such as toxoid vaccine, animal type, levamisole dose used, uptake time and number of replicates) are believed to play a role among the reasons for this decline in lymphocytes.

In current study, the administration of levamisol dramatically increased the amount of serum IgG. Similar finding with the outcome of this research have reported by some researcher in cattle with the Hemorrhagic septicemia-Pasteurella multocida (Sharma et al. 1990), Brucella S-19 (Sajid et al. 2006), foot and mouth vaccine (Rao et al. 2017), bovine viral diarrhea-BVD (Sayed-Ahmed et al. 2015), FMD vaccine in buffaloes (Qureshi et al. 2000), Enterotoxemia vaccine in sheep (Rashid and Yüksek 2019), FMD vaccine in sheep (Abdullah and Başbuğan 2020), blue tongue (Stelletta et al. 2004), sheep pox (Rao Dabbir and Nanjundaiah 2020), plague in goat-peste des petits ruminants-PPR (Undiandeye et al. 2014, Das et al. 2016), parvovirus in dogs (Mojžišová et al. 2004), and inactivated influenza vaccine in chickens (Ismail et al. 2018). The results of the present study are consistent with the results of the research described above. The level of serum IgG in the levamisole-vaccine group increased significantly compared to the vaccine group alone. This may be due to the fact that levamisole indirectly stimulates the humoral response by sensitizing phagocytes to antigens and increasing the number of T lymphocytes. As evidenced by the above studies, levamisole is used to support the immune system in vaccines and infections in humans and animals. Levamisole shows

immunomodulatory and immunostimulant effects when used repeatedly in low doses (usually one third of the treatment dose-2.5 mg/kg) before, during or after vaccination in different animal species (Baydan 1995, Sajid et al. 2006, Rao Dabbir and Nanjundaiah 2020). In one study, higher doses of anthelmintic and repeated levamisole did not increase antibody levels in bovine and ovine animals infected with the leukaemia virus. When levamisole is used to reinforce the immune system, it should be used in immunostimulating and repeated doses (Van Der Maaten et al. 1983). In this study, administration of levamisole at 2.5 mg/kg three times every three days prior to anthrax vaccination in cattle led to an increase in serum IgG. Based on this information, levamisole used at a dose of 2.5 mg/kg boosted the immune system in cattle vaccinated against anthrax.

CONCLUSION

In conclusion, levamisole, which was administered to cattle at a dose of 2.5 mg/kg three times with three-day intervals, increased the number of neutrophils, monocytes and lymphocytes on the 7th and 14th days after anthrax vaccine. The lymphocyte count increased on the 28th, 35th, and 45th days. In addition, while the mean serum IgG amount of the 7th day vaccine group was 98.90 ± 22.99 μg/mL, and the mean of the vaccine-levamisole group was 232.50 ± 69.49 μg/mL. The mean amount of antibodies in the levamisole-vaccine group on the 14th, 28th, 35th, and 45th days showed a significant increase ($P<0.05$) in comparison with the vaccine group. There was no difference in the mean antibody quantity between the control and levamisole groups. As we know, anthrax threatens both human and cattle health. Given that the disease is zoonotic, meat obtained from dead animals is not eaten. Therefore, effective disease control is important from a health and economic point of view. It is recommended to boost the immune system by using levamisole prior to anthrax vaccination in cattle. It is believed that this practice will be used to strengthen immunity against anthrax and to protect human and animal health.

Ethics Committee Information: This study was approved by the Committee for Ethics of the University of Kafkas (date of decision 27.11.2020 and number 2020-156) and the Ministry of Agriculture and Forestry (letter of 30.10.2020 and number E-3056045).

Conflict of Interest: The author declares that there is no conflict of interest.

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RESEARCH ARTICLE

The Effects of Toltrazuril Administration on Serum Oxidative Stress Levels and Serum Haptoglobin Levels in the Treatment of Acute Natural Coccidiosis in Honamli Kids

Kemal VAROL[1]*, Mustafa ESER[2]

[1]*Burdur Mehmet Akif Ersoy University, Food, Agriculture and Livestock Vocational College of Burdur, Department of Veterinary, 15030, Burdur, Turkey*

[2]*Anadolu University Open Education Faculty Health Programs, Yunusemre Campus, TR-26470 Eskisehir, Turkey*

ABSTRACT

Coccidiosis caused by *Eimeria* species is one of the factors causing diarrhea in lambs and kids. Although it is known that toltrazuril is quite effective in the treatment of acute coccidiosis in lambs and kids, there is limited information on how it affects the animals. Therefore, in this study, it was aimed to determine the effects of toltrazuril application in the treatment of acute natural coccidiosis in Honamlı kids, serum oxidative stress levels, serum haptoglobin levels and hematological parameters. The material of this study was 10 Honamlı male kids, 20-30 days old, with acute natural coccidiosis, in a private farm. Toltrazuril was administered at a single dose of 20 mg/kg in the treatment of coccidiosis. In the findings, A statistical difference ($p<0.05$) was determined between pre-treatment and post-treatment measurements of white blood cell (WBC), lymphocyte count, lymphocyte %, neutrophil count, neutrophil %, eosinophil count, eosinophil %, basophil count, basophil %, monocyte count, monocyte %, red blood cell (RBC) count, mean corpuscular volume (MCV) count, mean corpuscular hemoglobin (MCH) counti mean corpuscular hemoglobin concentration (MCHC) count values. In addition, a statistical difference ($p<0.05$) was determined between pre-treatment and post-treatment measurements of total oxidant status (TOS) and oxidative stress index (OSI) values. In conclusion, in this study, it was determined that toltrazuril was effective in the treatment of kids with acute coccidiosis, and 7 days after the application of toltrazuril, the haptoglobin (Hp) value increased and total antioxidant status (TAS), TOS and OSI values decreased.

Keywords: Coccidiosis, Haptoglobin, Hematology, Honamli kid, OSI, TAS, Toltrazuril, TOS

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Honamlı Irkı Oğlakların Akut Doğal Koksidiyozisinin Tedavisinde Toltrazuril Uygulamasının Serum Oksidatif Stres Düzeyleri ve Serum Haptoglobin Düzeyleri Üzerine Etkisi

ÖZ

Eimeria türlerinin neden olduğu koksidiyoziste kuzu ve oğlaklarda diyareye neden olan etkenlerden biridir. Kuzu ve oğlaklarında akut koksidiyozis tedavisinde toltrazuril'in oldukça etkili olduğu bilinse de canlıda nasıl etkiler oluşturduğu ile ilgili bilgiler sınırlıdır. Bu nedenle bu çalışmada Honamlı ırkı oğlakların akut doğal koksidiyozisinin tedavisinde toltrazuril uygulamasının hayvanda oluşturduğu etkilerin, serum oksidatif stres düzeyleri, serum haptoglobin düzeyleri ve hematoljik parametreler belirlenerek ortaya konması amaçlanmıştır. Bu çalışmanın metaryalini özel bir işletmede bulunan, 20-30 günlük, akut doğal koksidiyozisli 10 adet Honamlı ırkı erkek oğlak oluşturmuştur. Koksidiyozisin tedavisinde Tek 20 mg/kg dozunda toltrazuril uygulanmıştır. Bulgularda, beyaz kan hücresi (WBC), Lenfosit sayısı, Lenfosit %, Nötrofil sayısı, Nötrofil %, Eozinofil sayısı, Eozinofil %, Bazofil sayısı, Bazofil %, Monosit sayısı, Monosit %, kırmızı kan hücresi (RBC), ortalama korpusküler volüm (MCV), ortalama korpusküler hemoglobin (MCH) ortalama korpusküler Hemoglobin konsantrasyonu (MCHC) sayısı değerlerinin tedavi öncesi ve tedavi sonrası ölçümleri arasında istatistiksel bir fark ($p<0.05$) belirlenmiştir. Buna ek olarak total oksidant statü (TOS) ve okidatif stres indeksi (OSI) değerlerinin tedavi öncesi ve tedavi sonrası ölçümleri arasında da istatistiksel fark ($p<0.05$) belirlenmiştir. Sonuç olarak bu çalışmada akut koksidiyozisli oğlakların tedavisinde toltrazuril'in etkili olduğu ve toltrazuril uygulamasından 7 gün sonra haptoglobin (Hp) değerinin yükseldiği, total antioksidant statü (TAS), TOS ve OSI değerlerinin düştüğü belirlenmiştir.

Anahtar Kelimeler: Haptoglobin, Hematoloji, Honamlı oğlak, Koksidiyozis, OSI, TAS, Toltrazuril, TOS

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ORCID ID; *KV: 0000-0002-3057-2865, ME: 0000-0003-1542-2989*

*Corresponding author e-mail: kmlvrl@yahoo.com

INTRODUCTION

The acute phase response develops with disruption of homeostasis in vivo and is stimulated by interleukins and proinflammatory cytokines released from activated leukocytes at the site of tissue damage. In addition, these cytokines are responsible for the production of acute phase proteins, which are glycoproteins, in the liver (Eckersall 2000). Haptoglobin (Hp) is one of these acute phase proteins and is one of the most important acute phase proteins for ruminants. Haptoglobin is widely used to diagnose many inflammatory diseases (pasteurellosis, pneumonia and foot and mouth etc.) (Eckersall 2000, Ganheim et al. 2003).

Oxidative stress is the event that the balance between oxidant and antioxidant substances in the organism, is disrupted in favor of oxidant substances. This condition is considered pathological in vivo. The sum of oxidative stress resulting from this imbalance is shown as total oxidative stress or total oxidant status (TOS). Oxidative stress, on the other hand, occurs as a result of the production of excessive reactive oxygen or nitrogen substances in the body or the failure of the antioxidant buffer systems to work properly. Total antioxidant status (TAS) is an indicator of the capacity of antioxidant substances in the body to protect cellular membranes and other cellular structures against damage by oxidants and to prevent the formation of oxidants (Mac Kinnon et al. 1999, Mert et al. 2019). Determining the total antioxidant level may provide better results than measuring each antioxidant substances separately. Because TAS reveals the total activity of all substances with antioxidant properties in the serum (Erel 2004, Mert et al. 2019).

The most important diseases observed in neonatal lambs and kids are diarrheal diseases. Coccidiosis caused by *Eimeria* species is one of the causative agents of diarrhea in lambs and kids. It is stated that coccidiosis is very common in the worldwide and in Turkey, causing huge economic losses in sheep and goat farms (Çimtay and Sevgili 2003, Gauly et al. 2004, Iqbal et al. 2013, Ok et al. 2019). However, there is insufficient scientific data about the prevalence, risk factors and economic losses of the disease in sheep and goats compared to cattle. Of the 15 sheep and 13 goat *Eimeria* species reported so far, 12 sheep and 9 goat *Eimeria* species were found in Turkey (Karaer et al. 2012). Lambs and kids are more susceptible to coccidiosis in the 3-8 week period. In addition, the mortality rate (>58 %) is quite high in lambs and kids (Jalila et al. 1998, Ok et al. 2019). In lambs and kids, *Eimeria* species cause anorexia, poor performance, weight loss, dysentery, bloody diarrhea, dehydration, anemia, coma and death (Jalila et al. 1998, Öcal et al. 2007, Ok et al. 2019).

Enteritis and colitis are typical pathological lesions in lamb and kid coccidiosis. In lambs and kids, clinically a general depression, fatigue, loss of appetite, contamination of hair and wool with feces, matte color of the fleece and weakening are the prominent symptoms. Diarrhea with abundant watery, which is not continuous and recurring at intervals, diarrhea with mostly mucus and sometimes blood, and dysentery draw attention. In addition, tenesmus and prolapse recti are observed in advanced cases. In mild cases, symptoms such as dehydration, anemia, weight loss and wool loss are seen, while in severe cases fever, muscle spasms and nervous symptoms are also seen. Such cases can result in death if left untreated. Neurological symptoms characterized by ataxia, incontinence paresis, tremors, muscle spasms, tremors, convulsions, depression and dehydration were observed clinically, especially in 1-2 month-old kids (Iqbal et al. 2013, Karaer et al. 2012).

Oral or parenteral anticoccidial drugs are administered for the treatment of acute clinical coccidiosis. Sulfaquinoxaline, sulfamethazine, sulfaguanidine, sulfadimethoxine, sulfadimidine, nitrofurazone, amprolium, monensin, halofuginone, toltrazuril and diclazuril and combinations of these drugs are the most commonly used anticoccidial agents, especially in companion animals and livestock (Diaferia et al. 2013, Cartier et al. 1992, Mundt et al. 2007, Iqbal et al. 2013, Odden et al. 2018, Ok et al. 2019). The fact that the treatment period, 3-5 days, of amprolium and sulfadimethoxine, which is effective in the treatment of coccidiosis, increases the workforce (Ghanem and El-Raof 2005, Cartier et al. 1992). The use of triazinones (toltrazuril, diclazuril) in a single dose and being effective in treatment provides an important advantage (Öcal et al. 2007, Ok et al. 2019).

Although it is known that toltrazuril is quite effective in the treatment of acute coccidiosis in lambs and kids, there is limited information on how it affects the animals (Karaer et al. 2012, Iqbal et al. 2013, Ok et al. 2019). Therefore, in this study, the effects of toltrazuril application in the treatment of acute natural coccidiosis in Honamlı kids will be revealed by determining serum oxidative stress levels, serum haptoglobin levels and hematological parameters.

MATERIALS and METHODS

Study design: Prospective study

This study was carried out with the permission of the Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee, dated 10.02.2022 and numbered 98/856. Sample size in this study could not be reached to the numbers obtained from power analyze. So, this study based on convenience sampling. The material of this study was 10 Honamlı male kids, 20-30 days old, with acute natural coccidiosis, in a private farm. Toltrazuril (Baycox 5 %/Bayer) was administered at a single dose of 20 mg/kg in the treatment of coccidiosis.

Blood was drawn 2 times in total, just before the treatment and 7 days after the application. Blood samples taken at the beginning of the treatment formed the control of the study.

The diagnosis of coccidiosis was made on the basis of examination of stool samples taken from the rectal route from affected kids. It was examined in terms of coccidia oocysts by flotation method. The number of oocysts in one gram of stool (OpG) was determined in stool samples in which oocysts were detected in the flotation technique. In cases with clinically bloody diarrhea and pathogenic species, OpG over 2000 were included in the study.

Blood collection process; with the help of a holder from the vena jugular of the animals, by complying with the conditions of asepsis and antisepsis; 8 ml blood sample was taken into gel tubes (BD Vacutainer®/China) to obtain blood serum, and 2 ml blood sample was taken into vacuum tube with K3EDTA (BD Vacutainer®/China) to determine hematological parameters. In order to extract the serum from the collected blood, the blood samples was centrifujed at 3000 rpm for 15 minutes, and the extracted serums were stored at -20°C until the tests were performed. To determine the hematological parameters, blood samples were analyzed in a hematology device (Mindray BC-5000 Vet/China) in a private clinic and whole blood parameters were determined.

TAS and TOS were determined according to erel's metodhs(2004,2005). Oxidative stress index (OSI) was calculated using the formula [TOS (μmol H2O2 equivalent/L)/10 x TAS (mmol Trolox equivalent/L)] (Karababa et al. 2013). OSI will be calculated from the ratio. Serum Haptoglobin levels (BT E0099Go), which is one of the acute phase proteins, were determined by ELISA kit.

Statistical analysis

IBM SPSS 22.0 for Windows package program was used to evaluate the study data. The normal distribution of the groups in the analyzes was evaluated by using the Shapiro-Wilk test. In the case of normal distribution, paired samples t test test was used for comparisons between measurements. A p value of <0.05 was considered statistically significant.

RESULTS

Clinical findings such as loss of appetite, weakness, and bloody diarrhea were observed in the kids with diarrhea, which was the material of this study. In addition, an increase in body temperature, respiratory rate and heart rate was detected. From the 24th hour following the treatment applied to the kids, an increase in appetite was observed, as well as the disappearance of symptoms such as loss of appetite and weakness. The consistency of the stool improved following 24 hours of the treatment. At the 48th hour after the treatment, it was determined that the kids were alert and the feces were completely improved.

When the measurements were examined before toltrazuril application (before treatment) and 7 days after toltrazuril application (after treatment); a statistical difference ($p<0.05$) was determined between the pre-treatment and post-treatment measurements of white blood cell (WBC), lymphocyte, monocyte, lymphocyte %, monocyte %, red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), neutrophil, eosinophil, basophil, neutrophil %, eosinophil % and basophil % values (Table 1).

No statistical difference ($p>0.05$) was found in the comparisons between the measurements of HGB, HCT and MCH values (Table 1).

Statistical difference ($p<0.05$) was determined between pre-treatment and post-treatment measurements of TOS and OSI values (Table 2 and Figure 1C, D).

No statistical difference ($p>0.05$) was found in the comparisons between the measurements of the Hp and TAS values (Table 2 and Figure 1A, B).

Correlation findings are given in Table 3.

Table 1. Hematological parameters before and 7 days after toltrazuril application.

Parameter	Before treatment (*n=10*) $\bar{x}\pm sd$	7 days after treatment (*n=10*) $\bar{x}\pm sd$	p
WBC (10^9/L)	14,71 ±2,13[a]	17,39 ±2,85[b]	0,013
Lymphocyte (10^9/L)	6,60±2,24[a]	15,93±4,84[b]	<0,001
Monocyte (10^9/L)	1,75±0,48[a]	0,22±0,037[b]	<0,001
Lymphocyte (%)	0,45±0,11[a]	0,91±0,02[b]	<0,001
Monocyte (%)	0,12±0,04[a]	0,01±0,003[b]	<0,001
RBC (10^{12}/L)	14,37±1,81[a]	18,68±4,96[b]	0,050
HGB (g/dL)	9,11±0,68[a]	10,71±3,14[a]	0,164
HCT (%)	25,68±1,64[a]	26,46±7,03[a]	0,721
MCV (fL)	18,14±2,02[a]	14,39±2,53[b]	0,010
MCH(pg)	6,41±0,54[a]	5,78±0,64[a]	0,081
MCHC (g/L)	354,50±16,00[a]	406,70±51,00[b]	0,018
RDW (fL)	35,35±9,62[a]	23,23±9,15[b]	0,009
Neutrophil (10^9/L)	5,67±2,55[a]	0,66±0,18[b]	<0,001
Eosinophil (10^9/L)	0,24±0,07[a]	0,33±0,07[b]	0,025
Basophil (10^9/L)	0,37±0,12[a]	0,24±0,15[b]	0,007
Neutrophil (%)	0,38±0,10[a]	0,04±0,02[b]	<0,001
Eosinophil (%)	0,017±0,005[a]	0,019±0,003[a]	0,175
Basophil (%)	0,02±0,007[a]	0,01±0,007[b]	0,002

White Blood Cell (WBC), Red Blood Cell (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW).

Table 2. Hp, TAS, TOS and OSI levels before and 7 days after toltrazuril application.

Parameter	Before treatment (*n=10*) $\bar{x}\pm sd$	7 days after treatment (*n=10*) $\bar{x}\pm sd$	p
Hp (mg/L or µg/mL)	58,67 ±11,00[a]	65,86 ±13,43[b]	0,237
TAS (mmol/L)	1,47±0,15[a]	1,41±0,15[a]	0,392
TOS (µmol/L)	16,23±1,48[a]	7,15±3,37[b]	<0,001
OSI (arbitrary unit)	1,10±0,11[a]	0,50±0,21[b]	<0,001

Haptoglobin (Hp) Total Antioxidant Level/Ltatus (TAS), Total Oxidant Level/Status (TOS) Oxidative Stress Index (OSI).

Figure 1: Hp, TAS, TOS and OSI levels before and 7 days after toltrazuril application.

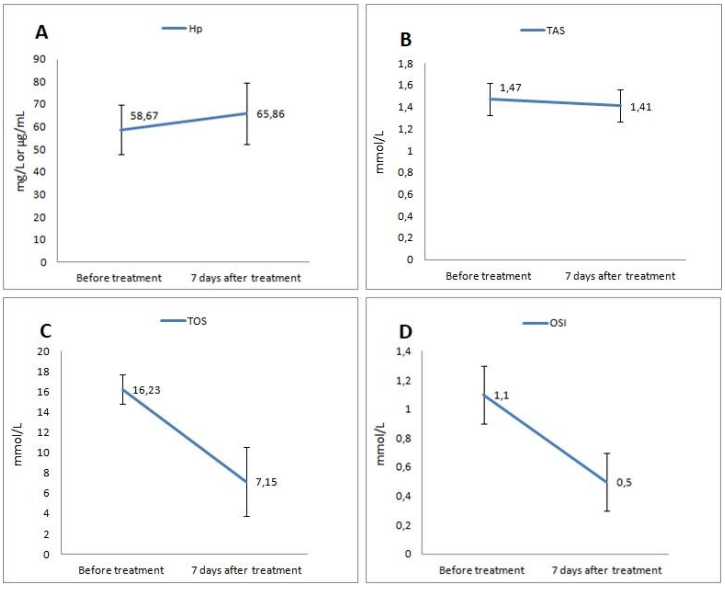

Table 3. Correlation findings between hematological parameters, Hp, TAS, TOS and OSI levels.

Pearson Correlation		WBC	LYMP	MON	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	NEUT	EOS	BAS	HP	TAS	TOS	OSI
WBC	**Pearson Correlation**	1	,807**	-,531*	,480*	,437	,221	-,444*	-,238	,582**	-,087	-,331	,547*	,055	,178	-,287	-,474*	-,419
	Sig. (2-tailed)		,000	,016	,032	,054	,350	,050	,313	,007	,715	,154	,012	,819	,453	,219	,035	,066
LYMP	**Pearson Correlation**	,807**	1	-,774**	,752**	,662**	,408	-,628**	-,405	,675**	-,246	-,630**	,709**	-,207	,365	-,248	-,731**	-,727**
	Sig. (2-tailed)	,000		,000	,000	,001	,074	,003	,077	,001	,296	,003	,000	,382	,114	,291	,000	,000
MON	**Pearson Correlation**	-,531*	-,774**	1	-,442	-,272	-,044	,575**	,467*	-,494*	,569**	,828**	-,450*	,425	-,260	,314	,854**	,824**
	Sig. (2-tailed)	,016	,000		,051	,246	,852	,008	,038	,027	,009	,000	,047	,062	,268	,177	,000	,000
RBC	**Pearson Correlation**	,480*	,752**	-,442	1	,892**	,672**	-,654**	-,473*	,632**	-,139	-,349	,505*	-,284	,113	-,017	-,373	-,404
	Sig. (2-tailed)	,032	,000	,051		,000	,001	,002	,035	,003	,560	,132	,023	,224	,635	,945	,106	,077
HGB	**Pearson Correlation**	,437	,662**	-,272	,892**	1	,889**	-,292	-,034	,449*	,201	-,236	,320	-,162	,336	-,158	-,362	-,357
	Sig. (2-tailed)	,054	,001	,246	,000		,000	,212	,888	,047	,397	,316	,169	,495	,148	,505	,117	,122
HCT	**Pearson Correlation**	,221	,408	-,044	,672**	,889**	1	,105	,233	-,003	,424	-,018	,125	,027	,288	-,114	-,140	-,123
	Sig. (2-tailed)	,350	,074	,852	,001	,000		,659	,323	,990	,063	,939	,599	,910	,218	,633	,556	,605
MCV	**Pearson Correlation**	-,444*	-,628**	,575**	-,654**	-,292	,105	1	,893**	-,829**	,659**	,454*	-,563**	,426	,136	-,146	,409	,485*
	Sig. (2-tailed)	,050	,003	,008	,002	,212	,659		,000	,000	,002	,044	,010	,061	,566	,538	,073	,030
MCH	**Pearson Correlation**	-,238	-,405	,467*	-,473*	-,034	,233	,893**	1	-,511*	,731**	,319	-,485*	,352	,366	-,324	,168	,261
	Sig. (2-tailed)	,313	,077	,038	,035	,888	,323	,000		,021	,000	,171	,030	,129	,112	,163	,478	,266
MCHC	**Pearson Correlation**	,582**	,675**	-,494*	,632**	,449*	-,003	-,829**	-,511*	1	-,356	-,453*	,494*	-,362	,176	-,131	-,505*	-,524*
	Sig. (2-tailed)	,007	,001	,027	,003	,047	,990	,000	,021		,123	,045	,027	,117	,458	,581	,023	,018
RDW	**Pearson Correlation**	-,087	-,246	,569**	-,139	,201	,424	,659**	,731**	-,356	1	,370	-,161	,581**	,159	-,221	,407	,484*
	Sig. (2-tailed)	,715	,296	,009	,560	,397	,063	,002	,000	,123		,108	,499	,007	,504	,348	,075	,031
NEUT	**Pearson Correlation**	-,331	-,630**	,828**	-,349	-,236	-,018	,454*	,319	-,453*	,370	1	-,384	,543*	-,328	,257	,790**	,784**
	Sig. (2-tailed)	,154	,003	,000	,132	,316	,939	,044	,171	,045	,108		,094	,013	,158	,275	,000	,000
EOS	**Pearson Correlation**	,547*	,709**	-,450*	,505*	,320	,125	-,563**	-,485*	,494*	-,161	-,384	1	,153	,003	,029	-,359	-,403
	Sig. (2-tailed)	,012	,000	,047	,023	,169	,599	,010	,030	,027	,499	,094		,520	,990	,903	,120	,078
BAS	**Pearson Correlation**	,055	-,207	,425	-,284	-,162	,027	,426	,352	-,362	,581**	,543*	,153	1	-,181	-,013	,451*	,486*
	Sig. (2-tailed)	,819	,382	,062	,224	,495	,910	,061	,129	,117	,007	,013	,520		,446	,956	,046	,030
Hp	**Pearson Correlation**	,178	,365	-,260	,113	,336	,288	,136	,366	,176	,159	-,328	,003	-,181	1	-,483*	-,533*	-,478*
	Sig. (2-tailed)	,453	,114	,268	,635	,148	,218	,566	,112	,458	,504	,158	,990	,446		,031	,015	,033
TAS	**Pearson Correlation**	-,287	-,248	,314	-,017	-,158	-,114	-,146	-,324	-,131	-,221	,257	,029	-,013	-,483*	1	,354	,159
	Sig. (2-tailed)	,219	,291	,177	,945	,505	,633	,538	,163	,581	,348	,275	,903	,956	,031		,126	,503
TOS	**Pearson Correlation**	-,474*	-,731**	,854**	-,373	-,362	-,140	,409	,168	-,505*	,407	,790**	-,359	,451*	-,533*	,354	1	,975**
	Sig. (2-tailed)	,035	,000	,000	,106	,117	,556	,073	,478	,023	,075	,000	,120	,046	,015	,126		,000
OSI	**Pearson Correlation**	-,419	-,727**	,824**	-,404	-,357	-,123	,485*	,261	-,524*	,484*	,784**	-,403	,486*	-,478*	,159	,975**	1
	Sig. (2-tailed)	,066	,000	,000	,077	,122	,605	,030	,266	,018	,031	,000	,078	,030	,033	,503	,000	

**. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Toltrazuril is a drug of the Triazinon group, derived from triazine by trimerization of nitrile. It is suggested that toltrazuril exhibits antimicrobial, antiprotozoal, anticonvulsant, antihemostatic, antitumor, anti-inflammatory and analgesic properties (Harder and Haberkorn 1989). Clinically, toltrazuril is used in the treatment of many protozoal diseases (*Isospora* spp. neosporosis, hepatozoonosis, sarcocystosis, toxoplasmosis, etc.) as well as the treatment of coccidiosis caused by *Eimeria* spp. (Al-Qadri et al. 2020).

The liver is responsible for the metabolism of toltrazuril. The excretion of toltrazuril occurs through the feces. Very little of it is excreted through the kidneys (Perez et al. 2008). The absorption and elimination half-life following oral administration of toltrazuril varies with species. Accordingly, when Toltrazuri is administered at a dose range of 15-20 mg/kg in rats, pigs, calves and sheep, the half-life is 23, 148, 154 and 160 hours, respectively (Dirikolu et al. 2009, Soliman, 2015, Al-Qadri et al. 2020).

As can be seen, due to the long half-life of toltrazuril in the blood, it has a very long and good effect. Therefore, the most important advantage of toltrazuril is that it is effective in all stages of *Eimeria* causing sheep and goat coccidiosis (Mundt et al. 2009, Ghanem and Abd El-Raof 2005, Le Sueur et al. 2009).

Ocal et al. (2007) reported that toltrazuril was highly effective in the treatment of acute coccidiosis in hair goat kids. Iqbal et al. (2013) determined that toltrazuril was more effective than amprolium in the treatment of intestinal coccidiosis of goats. Mundt et al. (2009) reported that toltrazuril is quite effective in the treatment and metaphylaxis of lamb coccidiosis. Ok et al. (2019) reported that lambs and kids started to respond to treatment 24 hours after a single dose of toltrazuril and diarrhea slightly darkened. In the same study, they reported that after 48 hours, the lambs and kids were voracious, lively, standing and interested in the environment, and the feces were completely solid.

In our study, following the application of toltrazuril, it was observed that the kids responded to the treatment after 24 hours and completely recovered after 72 hours. In addition, it was observed that the blood in the feces of the kids decreased in the 24th hour and completely disappeared at the 48th hour. The disappearance of bleeding in the feces of kids in a short time may be related to toltrazuril's antiprotozoal activity as well as its antihemostatic property. The increase in RBC, HGB and HCT values also supports this results.

It is reported that TOS, TAS and OSI may change in cases of local and/or systemic inflammation or infection and can be used as non-invasive markers (Celi and Gabai 2015, Merhan et al. 2017b, Aydoğdu et al. 2018). Studies have reported that serum TOS levels increase in cases of inflammation (Çiçek et al. 2012, Kırmızıgül et al. 2016, Ertaş and Kırmızıgül 2021). Oxidative stress plays a role in the pathogenesis of many diseases (Miller et al. 1993). In parasite infections, the host organism forms a response mechanism against parasites by means of free radicals that cause oxidative stress (Woodbury et al. 1984). There are many studies showing that oxidative stress occurs in animals infected with the parasite (Şimşek et al. 2006, Saleh 2008, Saleh et al. 2009, Merhan et al. 2017a, Bozukluhan et al. 2017, Gültekin et al. 2017, Kozan et al. 2010).

In our study, it was observed that oxidative stress increased clearly by increasing TOS and OSI values in acute coccidiosis in kids. After 7 days of treatment with toltrazuril, TOS and OSI values decreased, and oxidative stress was found to be decreased and statistically significant ($p<0.05$) (Table 2 and Figure 1C, D).

Haptoglobin is a valuable indicator of inflammation in goats (Gonzalez et al. 2008). On the other hand, Gonzalez et al. (2008) reported that the mean Hp value in healthy goats was 41.6 mg/L. El-Deeb et al. (2020) determined the average Hp value as 93 mg/L in healthy goats.

In our study, the Hp value was determined as 58.67 mg/L before the treatment and 65.86 mg/L after the treatment. Although there was a slight increase on the 7th day after treatment compared to before treatment, no statistical difference ($p>0.05$) was detected (Table 2 and Figure 1A).

Acute inflammation is characterized by vascular changes, edema, and neutrophilic infiltration. Mononuclear cell infiltration rich in macrophages, lymphocytes and plasma cells is seen in chronic inflammation (Şentürk 2013). In our current study, since chronic inflammation developed on the 7th day after treatment; WBC, lymphocyte and Eosinophil values increased on the 7 days after treatment compared to before treatment, and it was found to be statistically significant ($p<0.05$). It was observed that Monocyte, Neutrophil and Basophil values decreased on the 7 days after treatment compared to before treatment, and was statistically significant ($p<0.05$) (Table 1).

Macrophages, neutrophils, and other phagocytic cells generate large amounts of toxic reactive oxygen species and reactive nitrogen species, which directly kill pathogens. TOS can be used as an indicator for oxidants produced by the organism and taken up by environmental factors (Macun et al. 2018).

In the light of this information and considering the inflammation process; while TOS and OSI values were negatively correlated with WBC, lymphocyte and eosinophil values, it was observed that they were

positively correlated with WBC, lymphocyte and eosinophil values (Table 3).

Antioxidants eliminate the harmful effects of free radicals. TAS defines all antioxidants. In cases of inflammation, the serum TAS level decreases (Ertaş and Kırmızıgül 2021). Although not statistically significant, a decrease in TAS value was observed at the end of 7 days after treatment compared to before treatment (Table 2 and Figure 1B). Despite complete recurrence after treatment with toltrazuril, the increase in inflammation may suggest that toltrazuril does not show anti-inflammatory properties in coccidiosis cases. Therefore, this situation should be clarified by conducting new studies on the anti-inflammatory properties of toltrazuril.

In conclusion, in this study, it was determined that toltrazuril was effective in the treatment of kids with acute coccidiosis, and 7 days after the application of toltrazuril, the Hp value increased and TAS, TOS and OSI values decreased. In addition, the effectiveness of toltrazuril, which has antimicrobial, antiprotozoal, anticonvulsant, antihemostatic, antitumor, anti-inflammatory, analgesic properties and a rather long half-life, should be investigated with more comprehensive studies.

Limitations of the study

In the current study, the lack of the molecular analysis to diagnosing *Eimeria* Spp. in fecal samples is one of the limitations of the study. The second limitation is the small sample size for the study group.

ETHICAL RULES

Ethics Committee Information: This study was carried out with the permission of Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee, dated 10.02.2022 and registration number 98/856. In addition, the authors declared that they comply with the Research and Publication Ethics.

Conflict of Interest: The authors declared that there are no actual, potential or perceived conflicts of interest for this article.

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The authors declared that they contributed equally to the article.

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CASE REPORT

Gastric Bleeding Case Associated with Hypocalcemia in A Dog

Beste SARAÇOĞLU[1*], Doğan Can HANEY[2], Volkan YAPRAKÇI[3], Turan CİVELEK[4]

[1]*Afyon Kocatepe University, Veterinary Faculty, Department of Wild Animal Diseases and Ecology, 03200, Afyonkarahisar, Turkey*
[2]*Muğla Sıtkı Koçman University, Milas Veterinary Faculty, Department of Internal Medicine, 48210, Milas-Muğla, Turkey*
[3]*Afyon Kocatepe Üniversitesi, Veterinary Faculty, Department of Surgery, 03200, Afyonkarahisar, Turkey*
[4]*Afyon Kocatepe Üniversitesi, Veterinary Faculty, Department of Internal Medicine, 03200, Afyonkarahisar, Turkey*

ABSTRACT

The presented case report material was composed of a 4-year-old Golden Retriever dog brought to Afyon Kocatepe University Animal Hospital with complaints of weakness, anorexia, severe contraction and incoordination 1.5 months after giving birth. Due to biochemical analysis performed within the scope of anamnesis and clinical symptoms, serum calcium level was measured as 5.81 mg/dL and the patient was diagnosed with hypocalcemia (eclampsia). After the appropriate treatment procedure, all vital findings returned to normal, moderate hematemesis was observed 4 hours after the treatment. It was thought that the effect of hypocalcemia on the coagulation mechanism may cause gastric bleeding. In the biochemical measurement repeated 24 hours after the treatment, serum calcium level was determined as 7.70 mg/dL. In the subsequent follow-ups, no evidence of hematamesis was found and no recurrence was observed. The risk of bleeding disorders in dogs with hypocalcemia is significant and should be considered.

Key Words: Phosphorus, gastrointestinal bleeding, hypocalcemia, calcium, dog.

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Bir Köpekte Hipokalsemi İle İlişkili Gastrik Kanama Olgusu

ÖZ

Sunulan vaka raporu materyalini doğum yaptıktan 1.5 ay sonra halsizlik, iştahsızlık, şiddetli kasılma ve inkoordinasyon şikâyeti ile Afyon Kocatepe Üniversitesi Hayvan Hastanesine getirilen 4 yaşlı Golden Retriever ırkı köpek oluşturdu. Anamnez ve klinik belirtiler kapsamında gerçekleştirilen biyokimyasal analiz sonucu serum kalsiyum düzeyi 5.81 miligram/desilitre (mg/dL) olarak belirlenen hastaya hipokalsemi (eklampsia) tanısı kondu. Uygun tedavi prosedürü sonrası tüm vital bulguları normale dönen hastada, tedaviden 4 saat sonra orta şiddetli hematemesis gözlendi. Gastrik kanamaya hipokalseminin pıhtılaşma mekanizması üzerindeki etkisinin neden olabileceği düşünüldü. Tedaviden 24 saat sonra tekrarlanan biyokimyasal ölçümde serum kalsiyum düzeyi 7.70 mg/dL olarak belirlendi. Sonraki takiplerde hematamesis bulgusuna rastlanmadı, nüks gözlenmedi. Köpeklerde hipokalsemi olgularında kanama bozukluğu riski önemlidir ve dikkate alınmalıdır.

Anahtar Kelimeler: Fosfor, gastrointestinal kanama, hipokalsemi, kalsiyum, köpek.

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ORCID ID; *BS: 0000-0002-1006-2841, DCH: 0000-0001-9737-6722, MVY: 0000-0003-2793-4295, TC: 0000-0002-4819-3484*
*Corresponding author e-mail: beste_saracoglu@hotmail.com

INTRODUCTION

Eclampsia is an acute disease with high mortality, characterized by a blood calcium level lower than 7mg/dL (hypocalcemia) in dogs. It is usually seen during pregnancy or between 1st and 3rd weeks of lactation. However, as stated in the presented case report, dogs that continue to breastfeed can still have the disease 1.5 months after birth (Dimitrov et al., 2016). Eclampsia is triggered by the increase in the need for calf calcium in the last period of pregnancy and the first milk release after birth, and the disease picture emerges as a result of the decrease in maternal blood calcium level. Since the calcium level in breast milk is kept at a normal level, offspring are not affected by hypocalcemia (Pathan et al., 2011). Animals that continue to breastfeed are highly susceptible to changes in blood calcium levels (Moe, 2008). Undiagnosed hypocalcemia causes clinical symptoms that require urgent treatment. Therefore, blood ionized calcium levels should be measured routinely in all suspected patients. Although this disease is usually seen in small breed dogs, it is also found in medium breed dogs with multiple puppies. Excessive calcium loss, atrophy of the parathyroid gland, and calcium deficiency play a role in the etiology of the disease (Drobatz et al., 2000). Eclampsia is an easy disease to diagnose with its typical clinical findings. At the onset of the disease, affected dogs are restless and nervous. In a short time, they start walking with difficulty and shaking. In the last phase of the disease, the legs contract and the dog becomes unable to walk. Severe muscle spasms, nystagmus and dyspnea may be seen. The patient's body temperature is often above 40°C. Accordingly, the respiratory rate also increases. Clinical symptoms sit very quickly, and ataxia, tremors and tetany are observed on average 12 hours after the first symptoms such as restlessness and aggression. Eclampsia is diagnosed by measuring blood calcium, phosphorus and glucose levels. Dogs with serum calcium levels below 7 mg/dL are diagnosed with eclampsia. At the same time, serum phosphorus level is generally determined to be low in these patients (Pathan et al., 2011). Apart from puerperal eclampsia, the most common causes of hypocalcemia in dogs are hypoproteinemia and hypoalbuminemia (Thomas et al., 1995). Calcium is responsible for blood coagulation, mineralization of the skeletal system, transmission between nerve cells, muscle contraction and intracellular communication. The role of calcium in blood plasma in coagulation is to convert prothrombin produced in the liver to thrombin. Thrombin, on the other hand, provides coagulation by converting fibrinogen to fibrin. Since the blood calcium level is low in an animal with eclampsia, prothrombin cannot turn into thrombin and fibrinogen cannot turn into fibrin, and coagulation metabolism is impaired (Dzik et al., 1988). Calcium ions are involved in the regulation of the coagulation process, which plays an important role in hemostasis (Varga-Szabo et al., 2009; Braun et al., 2011). Calcium ions are responsible for the full activation of various coagulation factors, including coagulation factor XIII, apart from platelet activation (Ambrus et al., 2001). The aim of this case report is to show that dogs with eclampsia can develop hematemesis and to emphasize the importance of this issue.

CASE HISTORY

Case Submission

The material of this case report was composed of a 4 year-old and 20 kg live weight Golden Retriever dog brought to Afyon Kocatepe University Animal Hospital. In the anamnesis, it was informed that the patient gave birth 1.5 months ago, had 6 puppies and the puppies are still sucking their mother. In the clinical examination of the patient who applied to our clinic with complaints of tremor, severe contraction and incoordination, her body temperature was measured as 42.5°C. Symptoms of hyperpnea (45 beats/minute), tachypnea (190 beats/minute) and agony were detected in the patient. Before the treatment complete blood count (Human, HumaCount80 TS) and calcium and phosphorus levels (Mindray BS120) were measured by blood samples taken from vena (V.) cephalica into anticoagulant and gel tubes (Tables 1-2). Whose serum calcium level was measured as 5.81 mg/dL in the dog and, it was diagnosed with hypocalcemia in the light of clinical findings. After the treatment, in order to control, blood samples were taken and serum calcium and phosphorus levels were detected (Table 2).

Table 1. Hemogram results before treatment (Fielder, 2015).

	RESULT	REFERENCE RANGE
WBC	19.13 10^3 /µl	5.0–14.1
LYM %	58.4 %	8–21
GRA %	40.9 %	62.0-87.0
LYM	11.18 10^3 /µl	0.4–2.9
GRA	7.83 10^3 /µl	3.00-12.00
Hb	12.9 g/dl	11.9–18.9
MCH	24.9 pg	21.0–26.2
MCHC	42.8 g/dl	32.0–36.3
RBC	5.16 10^3 /µl	4.95–7.87
MCV	58.3 fl	66–77
HCT	30.13 %	35–57
PLT	138 10^3 /µl	211–621
MPV	9.7 fl	6.1–10.1

Table 2. Serum Ca and P levels before and after treatment (Fielder, 2015).

	RESULT		REFERENCE RANGE
	Before Treatment	**After Treatment**	
Ca	5.81 mg/dL	7.70 mg/dL	9.1–11.7
P	-	2.85 mg/dL	2.9–5.3

Diagnosis and Treatment

The diagnosis of the disease were made by anamnesis, clinical findings, serum biochemical analysis and evaluation of the response to treatment. The fact that the serum calcium concentration measured before the treatment is lower than 7 mg/dL confirms the diagnosis (Hall, 2015). The first symptom of eclampsia is usually restlessness. Later, the animal has gait disturbances and inability to walk, convulsions, hyperpnea, tachycardia and an increase in body temperature. The pupillary reflex is generally decreased and the pupils are dilated (Austad et al., 1976).

In the presented case report, caffeine (Kafedif®, Ceva, Turkey) at a dose of 5-15 mg/kg (0.8 ml) was applied subcutaneously (SC) for the first time in the treatment of the disease. In the patient 9 ml of calcium (Calcicaf, Provet, Turkey) and 250 ml of 5% dextrose solution were intravenous (IV) applied as diluted by 500 ml 0.9% isotonic sodium chloride solution. Upon the intense blood vomiting observed in the patient 4 hours after the treatment, 1.6 ml Ranitidine (Raniver, Osel, Turkey) İntramuscular (IM), 4 ml Metoclopromide HCl (Metpamid, Sifar, Turkey) IM, 2.5 ml Tranexamic acid (Transamine, Actavis, Turkey) IM and 2 ml vitamin K (Hemadur-K, Alke, Turkey) IM were administered. It was thought that this bleeding was caused by the effect of serum calcium level on the coagulation mechanism. 5% Dextrose (250 ml), Ranitidine, Metoclopromide HCl and Tranexamic acid treatment were continued at the defined doses for two days. In addition to this treatment, the use of oral calcium preparations is also recommended in the treatment of the disease (Austad et al., 1976). Barbiturates and tranquilizers can also be used to relieve contractions in the treatment of eclampsia (Bloom, 1968).

DISCUSSION

A decrease in blood calcium concentration below 7 mg/dL is defined as puerperal hypocalcemia (eclampsia) (Hall, 2015). Carlstrom measured the blood calcium level of 5-7 mg/dL in dogs with eclampsia for the first time in 1929 (Carlstrplm, 1929). According to a study by Bentinck-Smith in 1971, normal serum calcium level was determined as 9-11.5 mg/dL, Mg level 1.7-2.9 mg/dL, inorganic P level 2.5-5 mg/dL, glucose level 60-100 mg/dL in non-lactating female dogs. Similarly, in the presented case, the serum calcium level was measured as 5.81 mg/dL, initially. The main reason for this decrease in calcium levels is breastfeeding. The first urgent application in the treatment of the disease is to increase the falling blood calcium level. Calcium preparations used for this purpose are organic calcium compounds such as calcium barogluconate or calcium gluconate. The drug to be used is administered IV slowly until the contractions stop (Austad et al., 1976). In the general treatment protocol, 10% calcium gluconate (0.5-1.5 ml/kg) is administered at a dose. After the application, rapid clinical recovery and muscle relaxation are seen within

the first 15 minutes. In the presented case report, 0.5-1.5 ml/kg of calcium was administered to the patient by IV route and a decrease in the intensity of muscle contractions was observed in a short time. The determined dose of calcium gluconate can be given by diluting in 0.9% isotonic (Hall, 2015). The calcium preparation we gave in the treatment was diluted in 0.9% istonic and used in our patient. Parathyroid gland hormones such as parathyroid hormone (PTH) are effective in regulating calcium metabolism in the body. In the deficiency of these hormones, neural symptoms along with muscle contraction and muscle spasm and convulsions are seen after a few days (McDonald, 1965). The oscillations of impulses in dogs occur at the neuromuscular junction in hypocalcemia, low blood calcium concentration decreases the activation level of sodium channels. The reason for this interaction is that calcium ions bind to the outer surface of sodium channels. Sodium channels are activated much less than normal levels, and this event facilitates the stimulation of nerve fibers. Sometimes nerve fibers are repeatedly stimulated without rest. In dogs with hypocalcemia, severe muscle contractions and muscle spasms occur when motor nerve fibers are stimulated spontaneously and repeatedly without rest (Hall, 2015).

Due to severe muscle contraction, an excessive increase in body temperature occurs. Extra antipyretic agents are not required for this increase in body temperature. Fever usually improves with hypocalcemia treatment (Hall, 2015). The body temperature of our patient in the case presentation was measured as 42.5 oC. After the first treatment of hypocalcemia, the body temperature of our patient was measured as 39.5 oC.

Too fast calcium administration may lead to bradycardia. During calcium administration, heart rate should be monitorize in order to prevent bradycardia and arrhythmia. In the event of an arrhythmia, calcium therapy should be interrupted until the heart rhythm returns to normal (Hall, 2015). Our patient was continuously monitorized during the treatment. No adverse events developed associated with caffeine injection.

Severe hyperpnea findings can also be seen in the disease. Hyperpnea causes respiratory alkalosis. Respiratory alkalose, on the other hand, promotes the binding of serum calcium to proteins, causing exacerbation of hypocalcemia (Hall, 2015). Similarly, when the patient, who was the subject of the case report, applied to our hospital, a respiratory rate of 45/min hyperpnea was detected.

In a study conducted by Moretti et al in 2016, it was stated that calcium is an important cofactor of the coagulation mechanism and may play a role in the pathophysiology of intracerebral hemorrhage. Hypocalcemia was diagnosed in 229 of 2103 patients with intracerebral hemorrhage included in this study. Hypocalcemia is associated with the degree of bleeding in patients with intracerebral bleeding. Calcium plays a role in vascular reactivity. Hypocalcemia causes higher blood pressure due to increased arterial vascular tone (Morotti et al., 2016). A certain level of calcium is required in the blood coagulation mechanism. Since prothrombin cannot turn into thrombin and fibrinogen in the absence of calcium in the blood, impairments occur in coagulation metabolism and bleeding occurs in various organs or parts of the body (Dzik et al., 1988). Similarly, in this case report, it was evaluated that gastrointestinal system bleeding was observed in our patient due to a possible effect of calcium deficiency on the blood coagulation mechanism.

In a study conducted by Drobatz et al. in 2000, various abnormal clinical findings were detected in 29 of 31 dogs with eclampsia. Multiple abnormal clinical signs were reported in most dogs affected in this study. According to the study, typical eclampsia symptoms such as tremors, spasm, gait disturbance, and nystagmus were observed in 23 of the 29 dogs. Further, in this study, hyperpnea in 15 animals, behavioral disorders in 14 animals, weakness in 6 animals, vomiting in 3 animals, diarrhea in 3 animals and aggression in 2 animals (Drobatz et al., 2000). Similarly, a significant part of these findings were detected during the preliminary clinical examination in the presented case report.

While Mayer (1968) emphasized that eclampsia develops due to calcium and vitamin D deficiency during pregnancy and breastfeeding. Nesvadba (1971) stated that overfeeding with animal proteins such as egg and meat may cause impairment of calcium metabolism and hypocalcemia. Vitamin D supplements increase the absorption of calcium from the intestines. Vitamin D supplementation administered at a dose of 0.03-0.06 mcg/kg to dogs with eclampsia may play a role in preventing hypocalcemia (Hall, 2015). Again, phytate compounds bind ionized calcium to make it biologically unusable and are recommended as a support for hypocalcemia treatment (Resnick, 1964). For patients who will continue breastfeeding after hypocalcemia, the use of oral calcium preparations at a dose of 25-50 mg / kg 3-4 times a day has been recommended. If the puppies are large enough, they should be weaned. Puppies should not be allowed to suckle for 24 hours after an eclampsia attack to prevent relapse (Wikstrom, 1974). In this case, the puppies were prevented from sucking.

CONCLUSION

In the presented case report, hypocalcemia due to calcium deficiency was diagnosed in our patient due to prolonged breastfeeding. Breastfeeding was discontinued in the patient whose general condition improved after treatment, and the patient's diet regimen was changed. A proprietary formula containing calcium and vitamin D and phytate-

containing cereals (soybean, legume) was proposed. The patient was prescribed a balanced feed supplement (VMP tablet) containing vitamins, proteins and minerals. No recurrence was detected. Feeding is important in dogs in the post-pregnancy period. In order to avoid possible cases of hypocalcemia and serious complications such as GIS bleeding, attention should be paid to maternal feeding during this period. During the lactation period, the use of a balanced and period-appropriate diet and feed supplement and etc. should be recommended. The risk of bleeding disorders in dogs with hypocalcemia should not be clinically ignored and taken into account by veterinarians.

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

Conflict of Interest: The authors declared that there are no actual, potential or perceived conflicts of interest for this article.

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