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RESEARCH ARTICLES

CASE REPORT

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RESEARCH ARTICLE

Molecular Prevalence and Phylogenetic Characterization of *Blastocystis* in Cattle in Kayseri Province, Turkey

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ABSTRACT

Blastocystis is one of the most common emerging zoonotic parasites in humans and animals. This study aimed to determine the molecular prevalence and subtype of *Blastocystis* in cattle (*Bos taurus*). A total of 150 fresh fecal samples were collected from slaughtered cattle from various slaughterhouses in Kayseri Province, Central Anatolia. Genomic DNA was extracted from all samples and used in PCR analyses of the small subunit ribosomal RNA (SSU rRNA) gene of *Blastocystis*. *Blastocystis* positive samples were sequenced for identify subtypes. Obtained sequences were assembled with suitable genetic software, then phylogenetic relationships were revealed. According to PCR analyses, overall prevalence of *Blastocystis* was determined as 58.7%. The sequence analyses of the PCR product gene revealed the presence of one known livestock-specific subtype, ST10. Phylogenetic analysis revealed that ST10 isolates the characterized in the study were clustered with isolates identified previously from cattle. Molecular characterization and subtype of *Blastocystis* sp. in slaughtered cattle in slaughterhouses were obtained data with this study.
Keywords: *Blastocystis*, Cattle, Molecular Prevalence, Phylogenetic Characterization, Subtype

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Kayseri Yöresinde Sığırlarda *Blastocystis*'in Moleküler Prevalansı ve Filogenetik Karakterizasyonu

ÖZ

Blastocystis insanlarda ve hayvanlarda en yaygın bulunan zoonotik parazitlerden biridir. Bu çalışmada, sığırlarda (*Bos taurus*) *Blastocystis*'in moleküler prevalansı ve alt tiplerinin belirlenmesi amaçlanmıştır. Kayseri yöresinde bulunan çeşitli kesimhanelerde kesilen toplam 150 sığırdan taze dışkı örnekleri toplanmıştır. Dışkı örneklerinden genomik DNA ekstraksiyonu yapılmış ve DNA örnekleri *Blastocystis*'in small subunit ribosomal RNA (SSU rRNA) geninin amplifikasyonunda kullanılmıştır. Tüm PCR pozitif ürünler alt tiplerin belirlenmesi için sekanslanmıştır. Elde edilen sekanslar uygun genetik yazılımlarla işlenerek genotipik yapıları ve sonrasında da filogenetik ilişkileri ortaya çıkarılmıştır. İncelemesi yapılan örneklerin SSU rRNA geninin PCR analizleri ile *Blastocystis*'in ortalama prevalansı %58,7 olarak belirlenmiştir. *Blastocystis* SSU rRNA geninin PCR ürünlerinin sekans analizleri sonucunda izolatların ruminant spesifik alt tip, ST10, içerisinde olduğu tespit edilmiştir. Filogenetik analizler çalışmada karakterize edilen ST10 izolatlarının daha önce sığırlarda identifiye edilen izolatlarla birlikte kümelendiğini ortaya koymuştur. Bu çalışma ile mezbahada kesilen sığırlarda *Blastocystis* sp.'nin moleküler karakterizasyonu ve alt tipi üzerine veriler elde edilmiştir.
Anahtar Kelimeler: Alt tip, *Blastocystis*, Filogenetik Karakterizasyon, Moleküler Prevalans, Sığır

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INTRODUCTION

Blastocystis are one of the most common anaerobic unicellular protozoan parasites that infect humans and many animals, including companion, wild, and domestic animals (Lee et al. 2019; Ren et al. 2019). Generally, *Blastocystis* sp. is transmitted by the fecal-oral route, especially via the consumption of infectious cysts from contaminated water or food (Ramírez et al. 2016; Lee et al. 2018).

The species are morphologically and genetically polymorphic protozoon (Yoshikawa et al. 2016; Maloney et al. 2019). Therefore, PCR-based molecular diagnostic tools have been widely used to identify the genetic diversity of *Blastocystis* in the different host species. To date, based on sequences analyses of the full length small subunit (SSU) rRNA gene in humans and different animal species, 22 subtypes (STs, ST1-ST17, ST21, and ST23-26) have been identified (Alfellani et al. 2013; Zhao et al. 2017; Maloney et al. 2019; Stensvold and Clark, 2020). Among them, ST-1 to ST-8 and ST-12 have been identified in both humans and animals, however, ST9 has been determined only in humans (Clark et al. 2013; Ramírez et al. 2016; Stensvold and Clark, 2016). ST10,11,13-17, 21, and 23-26 were only found in animals (Stensvold et al. 2009; Parkar et al. 2010; Valença-Barbosa et al. 2019; Stensvold and Clark, 2020). Recently, various studies have shown that *Blastocystis* is detected among people who have close contact with animals such as animal handlers, namely zoo-keepers, research institutions, and abattoir workers (Salim et al. 1999; Abe et al. 2002; Parkar et al. 2010; Li et al. 2018). Thus, there is reported that close contact with infected animals may be important for zoonotic transmission of *Blastocystis* infection (Lee et al. 2018).

Blastocystis infection is one of the important and common parasitic diseases causing acute or chronic gastrointestinal symptoms in humans in Turkey (Beyhan et al. 2015). The epidemiological status and molecular characterization of *Blastocystis* in cattle are somewhat limited. Only two studies have been done on a molecular survey of this parasite in cattle in Turkey (Aynur et al. 2019; Onder et al. 2021). In these studies, ST10 and ST14 subtypes were reported in cattle (Aynur et al. 2019; Onder et al. 2021). However, no information is present on the molecular characterization of *Blastocystis* in slaughtered cattle in slaughterhouses. Therefore, this study aimed to determine the genetic characterization and subtype distribution of *Blastocystis* in cattle that were brought for slaughter from different regions of Turkey.

MATERIAL AND METHODS

Study area and collection of fecal samples

For present study, Ethics Committees of Animal Experiments dated 05.06.2009 and "Research scope made to animals for diagnosis and treatment purposes other than, clinical applications, studies with dead animal or dead animal tissue, slaughterhouse materials, waste fetuses, milking, collection of feces or litter samples, blood collection, sampling with swap etc. ethics committee approval was not obtained based on the decision no 12.

This study was conducted on various cattle abattoirs located in Kayseri province. These abattoirs provide the beef requirements of the inhabitants of Turkey. The cattle were brought for slaughter from different regions of Turkey. The daily cattle slaughter in the slaughterhouse is 100-150 head/day. The slaughterhouses were visited several times between February and April 2019.

In total, 150 fresh fecal samples were collected from slaughtered cattle. Fecal samples were taken from the intestines of the slaughtered cattle around 7:00-9:00 am following the slaughtering process. Each fecal sample was placed in separate plastic containers, labeled with the age and breed, and date of collection. All samples were transported to the laboratory on ice packs and stored at 4°C until DNA extraction.

DNA extraction and PCR amplification

Total DNA was extracted from each fecal sample by using the QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) implementing the manufacturer's protocol. All extracted genomic DNA (gDNA) was eluted in 50 µL of AE elution buffer and stored at -20°C until further PCR analyses.

All gDNA samples were examined with PCR analyses to determine of the *Blastocystis* by amplifying a ~600 bp fragment of the SSU rRNA gene using the primers RD5 (5-ATCTGGTTGATCCTGGCCAGT-3) and BhRDr (5-GAGCTTTTTAACTGCAACAACG-3) (Ramírez et al. 2014). Each PCR reaction solution was carried out in a 25 µl total volume, a mix containing 12.5 µl Dream Taq Hot Start PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 2 µl of genomic DNA (10-30 ng/µL), 8 µl distilled water, and 1.25 µl forward and reverse primers (10 pmol/µl). The PCR amplifications were performed an initial denaturation at 95°C for 3 min, followed by 35 cycles at 95°C for 30 sec, 56°C for 30 sec, and 72°C for 30 sec, and a final extension at 72°C for 5 min in a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA). For each PCR amplification, *B. hominis* positive DNA samples and distilled water were used as a positive and negative control, respectively. The PCR product (10µl) was analyzed by 1.5% agarose gels electrophoresis with ethidium bromide-stained for 30 min at 135 V. Bands was visualized by Fusion FX Gel Documentation System (Vilber Lourmat, Collégien, France).

Nucleotide sequencing and phylogenetic analysis
All PCR positive amplicons were purified from agarose gel using a High Pure PCR Product Purification Kit (Roche, Mannheim, Germany). The gel-purified products were sequenced bidirectionally with the same PCR primers (Macrogen, the Netherlands). The obtained chromatograms were analyzed to obtain the consensus sequence with the Geneious Prime 2020.0.3 software (https://www.geneious.com). These consensus sequences were assembled and edited using the Geneious Prime 2020.0.3 software. The resulting SSU rRNA gene sequences were compared with the reference sequences available in the GenBank database using BLASTn, to determine the subtypes of the *Blastocystis* sp. The haplotype and nucleotide diversity were calculated using DnaSP software version 5.10.01 (Librado and Rozas, 2009).

A phylogenetic analysis of all nucleotide sequences obtained in this study was carried out together with reference sequences obtained from GenBank. Phylogenetic reconstruction based on the SSU rRNA dataset of *Blastocystis* was utilized by the Maximum Likelihood method (ML) with 1000 replicates bootstrap values, using the Mega 7 software (Kumar et al. 2016). The best-fit DNA-substitution model for ML based on the lowest Akaike information criterion (AIC) algorithm was selected as HKY+G + I using jModeltest v.0.1.1 (Posada, 2008). The nucleotide sequences obtained in the study were deposited in the GenBank database under accession numbers MK966392-94.

Statistical Analysis
The statistical differences in the prevalence of *Blastocystis* between age group and breed were analyzed by a Chi-square test with the software SPSS 21. The differences were considered statistically significant when $p<0.001$.

RESULTS

Molecular prevalence of *Blastocystis* sp. in cattle
Of 150 cattle, 88 (58.7%) were *Blastocystis*-positive according to PCR analyses of the SSU rRNA gene. The prevalence of *Blastocystis* infection related to age and breed of cattle is presented in Table 1.

Table 1. Distribution and statistical analysis of cattle with *Blastocystis* positivity according to age and breed groups

Parameters	No. of samples examined	No. of positive (%)	*p-value*
Age groups			
1-3 years	72	31 (43.0)	0.000[a]
>3 years	78	57 (73.0)	
Breed group			
Holstein	35	19 (54.3)	0.921
Brown Swiss	53	34 (64.2)	
Simmental	12	7 (58.3)	
Crossbreed	24	13 (54.2)	
Aberdeen-Angus	18	11 (61.1)	
Limousin	8	4 (50.0)	
Total	150	88 (58.7)	

a: statistically significant ($p<0.001$)

The highest prevalence rate (73.0%) was detected in adult cattle 3 years old and older. However, the cattle between 1 and 3 years old were observed low infection prevalence (43.0%). Statistically, significant difference ($p<0.001$) was determined between infection with *Blastocystis* and >3 years adult cattle. In addition, the lower infection was detected in Limousin and Simmental breeds, respectively, than in other breeds. There was no significant difference ($p>0.001$) between breeds of cattle and *Blastocystis* infection. Due to all slaughtered cattle were males, the analysis did not provide any results in terms of the gender of the cattle.

Nucleotide sequence and phylogenetic analyses

The final length of the contig nucleotide sequences of the SSU rRNA gene region were 568 bp. No intraspecific nucleotide differences were detected in the sequence analyses of partial SSU rRNA gene region. Nucleotide sequence analyses of the 88 SSU rRNA-positive samples revealed the presence of subtype ST10. Our sequences showed 99.47-100% identity to that of the SSU rRNA sequences of *Blastocystis* recorded previously from the cattle (Fig.1).

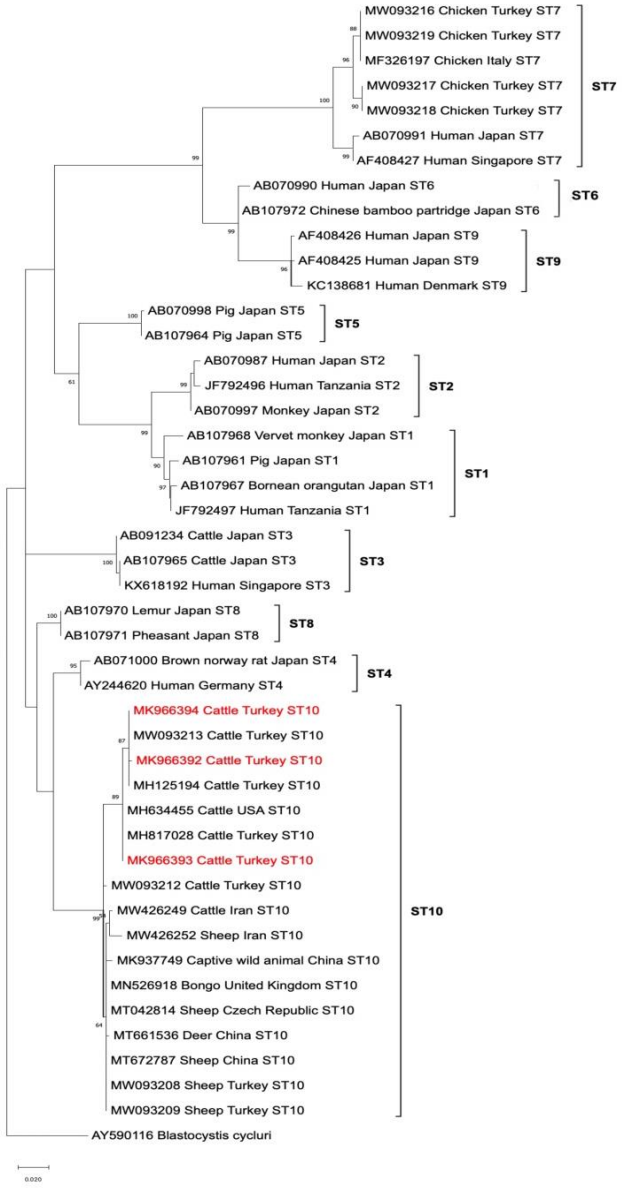

Fig. 1. Phylogenetic relationships between our *Blastocystis* (MK966392-94) isolates and other *Blastocystis* isolates as inferred by maximum likelihood obtained from SSU rRNA gene. Numbers at the nodes represent the Bootstrap values (1000 replicates). The sequences are given as GenBank accession number, host, country, and subtype. Nucleotide sequences determined in this study are indicated in red. The scale bar represents 0.02 substitutions per nucleotide position.

DISCUSSION

Blastocystis is an important pathogen commonly detected in humans and animals (Clark et al. 2013; Maloney et al. 2019). Most studies on *Blastocystis* in Turkey are performed on humans, farms and pet animals, laboratory rats, and environmental samples (Koltas and Eroglu 2016; Dogan et al. 2017; Koloren et al. 2018; Malatyali et al. 2019, Malatyali et al. 2021; Onder et al. 2021). In the present study, we analyzed the commonly used SSU rRNA gene to molecularly characterize and genotyping of *Blastocystis*. The previous study conducted by Aynur et al. (2019) in the southwestern part of Turkey had resulted in 11.25% (9/80) of examined cows and cattle samples were *Blastocystis*-positive. Notably, recent surveys of *Blastocystis* infection in cattle from Central Anatolia and the Middle Black Sea Region of Turkey revealed lower prevalence rates, at 16.0% (32/200). We determined *Blastocystis* infection with an overall prevalence of 58.7% in the cattle in various slaughterhouses, which is higher than the rate reported in the previous studies (Aynur et al. 2019; Onder et al. 2021). *Blastocystis* sp. has been determined as the most prevalent species in calves and cattle in various countries such as Colombia (80%), Japan (37.5%, 26%, and 100%) USA (2.9% and 19%), Nepal (16.7%), China (27.6%, 10.3%, and 9.5%), Libya (41.7%), UK (22.6%), and Iran (9.6%) (Abe et al. 2002; Yoshikawa et al. 2003; Yoshikawa et al. 2004; Santin et al. 2011; Fayer et al. 2012; Lee et al. 2012; Alfellani et al. 2013; Ramírez et al. 2014; Badparva et al. 2015; Zhu et al. 2017; Wang et al. 2018; Maloney et al. 2019; Ren et al. 2019). It has been reported that these differences in the prevalence of *Blastocystis* infection may be related to some factors such as sample size, animal age, sampling season, farm management, geographical location, and feeding conditions (Zhu et al. 2017; Lee et al. 2018; Suwanti et al. 2020).

Blastocystis infection has been reported with a high prevalence in weaned, yearling, and adult cattle than pre-weaned calves (Zhu et al. 2017; Maloney et al. 2019). Higher infection rate was determined in >3 years adult cattle in this study. Data for older age groups are consistent with reports published previously, documenting higher rates of *Blastocystis* infection among older cattle from China (Zhu et al. 2017), the USA (Maloney et al. 2019), and Korea (Lee et al. 2018). It has been reported that lower prevalence observed in the age group between 1 and 3 years could be related to being the immune-protective effect of maternal antibodies and provided their food directly from the breast or bottle (Zhu et al. 2017; Maloney et al. 2019).

In the present study, ST10 was found to be more prevalent in slaughtered cattle. In contrast, two subtypes, ST10 and ST14, have been detected in cattle in the southwestern part of Turkey, with a larger proportion of ST14 (Aynur et al. 2019). Similarly, in another study conducted in Central Anatolia and the Middle Black Sea Region of Turkey, *Blastocystis* ST10

was reported to be more prevalent in farm animals including cattle and sheep (Onder et al. 2021).

The findings in the present study were highly similar to results of research by Santin et al. (2011) that reported that their cattle samples were only infected with ST10. *Blastocystis* ST10 is the predominant ST reported subtypes in cattle in the USA (Fayer et al. 2012), Denmark (Stensvold et al. 2009), China (Zhu et al. 2017; Wang et al. 2018), UK, Libya (Alfellani et al. 2013), Thailand (Santín et al. 2011), and Lebanon (Greige et al. 2019). Therefore, these data confirmed the hypothesis of Cian et al. (2017) that cattle may be natural hosts of *Blastocystis* sp. ST10. Interestingly, *Blastocystis* ST10 was also reported in dogs in France (Osman et al. 2015), cats in the USA (Ruaux and Stang, 2014), and dogs and birds in Malaysia (Noradilah et al. 2017).

The three sequences in our study were located in the same clade with *Blastocystis* ST10 sequences reported from cattle in the USA and Turkey. Our sequences within ST10 were highly similar (99.8-100%) to the sequences reported from cattle in the USA (MH634455) and Turkey (MH817028, MW093213, MH125194). However, this clade was a different clade of *Blastocystis* ST10 from other ruminants within ST10.

The present study contributes to molecular characterization and subtype distribution of *Blastocystis* in cattle in various slaughterhouses in Kayseri province, Turkey. The detection of this parasite in cattle slaughtered in slaughterhouses is a warning for the slaughterhouses, and it is highlighted that this pathogen should not be neglected. Therefore, preventive measures should be taken in slaughterhouses to prevent the risk of transmission of zoonotic *Blastocystis* subtypes to slaughterhouse workers who are in close contact with animals.

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

Ethical Approval: This study is not subject to the permission of HADYEK in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics.

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RESEARCH ARTICLE

Effects Of Anise Supplement To Ration Of Beef Cattle On Performance And Some Blood Parameters

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ABSTRACT

In the current study, 14 male Holstein calves aged 11-12 months were used from the Usak Cattle Breeders Association Research Farm. In this study lasting for 60 days, 14 cattle were used and divided into 2 groups as control and treatment. The feeding method, which is routinely applied in the enterprise, was applied to the control group. In addition to the same ration, 250 gr/head/day crushed anise seed powder feed additive was poured on the feed of the treatment group consisting of the other 7 animals. The live weight yields of all animals were weighed on the 30th and 60th days of the study, the individual daily live weight gains of the animals were calculated. Animals in the treatment group gained 286 gr more live weight per day. Some blood parameters (glucose, total protein, albumin, AST, GGT, cholesterol, TAS, TOS) were examined in blood samples taken from the tail vein at the beginning and end of the study. In the study, it was determined that the statistical difference between the control and experimental groups in terms of fattening performance and blood parameters was insignificant. The results of the current study have revealed that more comprehensive studies should be carried out on the addition of anise seed powder to cattle feed.

Keywords: Anise seed powder; Beef cattle; Blood parameter; Fattening performance; Oxidant-antioxidant balance

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Besi Sığırlarına Rasyona Anason İlavesinin Performans ve Bazı Kan Parametreleri Üzerine Etkileri Etkisi

ÖZ

Bu çalışma Uşak Damızlık Sığır Yetiştiriciler Birliği Araştırma Çiftliğinde 11-12 aylık yaşta 14 baş erkek Holştayn dana kullanılmıştır. 60 gün süren çalışmada 14 adet sığır kullanılmış olup kontrol ve deneme grubu olacak şekilde 2 gruba ayrılmıştır. Kontrol grubuna işletmede rutin olarak uygulanan besleme yöntemi uygulanmıştır. Diğer 7 hayvandan oluşan deneme grubuna ise aynı rasyona ilave olarak 250gr/baş/gün anason tohumu tozu yem üzerine dökülerek verilmiştir. Tüm hayvanların canlı ağırlık verimleri denemenin 30. ve 60. günlerinde tartılmıştır ve hayvanların bireysel günlük canlı ağırlık artışları hesaplanmıştır. Deneme gurubundaki hayvanlar günlük 286 g daha fazla canlı ağırlık kazanmışlardır. Denemenin başında ve sonunda kuyruk venasından alınan kan örneklerinde bazı kan parametreleri (glikoz, total protein, albümin, AST, GGT, kolesterol, TAS, TOS) incelenmiştir. Yapılan çalışmada elde edilen verilere göre kontrol ve deneme gurubu arasında besi performansı ve kan parametreleri bakımından istatistiksel olarak önemli bir fark bulunmadığı belirlenmiştir. Sonuç olarak bu çalışma ile sığır yemlerine anason tohumu ilavesiyle ilgili daha kapsamlı çalışmaların yapılması gerekliliği ortaya çıkmıştır.

Anahtar kelimeler: Anason; Besi sığırı; Kan parametre, Besi performansı; Oksidan-antioksidan denge

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INTRODUCTION

Nowadays, in order to meet the needs of the increasing world population, ways to obtain more efficiency from a unit animal are being investigated. With a conscious feeding system, high efficiency can be guaranteed and the most economical product acquisition environment will be provided. For this reason, a sector called "feed additives" was formed in order to meet the high and quality nutrient requirements and to increase the yield and feed utilization of the animal. The activation of resistant microorganisms, the accumulation of metabolic residues related to the products in the animal organism and the possibility of adversely affecting human health made the use of antibiotics as feed additives, which increase feed utilization and thus animal productivity, questionable. For this reason, the use of ionophore, antibiotics and similar products in animal feeding has been prohibited since 2005 with the decision taken by the European Council (Jouany and Morgavi, 2007; Özen et al., 2005; Tuncer, 2007).

As a result of these prohibitions, it has come to the fore that medicinal and aromatic herbs used in many areas can be alternative due to their various benefits. As a result of recent studies, it has been stated that medicinal and aromatic plants in animal nutrition contribute to increased appetite, stimulation of digestion, body weight gain, improvement in feed utilization rate, improvement in carcass quality, and the formation of a microflora suitable for digestion and health by preventing the effects of pathogenic microorganisms in the intestine (Güler and Dalkılıç, 2005; Kamel, 2001; Tipu et al., 2006). At the same time, this group, which is extremely effective in terms of being an alternative to antibiotics, has been put into use more effectively, making it possible to obtain animal products that are more economical and positive in terms of consumer health (Kutlu, 2001).

Nowadays, there is an increasing interest in medicinal and aromatic herbs and herbal extracts derived from them. The number of studies conducted to better define these plants, to determine the safe use amounts in animal production and the amount of mixture as feed additives is increasing worldwide with each day. (Esfahani et al.,2016). However, more research is needed in this area. Some studies have been conducted on how to benefit from the carminative, antiviral and antifungal effects of the anise plant (Pimpinella anisum L.), one of these medicinal and aromatic plants, and how it can be used as an additive in both food and animal nutrition due to these important properties (Göktaş and Gıdık, 2019).

Anise is a one-year herbaceous plant belonging to the Apiaceae family. There are a total of 29 species, 8 of which are endemic, belonging to Pimpinella genus in Turkey (Davis, 1972). It has aromatic and therapeutic properties due to its precious essential oil in the secretory channels of anise seeds.

Anise is an important spice and medicinal herb used in the pharmaceutical, perfume and food industries. Its essential oil has been reported to have antispasmodic, antioxidant, germicidal properties, to be used as insecticide or repellent in agricultural applications, and to have antifungal properties. In addition, anise is used in dyspeptic complaints, in the treatment of colds and as a mild expectorant (Haşimi et al., 2014). In addition, it has been reported that it facilitates digestion due to its diuretic and carminative properties, has an antispasmodic effect, and, is used in the treatment of mild digestive disorders, nausea, colic, dyspeptic headache and bloating (Janahmadi et al., 2006).

In the current study, it was aimed to reveal the effects of anise plant (Pimpinella anisum L.), one of the medicinal and aromatic plants, on some blood parameters, blood oxidant antioxidant balance and body weight gain as an additive in animal feed.

MATERIAL AND METHOD

The current research was carried out at the Uşak Cattle Breeders Association Research Farm, following the approval of the Uşak University Animal Experiments Local Ethics Committee (USAKHADYEK 2020 / 02-01).

In this study, 14 male Holstein calves aged 11-12 months were used. Although the enterprise is engaged in milk production, it was used to shelter and feed the male material. On average, 11.5 kg of roughage and 8.6 kg of concentrate were given in the morning and evening in the fattening (Table 1 and Table 2).

Table 1. Nutrient Composition of Coarse and Concentrated Feeds Used in the Study (in Dry Matter, %)

Feed materials	Cattle Fattening Feed,14HP, 2800 ME	Barley grain, crushed	Corn silage, 30-35% KM	Sugar beet pulp	Sunflower residues, 32% HP
KM Feed %	88.35	91.00	32.00	17.00	92.20
ME Mcal/kg	3.01	2.92	2.33	2.76	2.40
NEL Mcal/kg	1.69	1.76	1.38	1.38	1.40
HP KM%	15.90	12.40	8.80	11.20	35.60
RUP HP%	31.85	18.10	33.30	66.20	18.40
HY KM%	2.49	2.20	3.20	2.10	1.50
HK KM%	7.30	2.90	4.30	4.70	7.30
NDF KM%	21.29	20.80	45.00	45.80	36.00
ADF KM%	9.10	7.20	28.10	23.10	26.00
Ca KM%	1.19	0.06	0.28	0.87	0.48
P KM%	0.56	0.39	0.26	0.10	1.00

Table 2. Beginning and ending fattening rations

	Beginning fattening ration (First Month)	Ending fattening ration (Second Month)
Corn silage, 30-35% KM	11.10	11.92
Wheat straw	2.38	1.47
Cattle Fattening Feed,14HP, 2800 ME	3.03	5.00
Barley grain, crushed	0.42	0.41
Sugar beet pulp	0.40	1.96
Sunflower residues	0.20	0.56
The total weight of the ration, kg	18.97	21.32
KM kg of rations	9.92±0.01	10.66±0.01
ME Mcal/kg KM of rations	2.25	2.45

The animals in the study were weighed and assigned to the control and treatment groups in such a way that the average body weights of the animals in the groups would be equal.

The feeding method (morning- evening), which is routinely applied in the enterprise, was applied to the control group. In addition to the same ration, 250gr / head / day crushed anise seed powder feed additive was poured on the feed of the treatment group consisting of the other 7 animals in the morning.

The live weight yields of all animals were weighed monthly with the scale in the enterprise and the individual daily live weight gains of the animals were calculated. At the same time, some blood parameters (glucose, total protein, albumin, AST, GGT, cholesterol, TAS, TOS) were examined in blood serum samples at the beginning and end of the study.

Blood samples were taken from the sub-caudal vein (vena coxygea mediana) of the animals in the middle and at the end of the study. The samples taken were centrifuged in the centrifuge for 5 minutes; the extracted serums were placed in eppendorf tubes of 2 ml and stored in a deep freezer at -18 ° C until the date of analysis. After waiting to dissolve at room temperature and reach the appropriate analysis temperature, some blood parameters (glucose, total protein, albumin, AST, GGT, cholesterol) were measured using a BT-3000 Plus auto analyser

TAS levels and TOS levels in blood samples were determined at Uşak University Scientific Analysis AndTechnological Application And Research Center Laboratory using the Elisa device according to the methods reported by Erel (2004, 2005).

For the statistical calculations of the data of the groups obtained in the study and the significance of the differences between the mean values of the groups, Mann-Whitney U test was conducted. The level of significance was set to be $P>0.05$. For this purpose, SPSS 23.0 program package was used.

RESULTS

At the end of the treatment, it was observed that the coarse and concentrated feed to which anise seed powder was added was consumed in a shorter time in the treatment group. As a statistical evaluation could not be made due to group feeding, it could not be evaluated whether there was a difference between the groups in terms of feed consumption.

Table 3 shows that weight gain results obtained in different days of the study there were no statistically significant difference between the groups in terms of body weight gains calculated based on the weighing periods.

The difference in mean weight gains calculated on the basis of the weighing periods was insignificant in the first and second month. While the difference between the means was 3.28 kg in the first month, it was 8.57 kg in the second month. It was determined that the live weight gains of cattle fed with anise seed powder added feed were better (Table 4).

Table 3. Weight gain results obtained in different days of the study, kg

Groups	Weighing Days	$\bar{x}$	$S_{\bar{x}}$	Mean Runk	Sum of Rank	U	Z	P
0-31	Control	443.43	9.21	6.00	42.00	14.000	-1.357	P>0.05
	Anise seed	443.29	12.01	9.00	63.00			
32-60	Control	477.43	8.67	4.86	34.00	6.000	-2.38	P>0.05
	Anise seed	485.86	12.75	10.14	71.00			

P>0.05 is considered to be insignificant

Table 4. Mean weight gains obtained in different days of the study

Groups	Weighing Days	$\bar{x}$	$S_{\bar{x}}$	Mean Runk	Sum of Rank	U	Z	P
0-31	Control	31.29	1.82	6.00	42.00	14.000	-1.357	P>0.05
	Anise seed	34.57	1.05	9.00	63.00			
32-60	Control	34.00	2.89	4.86	34.00	6.000	-2.38	P>0.05
	Anise seed	42.57	1.42	10.14	71.00			

P>0.05 is considered to be insignificant

Table 5. Daily live weight gains obtained in different days of the study, kg

Groups	Weighing Days	$\bar{x}$	$S_{\bar{x}}$	Mean Runk	Sum of Rank	U	Z	P
0-31	Control	1.042	0.05	4.86	34.00	23.500	-0.128	P>0.05
	Anise seed	1.152	0.03	10.14	71.00			
32-60	Control	1.133	0.09	6.50	45.50	17.500	-0.895	P>0.05
	Anise seed	1.419	0.05	8.50	59.50			

P>0.05 is considered to be insignificant

As seen in table 5 was determined that the difference in the calculated daily live weight gains was insignificant. However, it was determined that the daily live weight gains of cattle fed with anise seed powder added feed (1.419) were higher than those in the control (1.133) group.

The group averages of the results of glucose, albumin, total cholesterol, GGT, AST, and total protein analyzes obtained from blood samples taken from male fattening cattle in two separate periods in the control and treatment groups are given in Table 6 and Table 7.

Table 6. Results of Some Biochemical Analyses Conducted on the Blood Samples Taken at the Beginning of the Study (n=14)

Parameters	Grups	$\bar{x}$	$S_{\bar{x}}$	Mean Runk	Sum of Rank	U	Z	P
Glucose, mg/dl	Control	93.40	2.24	6.64	46.50	18.500	-0.767	P>0.05
	Anise seed	94.42	3.06	8.36	58.50			
Albumin mg/dl	Control	3.52	0.08	4.36	30.50	2.500	-2.827	P<0.05
	Anise seed	3.54	0.05	10.64	74.50			
Total Cholesterol, mg/dl	Control	100.12	3.65	10.93	76.50	0.500	-3.07	P<0.05
	Anise seed	92.16	4.30	4.07	28.50			
GGT, mg/dl	Control	18.54	1.97	8.71	61.00	16.000	-1.086	P>0.05
	Anise seed	17.90	1.15	6.29	44.00			
AST, mg/dl	Control	72.18	3.07	7.95	66.50	10.500	-1.79	P>0.05
	Anise seed	70.67	3.64	5.50	38.50			
Total Protein, mg/dl	Control	6.74	0.18	7.36	51.50	23.500	-0.128	P>0.05
	Anise seed	6.75	0.12	7.64	53.50			
TAS (mmol Trolox Ekivalent/L)	Control	0.12	0.11	8.29	58.00	19.000	-0.709	P>0.05
	Anise seed	0.20	0.01	6.71	47.00			
TOS(µmol H2O2 Ekivalent/L)	Control	0.10	0.01	8.71	61.00	16.000	-1.086	P>0.05
	Anise seed	0.09	0.12	6.29	44.00			

P>0.05 is considered to be insignificant

Table 7. Results of Some Biochemical Analyses Conducted on the Blood Samples Taken at the End of the Study (n=14)

Parameters	Grups	$\bar{x}$	$S_{\bar{x}}$	Mean Runk	Sum of Rank	U	Z	P
Glucose, mg/dl	Control	42.30	1.90	4.000	28.00	0.000	-3.148	P<0.05
	Anise seed	51.82	4.40	11.00	77.00			
Albumin mg/dl	Control	3.50	0.08	7.29	51.00	23.000	-0.193	P>0.05
	Anise seed	3.52	0.07	7.71	54.00			
Total Cholesterol, mg/dl	Control	104.11	3.69	11.00	77.00	0.000	-3.151	P<0.05
	Anise seed	90.15	4.35	4.00	28.00			
GGT, mg/dl	Control	18.50	1.90	8.93	62.50	14.500	-1.285	P>0.05
	Anise seed	17.70	1.11	6.07	42.50			
AST, mg/dl	Control	72.22	3.09	9.50	66.50	10.500	-1.799	P>0.05
	Anise seed	70.44	3.66	5.50	38.50			
Total Protein, mg/dl	Control	6.76	0.21	7.07	49.50	21.500	-0.385	P>0.05
	Anise seed	6.79	0.10	7.93	55.50			
TAS (mmol Trolox Ekivalent/L)	Control	0.61	0.01	10.29	72.00	5.000	-2.492	P>0.05
	Anise seed	0.55	0.01	4.71	33.00			
TOS(µmol H2O2 Ekivalent/L)	Control	0.11	0.01	8.00	56.00	21.000	-0.447	P>0.05
	Anise seed	0.10	0.01	7.00	49.00			

P>0.05 is considered to be insignificant

It has been determined that the amount of anise seed powder added to the food reduces the amount of glucose and cholesterol in the blood and the difference between them is significant. In addition, it was determined that anise reduced the total antioxidant status and the amount added was not sufficient (Table 7).

DISCUSSION

The present study was conducted to determine whether anise, which is among the feed additives used to increase the feed utilization the quality of the products obtained, to ensure the healthy breeding of animals and to reduce the cost of the obtained product, has any effect on the increase in live weight.

In this study, in the blood samples taken as a result of the trial period lasting 60 days; It was found that adding anise to the food decreased blood levels from 94.42 mg / dl to 51.82 mg / dl in glucose level. It was found that the cholesterol level in the blood decreased from 92.16 mg / dl to 90.15 mg / dl. (Table 6,7). This result shows that adding 250gr / head / day anise to cattle feed has a positive effect on blood cell values of animals. In addition, while the difference between live weight averages in the first month was 3.28, it was 8.57 kg in the second month. It was determined that the live weight gains of cattle fed with anise added feed were better.

In previous studies performed by adding plant or herbal extracts to diet as feed additives, it was reported that medicinal plants or their extracts may cause changes in blood levels of some biochemical parameters in ruminant animals (Raghuvansi et al., 2007, Mahgoub et al., 2008)

In the current study, it was determined that while the effect of adding anise to the diet on blood levels was insignificant as a result of the analysis of the blood samples taken at the end of the 60-day application period, it was found to have an effect on body weight gain (Tables 5, 6, 7). In a recent study, Iftikhar et al. (2017) added anise to the diets of Damani goats and examined the blood biochemical profile and milk quality. As a result of the study, it was determined that Anise supplement increased feed intake, body weight gain, milk yield and significantly changed its composition. It has been determined that anise increases glucose and protein levels in the blood while decreasing cholesterol and triglyceride levels. The results obtained in the study are differend from the results of the present study. Cardoza et al. (2006) also found that feed intake and live weight of the calves with feed added anise increased. This finding concurs with the findings of the present study.

Kaya (2018) examined the effect of an herbal feed additive (Yumesa-Meat Plus) on the performance, some blood parameters and carcass characteristics in beef cattle. He divided 20 Holstein male calves into 2 groups and conducted his treatment for 90 days. Animals in the treatment group gained 177 g more live weight per day. There was no statistically significant difference between the control and experimental groups in terms of some blood parameters and carcass characteristics. The findings obtained in terms of protein, GGT and albumin parameters are in accordance with the study. The results of the present study supported the results of Kaya (2018).

In the study conducted by Gümüş and Şehu (2016), a control group and a yeast group were formed with 16 male Holstein breed cattle aged 5-6 months and live yeast culture was added to the diet of the yeast group cattle for 120 days. It was determined that the difference between the groups in terms of body weight gain was not significant. This finding is supports the relevant finding of the present study.

In the study conducted by Özcan C. (2015) on the use of wild arugula plant in ruminant feeding of a total of 32 Anatolian Merino lambs aged 3–4 months, it was determined that the difference between the groups in terms of live weight gain was not significant. This result concurs with the finding of the current study. In the study, it was also found that there was no significant difference in terms of blood parameters (glucose, total protein, cholesterol, AST). The fact that there is no difference between the groups in other parameters except glucose and cholesterol is consistent with current study.

In the study conducted by Sallam et al. (2018) by adding Anise seed and active dry yeast to the rations of Egyptian buffaloes; 4 groups were compared: a control group, a treatment group whose feed 50 gr anise seed was added to, a treatment group whose feed 20 gr active dry yeast was added to and a treatment group whose feed 10 gr active yeast and 25 gr anise seed were added to. Total cholesterol and triglyceride concentrations were found to have decreased in the treatment groups ($P<0.01$). The finding of a decrease in cholesterol in the blood is in accordance with the study.

In their study conducted on 18 Holstein cows in Baghdad University Animal Farm to examine the effect of anise on heat tolerance and some blood parameters, Shwayel and Al-Mafraji (2020) formed three groups: a control group, a treatment group whose feed 30 gr anise was added to and a treatment group whose feed 30 gr anise s processed in formaldehyde was added to. As a result, it was determined that the

addition of anise did not have any effect (triglyceride, GPT, HDL and heat tolerance) in the summer months in the treatment groups. The fact that there is no difference between the groups in other parameters except glucose and cholesterol is consistent with current study.
Esfahani et al. (2016) examined the effect of anise seed on performance, digestibility and infectious microbes in the intestines of lactating calves. A total of 24 Holstein calves were divided into three groups: a control group, a treatment group whose feed 0.25% anise was added to and a treatment group whose feed 0.5% anise was added to. The study lasted 60 days As a result, it is compatible with the present study in terms of better live weight gain in groups fed with anise supplemented feed.
Probably, when anise seed power is added to the feed, it adds more flavor to the feed content and causes the cattle to consume more and more fondly. In this case, more weight gain is provided in live weight.

CONCLUSION

In recent years, as consumers have turned towards healthy and safe food consumption, the use of medicinal and aromatic plants has come to the fore in the production of animal products, especially as yield enhancers.
Considering the average weight gains and daily live weight gains (286 gr) obtained in the second month in the treatment group whose feed anise seed powder was added to and low cost of anise, it can be argued that anise can help animal breeders to increase their income. In addition, observing that animals that consume anise seed powder added feed are calmer is indication of another positive effect of aromatic plants on beef cattle.
It has been concluded that anise seed powder can be used as an alternative feed additive to synthetic products to meet consumer demand in animal products, but this potential should be confirmed with different ruminant species and longitudinal studies. If the findings to be obtained in such studies support the findings of the current study, it will be possible for animal breeders and businesses producing animal feed additives to use anise seed powder and in this way the use of anise seed powder in ruminant animals will be more widespread.

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

Ethical Approval: The current research was carried out at the Uşak Cattle Breeders Association Research Farm, following the approval of the Uşak University Animal Experiments Local Ethics Committee (USAKHADYEK 2020 / 02-01).

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RESEARCH ARTICLE

Comparison of Fat and Carbohydrate Metabolisms in Chicken and Rat Liver

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ABSTRACT

The aim of this study is to reveal histological differences between prenatal and adult liver carbohydrate and fat metabolism in chickens and rats. In this study, 30 Wistar albino rats and 30 Ross breed broiler chickens were used. The rats and chickens were divided into groups of 10. These groups were categorized as adult, 14- and 18-day-old fetus groups. In this study, the fat and carbohydrate metabolisms in rat and chicken liver were studied by histological methods, and changes in adult and fetal periods were evaluated comparatively. In the light microscopic examinations of liver carbohydrate accumulation, hepatic glycogen accumulation was similar in the adult rats and chickens. When the hepatic glycogen amount was compared in the 14-day-old rat and chicken fetuses, while glycogen granules were found in most of the chicken fetal hepatocytes, almost no glycogen granules were found in the rat fetuses. This difference between the animals was found to be statistically significant ($p<0.001$). It was observed that glycogen accumulation in the 18-day-old rat fetuses was less than that in chicken fetuses. This difference between the animals was found to be statistically significant ($p=0.013$). Light microscopic examinations of liver lipid accumulation showed that similar ratios of lipid droplets were observed in the hepatocytes of adult rats and chickens. Significantly higher amounts of lipid droplets were detected in the 14- and 18-day-old chicken fetus hepatocytes compared with 14- and 18-day-old rat fetus hepatocytes, which was statistically significant ($p<0.001$).

Keywords: chicken, fetüs, hepatocyte, rat.

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Tavuk ve Rat Karaciğerinde Yağ ve Karbonhidrat Metabolizmalarının Karşılaştırılması

ÖZ

Bu çalışmanın amacı, tavuklarda ve ratlarda doğum öncesi ve erişkin dönem karaciğer karbonhidrat ve yağ metabolizması arasındaki histolojik farklılıkları ortaya koymaktır. Çalışmada, Wistar albino türü 30 adet rat ile 30 adet Ross ırkı broiler tavuk kullanıldı. Ratların ve tavukların her biri 10' arlı gruplara ayrıldı. Bu gruplar erişkin, 14 günlük ve 18 günlük fötuslar halinde düzenlendiler. Bu çalışmada, rat ve tavuk karaciğerindeki yağ ve karbonhidrat metabolizmaları histolojik yöntemlerle incelenmiş, erişkin ve fetal dönemlerdeki değişimler karşılaştırmalı olarak değerlendirilmiştir. Karaciğer karbonhidrat birikiminin ışık mikroskobik incelemelerinde, Erişkin dönem ratlar ile tavuklarda hepatik glikojen birikiminin benzer yoğunlukta olduğu görüldü. 14 günlük rat ve tavuk fötuslarında hepatik glikojen miktarı karşılaştırıldığında, tavuk fötus hepatositlerinin çoğunda glikojen granüllerine rastlanırken rat fötuslarında yok denecek kadar az miktarda glikojen granülüne rastlandı. Hayvanlar arasındaki bu fark istatistiksel olarak da anlamlı bulundu ($p<0.001$). 18 günlük rat fötuslarındaki glikojen birikiminin tavuk fötuslarına göre daha az miktarda olduğu gözlendi. Hayvanlar arasındaki bu fark istatistiksel olarak da anlamlı bulundu ($p=0.013$). Karaciğer lipid birikiminin ışık mikroskobik incelemelerinde, erişkin rat ve tavuk hepatositlerinde benzer oranlarda lipid damlacığı görüldü. 14 ve 18 günlük tavuk fötusu hepatositlerinde 14 ve 18 günlük rat fötuslarına oranla belirgin derecede fazla miktarda lipid damlacığı tespit edildi. Hayvanlar arasındaki bu farklar istatistiksel olarak da anlamlı bulundu ($p<0.001$).

Anahtar Kelimeler: fötus, hepatosit, rat, tavuk.

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INTRODUCTION

The liver, the body's factory, has important functions such as bile secretion, storage of substances such as glycogen, lipid, vitamins, and iron, purification of blood from metabolism residues and toxic substances, and hematopoiesis in the fetal and neonatal periods (Junqueira and Charneiro 2009, Petorak 1986). These functions of the liver occur in tandem with the morphological development of the organ in the prenatal and postnatal period (Aydın et al. 2000). In order for the fetus to grow and develop normally, resources such as carbon, nitrogen, ions and water are required. These resources are used in important biochemical events such as the formation of new tissues, the storage of molecules such as glycogen and fat, and the production of energy for growing tissues. Therefore, although the total nutritional requirement of the fetus varies depending on its growth rate, it also differs by species (Fowden 2001). The fetal growth in chickens occurs through physiological phenomena that take place in a closed system independent of the mother. In rats, on the other hand, fetal growth occurs when physiological requirements are met as a result of the mutual interaction of the maternal and placental environment with the fetus (King and Loke 1994).

The liver has many roles in carbohydrate and lipid metabolisms. A significant part of these tasks is performed by hepatocytes, which constitute approximately 80% of the organs (Mitra and Metcalf 2012). Glucose, the most important fuel for living things, is stored in hepatocytes in the form of glycogen. Glycogen deposits can be visualized with Periodic acid–Schiff (PAS) and Best's carmine stains. The lipid droplets stored in the form of triglycerides in hepatocytes can be visualized using dyes such as Sudan Black B and Oil Red O after appropriate detection (Junqueira and Charneiro 2009, Altınışık 2010, Kierszenbaum and Tres 2016).

The liver is a key organ involved in various metabolic processes that regulate the growth and productivity of living things. Considering the yield characteristics of animals, the importance of liver carbohydrate and fat metabolism is understood. When the fetal and adult periods are considered comparatively, it is thought that the findings obtained from the research can be a guide in the development of yield characteristics, and thanks to this information, new clues can be obtained at the level of basic information on fat and carbohydrate metabolism. In this study, to compare the fat and carbohydrate metabolisms in chicken and rat liver, liver sections of 14- and 18-day-old rat and chicken fetuses and adult rats and chickens were investigated by light microscopy after staining with relevant histological techniques.

MATERİAL AND METHODS

This study was approved by the Ankara University Animal Experiments Local Ethics Committee (dated: 14.03.2018, approval number: 2018-6-55). In the study, rats supplied from Ankara University Experimental Animal Production and Research Laboratory and broiler chickens obtained from Sökmenler Poultry Chicken Production and Marketing were used.

In the study, 30 Wistar albino rats and 30 Ross broiler chickens were used as study material. The rats were divided into 3 equal groups including 10 rats in each group. These groups were categorized as adult (6–8 weeks) rats, 14- and 18-day-old fetuses. The broiler were divided into 3 equal groups including 10 chickens in each group. These groups were categorized as adult (39–43 days) chickens, 14- and 18-day-old chicken fetuses. The rats were cared for and fed in Ankara University Experimental Animal Production and Research Laboratory. Animals were fed with standard pellet feed and tap water in 12 hours of light and 12 hours of darkness without restriction of feed and water. In the care and nutrition of the chickens, they were fed in a 24-hour light environment by giving the basic ration without any feed and water restriction in the Sökmenler Poultry Chick Production Center. Eggs were incubated in an incubator under optimal conditions (Incubation temperature 36.5-37.5C, Humidity: 85%). Considering the basic embryological information available on the development of rat and chicken fetal liver and literature reviews on the carbohydrate and fat metabolism of these animals (Elias 1955, Luzzatto 1981, Hamburger and Hamilton 1951, Suksaweang et al. 2004), liver tissue samples were obtained from 14- and 18-day-old fetuses.

On the 14th and 18th days of pregnancy the abdomen of the rats was opened under ketamine–xylazine anesthesia and their fetuses were removed from the dissected uterus. After their macroscopic examination, the abdomen of the fetuses was opened and their livers were removed. Tissue samples were also obtained from various parts of the liver from the adult rats under anesthesia.

The developmental stages of the embryos in the chicken fetuses were determined according to the Hamburger and Hamilton (1951) stages, the formation of organs was evaluated according to this scale, and embryos that were out of the scale or showing developmental disorders were not included in the experiment. The chicken eggs were carefully broken and the liver tissues of the fetuses were dissected. Tissue samples were taken from various parts of the liver after cervical dislocation of the adult chickens.

Light Microscopic Examinations

The liver samples obtained from the animals were placed in tissue plates in 10% buffered formol, formol–alcohol at + 4°C and Baker's 10% formol–calcium solutions at + 4°C for histological preparation. Both fetal and adult liver tissue samples, which were fixed in formol–alcohol for 24 h at + 4°C, were passed through 96% alcohol, absolute alcohol, methyl benzoate, and benzol series and blocked in paraplast. Best's carmine staining and PAS staining were performed on 5 μm sections taken from the blocks to visualize glycogen in hepatocytes. In Best's carmine and PAS staining, the specificity of the reaction was determined with control preparations. For this purpose, α-amylase that hydrolyzes glycogen (diastase digestion) was applied to control sections and the non-stained structures were evaluated as glycogen (Bancroft and Gamble 2002). The liver tissue samples fixed in 10% buffered formol for 24 h were washed with tap water for 24 h and then passed through graded alcohols, methyl benzoate, and benzol series and blocked in paraplast. Mallory's trichrome staining technique, modified by Crossmon for general histological examinations were applied on 5 μm sections taken from the blocks (Bancroft and Gamble 2002). The preparations obtained were examined under the Leica DM 2500 model research microscope and were photographed.

In contrast, 8–10 μm thick cryostat sections were taken from the tissue samples that were fixed in 10% formol–calcium solution for 24 h at + 4°C. The Sudan Black B and Oil Red O stainings were performed on these sections to visualize the oils (Bancroft and Gamble 2002). The preparations obtained were examined under the Leica DM 2500 model research microscope and were photographed.

Evaluation of Histological Data

Statistically, the level of carbohydrate accumulation was graded using a Likert scale ranging from 1 to 5. The grading was conducted by two different histologists. Glycogen granules seen in pink-red color in the hepatocyte cytoplasm of the liver sections stained by PAS were evaluated and graded as follows. Descriptive statistics for the obtained data were calculated. The statistical control of the difference between the species and the developmental periods of the same species in terms of the evaluation of carbohydrate accumulation was done with the Chi-Square Test. SPSS 14.01 package program was used.

In order to evaluate the fat accumulation in the liver statistically, with the Image J program, the fat droplets in a 40× (enlarged 400 times) area were counted manually. In the hepatocyte cytoplasm of the liver sections stained with Oil Red O Red, lipid droplets were counted. Descriptive statistics for the obtained data were calculated. Differences between species in terms of oil droplet count statistical control was done with Independent Samples t-Test. The statistical control of the difference between the developmental stages of the same species was done by One-Way Analysis of Variance. In case of any statistical difference between the groups with One-Way Analysis of Variance, the Tukey Test was applied as a post hoc analysis to determine which two groups caused the difference. SPSS 14.01 package program was used.

RESULTS

Light Microscopic Results

In the triple-stained liver sections of the adult rats and chickens, it was observed that the parenchyma of the organ consisted of polygon-shaped epithelial cell cords that anastomosed with each other. Between the cords were sinusoids opening into the vena centralis. The portal triad consisting of artery, vena, and bile duct in the connective tissue mass between the lobes, which were not very pronounced, showed a similar appearance in adult rats and chickens. In the triple-stained liver sections of the fetuses taken on the 14th and 18th days of the development of rats and chickens, vena centralis and sinusoids were seen in the center of the hepatic lobules whose borders could not be clearly identified. Small hepatocytes were found around the vena centralis that did not show regular localization as in adult hepatocytes (Figure 3.1).

In the adult rat and chicken PAS-stained liver sections, dense glycogen accumulation was observed in a large number of hepatocytes around the vena centralis and portal area. Best's carmine method used for visualization of glycogen in the liver showed the same results as PAS staining. In PAS and Best's carmine staining methods, the specificity of the reaction was determined by the application of α-amylase, which hydrolyzes glycogen (Figure 3.2). When the fetal livers obtained on the 14th day of development of rats and chickens were compared, it was observed that there were distinct glycogen granules in most of the chicken fetal hepatocytes, whereas they were almost absent in the rat fetuses. Best's carmine method showed the same results as PAS staining (Figure 3.3). When microscopic liver images of the rat fetuses (18 days old) and chicken fetuses (18 days old) were compared, glycogen accumulation in the rat fetuses was less than that in the chicken fetuses (Figure 3.4). The specificity of the reaction was determined by the application of α-amylase, which hydrolyzes glycogen.

In the Oil Red O and Sudan Black B stainings made to visualize fats, positive lipid droplets were found in the adult rat and chicken hepatocytes. When the liver

cryostat sections of the rats and chickens were compared, similar proportions of lipid droplets were found in hepatocytes (Figure 3.5). In the fetal livers obtained on the 14th day of the development of rats and chickens, the amount of lipid droplets in the chicken fetus hepatocytes was observed to be significantly higher than that in the rat fetus hepatocytes (Figure 3.6). When the liver cryostat sections of 18-day-old rat and chicken fetuses were compared, it was observed that lipid droplets in the fetal chicken hepatocytes were more than those in the fetal rat hepatocytes (Figure 3.7).

As a result of light microscopic examinations, while both glycogen granules and lipid droplets were seen in the adult rat hepatocytes, glycogen granules were almost never observed on the 14th day of fetal development. However, glycogen granules were prominent in the 18-day-old rat fetuses, although not as much as in the adult rats. The amount of hepatic lipid droplets in the 18-day-old rat fetuses was higher than that in the 14-day-old rats. While the number of lipid droplets in the adult rats was higher than that in the 14-day-old rat fetuses, it was similar to that in the 18-day-old rat fetuses (Figure 3.8).

As a result of light microscopic examinations, both lipid droplets and glycogen granules were found in the liver of all groups of chickens. The group of chickens ranked from highest to lowest based on the density of hepatic glycogen concentration was adult chickens, 18-day-old chicken fetuses, and 14-day-old chicken fetuses. The group of chickens ranked from highest to lowest based on the amount of hepatic lipid droplet accumulation was 18-day-old fetuses, 14-day-old fetuses, and adult chickens, respectively (Figure 3.9).

Statistical Results

The average number of lipid droplets was counted in the liver sections of the adult rats and chickens, 14- and 18-day-old rat and chicken fetuses using the ImageJ program. As a result of this, no statistically significant difference was found in terms of average fat droplet counts in the adult rat and chicken livers (Table 3.1). The average fat droplet count of 14- and 18-day-old chicken fetuses was found to be higher compared with that of 14- and 18-day-old rat fetuses ($p<0.001$; Table 3.2, $p<0.001$; Table 3.3).

The average fat droplet count of the adult rats was found to be statistically higher than that in rats on the 14th day of fetal development. However, no statistically significant difference was found between the adult rats and 18-day-old rat fetuses in terms of average fat droplet count. There is a statistically significant difference between the 14- and 18-day-old rat fetuses in terms of average fat droplet count ($p=0.009$; Table 3.4).

The average fat droplet count of the adult chickens was statistically lower than that of the 14- and 18-day-old chicken fetuses. The average fat droplet count of the chickens on the 18th day of fetal development was statistically higher than that of the 14-day-old rat fetuses ($p<0.001$; Table 3.5).

The liver tissue samples of the adult rats and chickens, 14- and 18-day-old rat and chicken fetuses were rated by two different histologists using a Likert scale ranging from 1 to 5. As a result of the evaluation, no statistically significant difference was found between the adult rats and chickens in terms of carbohydrate accumulation in the liver (Table 3.6). However, there was a significant difference in terms of carbohydrate accumulation between the 14- and 18-day-old rat fetuses and chicken fetuses, and it was found that hepatic glycogen density in the chicken fetuses was higher than that of the rat fetuses ($p<0.001$; (Table 3.7, $p=0.013$; Table 3.8).

The liver carbohydrate accumulation in adult rats was found to be higher compared with that in the 14- and 18-day-old rat fetuses. In addition, a statistically significant difference was observed between the 14-day-old rat fetuses and the 18-day-old rat fetuses in terms of carbohydrate accumulation, and more glycogen accumulation was observed in the 18-day-old rat fetus livers ($p<0.001$; Table 3.9).

A statistically significant difference was found in terms of carbohydrate accumulation between the adult chickens and the 14- and 18-day-old chickens ($p=0.044$; Table 3.10).

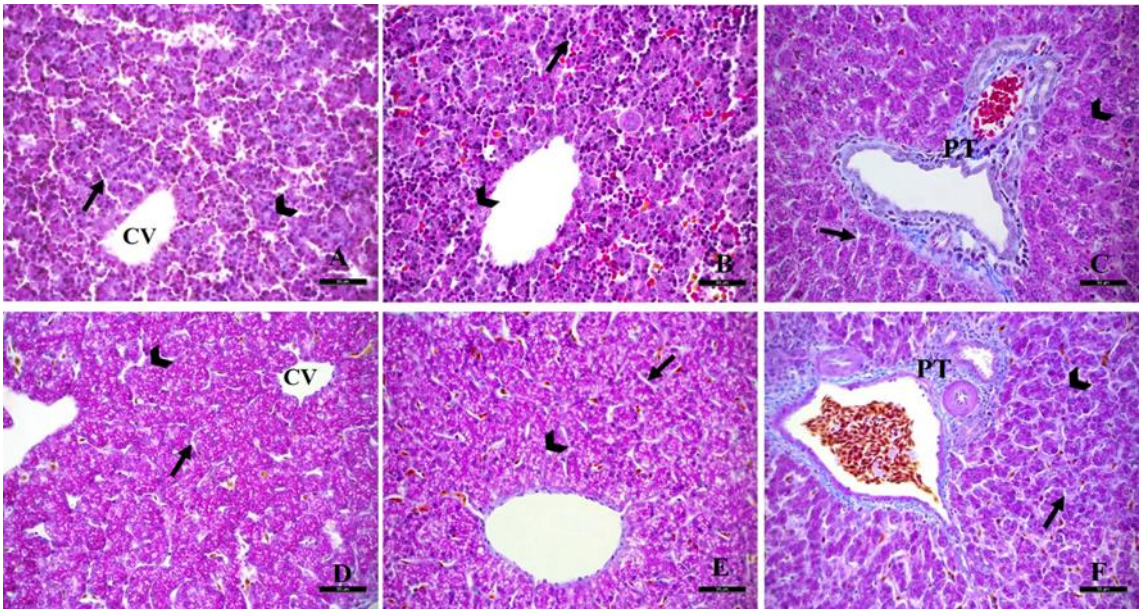

Figure 3.1: Liver sections of a 14-day-old rat (A), 18-day-old rat (B), adult rat (C), 14-day-old chicken (D) 18-day-old chicken (E) and adult chicken (F). PT: portal triad, Arrowheads: hepatocytes, arrows: sinusoids, cv: central vein. Mallory's trichrome. Bar: 50 µm.

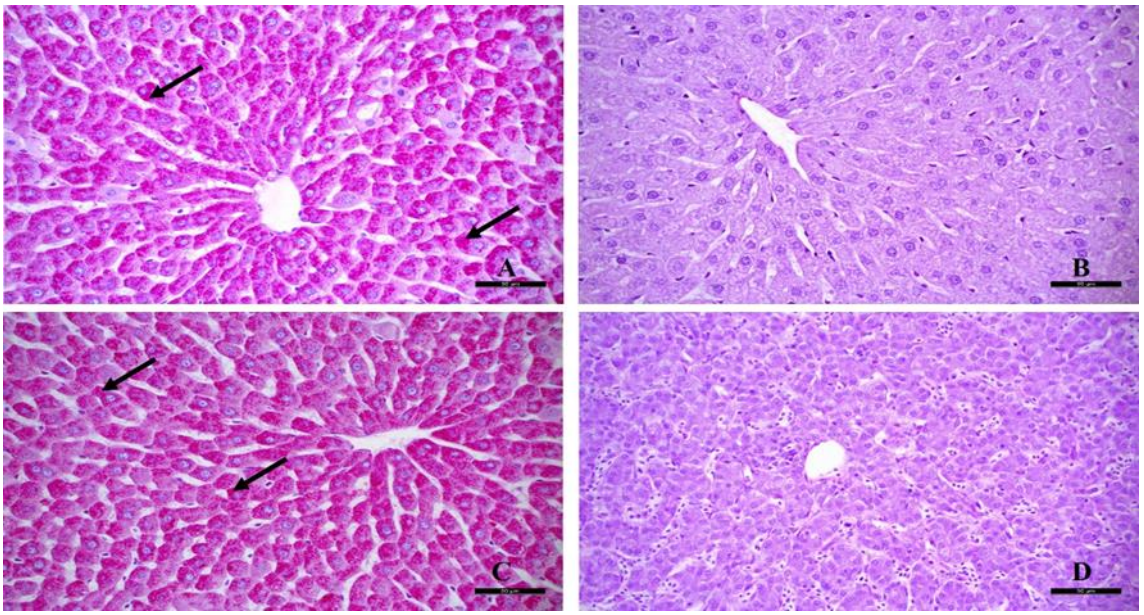

Figure 3.2: Strong PAS positive reaction (arrows) in adult rat (A) and adult chicken (C) hepatocytes. PAS. Bar: 50 µm. PAS negative reaction after diastase ingestion in adult rat (B) and adult chicken (D) hepatocytes. Diastase / PAS. Bar: 50 µm.

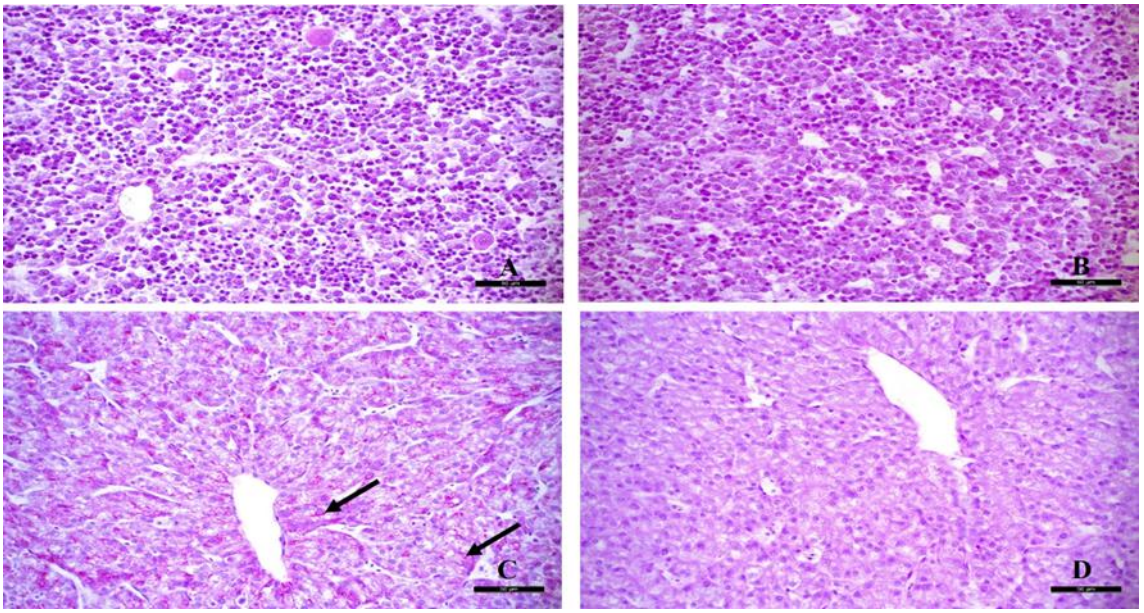

Figure 3.3: PAS negative reaction in 14-day-old rat hepatocytes (A), PAS-positive reaction (arrows) in 14-day-old chicken hepatocytes (C). PAS. Bar: 50 µm. PAS negative reaction in hepatocytes after diastase ingestion of 14-day rat (B) and 14-day chicken (D). Diastase / PAS. Bar: 50 µm.

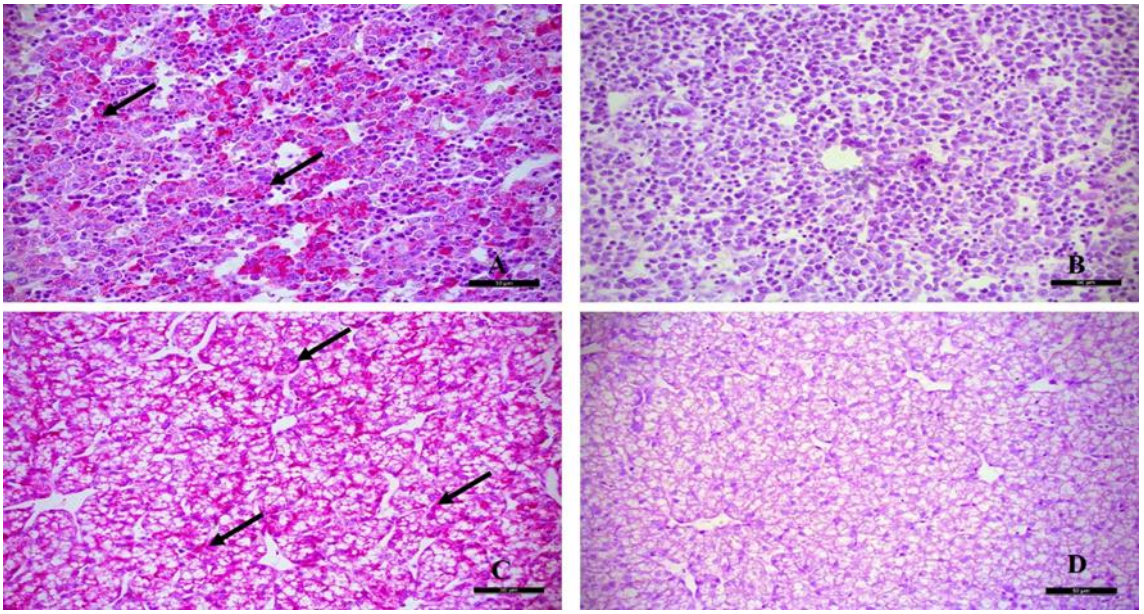

Figure 3.4: Glycogen accumulation (arrows) in 18-day-old rat (A) hepatocytes, dense glycogen accumulation (arrows) in 18-day-old chicken hepatocytes (C). Best Carmine. Bar: 50 µm. Best Carmine negative reaction after diastase digestion in 18 day-old rat (B) and 18 day-old chicken hepatocytes (D). Diastase / Best Carmine. Bar: 50 µm.

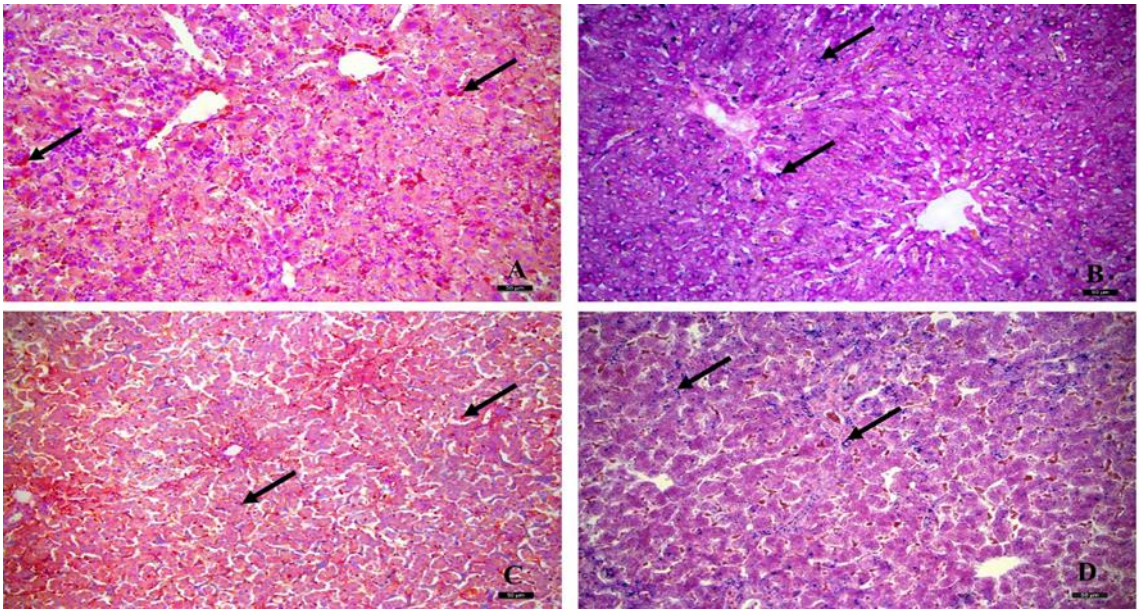

Figure 3.5: Positive lipid droplets (arrows) in adult rat (A, B) and adult chicken (C, D) hepatocytes. A, C: Oil Red O. Bar: 50 µm. B, D: Sudan Black B. Bar: 50 µm

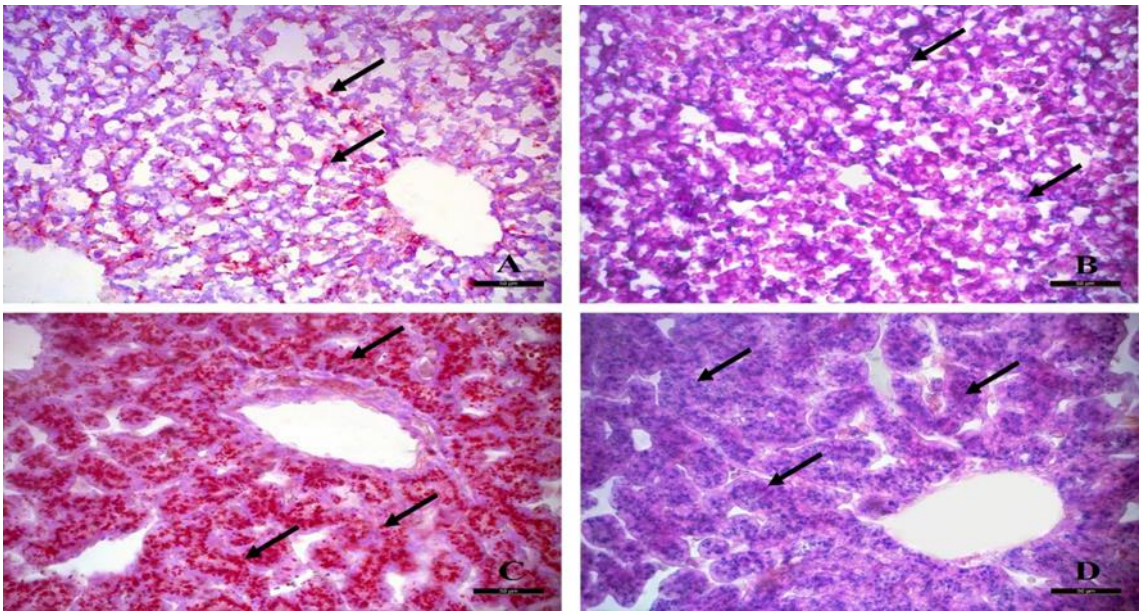

Figure 3.6: Cryostat sections of 14-day-old rat (A, B) and 14-day-old chicken (C, D). Oil Red O positive lipid droplets (arrows) in hepatocytes (A, C). Oil Red O. Bar: 50 µm. Sudan Black B positive lipid droplets (arrows) in hepatocytes (B, D). Sudan Black B. Bar: 50 µm.

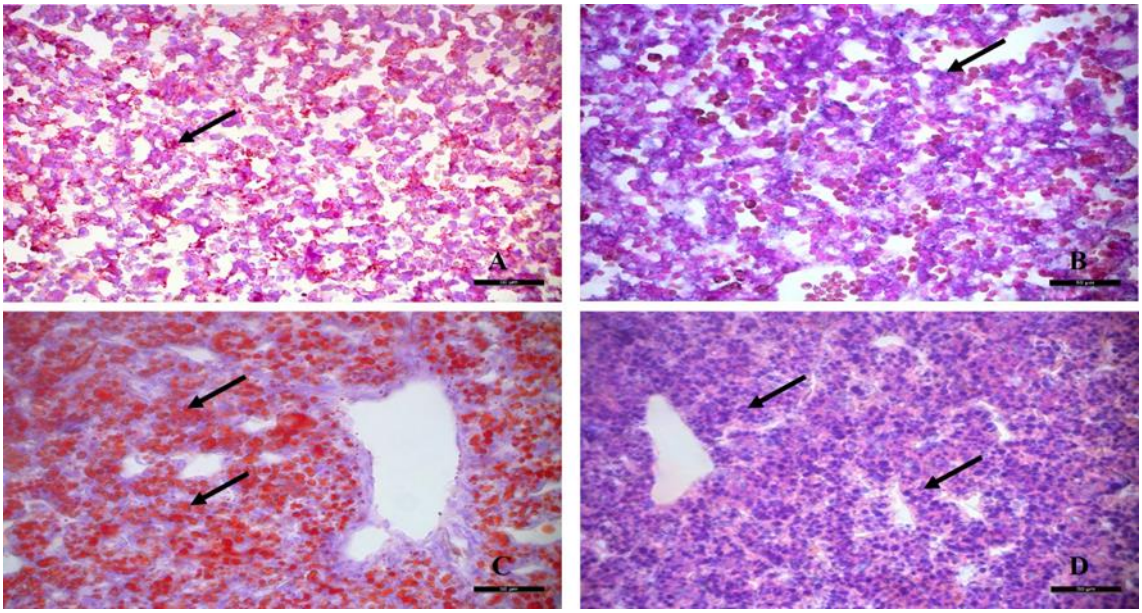

Figure 3.7: Positive lipid droplets (arrows) in of 18-day-old rat (A, B) and 18-day-old chicken (C, D) hepatocytes. A, C: Oil Red O. Bar: 50 µm. B, D: Sudan Black B. Bar: 50 µm.

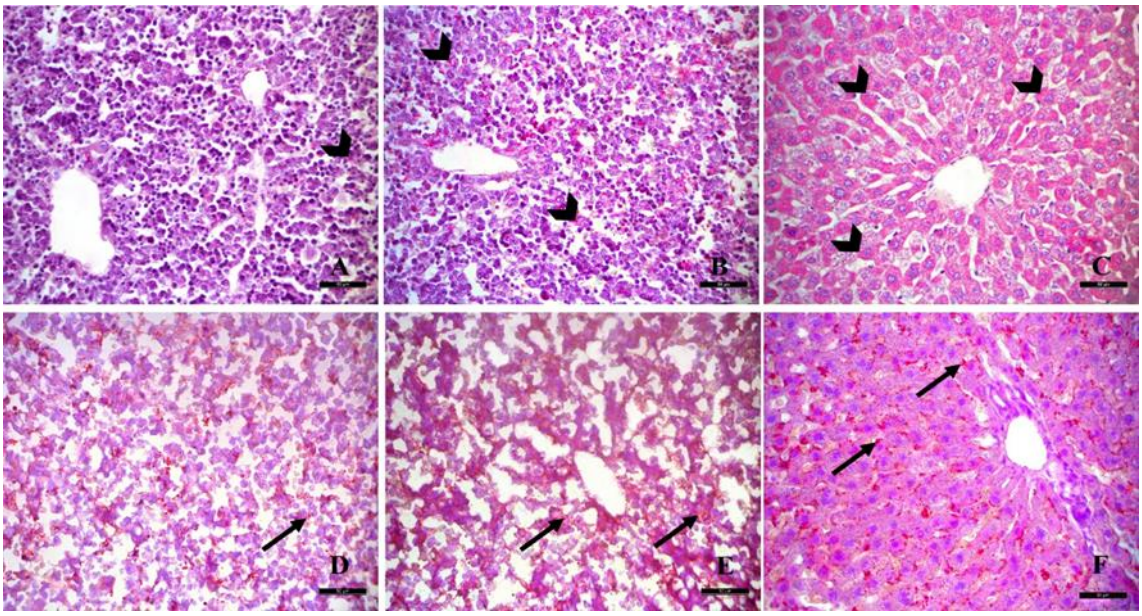

Figure 3.8: Liver sections of a 14-day-old rat (A, D), 18-day-old rat (B, E), and adult rat (C, F). B, C: dense glycogen accumulation (arrowheads) in hepatocytes. A: very little glycogen accumulation in hepatocytes (arrowhead). Best's carmine. Bar: 50 µm. D, E, F: positive lipid droplets (arrows) in hepatocytes. Oil Red O. Bar: 50 µm.

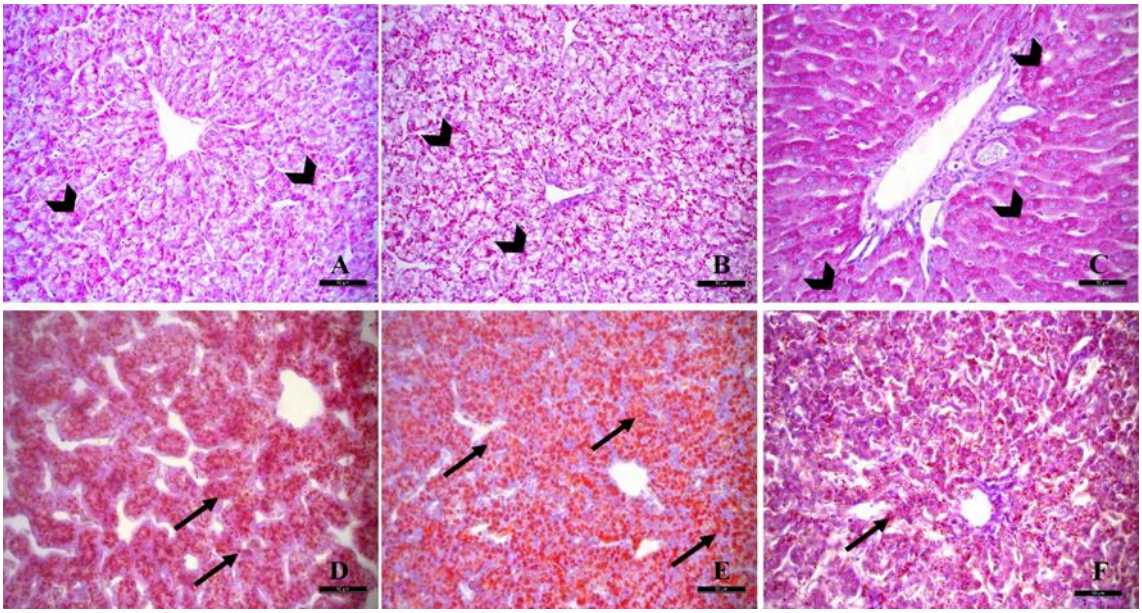

Figure 3.9: Liver sections of an adult chicken (C, F), 14-day-old chicken (A, D), and 18-day-old chicken (B, E). A, B, C: dense PAS positive reaction in hepatocytes (arrowheads). PAS. Bar: 50 μm. D, E, F: positive lipid droplets (arrows) in hepatocytes. Oil Red O. Bar: 50 μm.

Table 3.1. Hepatic lipid droplet count results in adult rat and adult chicken.

Group	N	Arithmetic Mean	Standard deviation	Standard error	Median	Maximum	Minimum	p
AR	10	297,5	84,1	26,59	314,05	395,70	141,80	0,130
AC	10	357,41	84,89	26,84	340,95	503,10	256,10	

AR, adult rat; AC, adult chicken; N, sample size.

Table 3.2. Hepatic lipid droplet count results in 14-day-old rat fetus and 14-day-old chicken fetus.

Group	N	Arithmetic Mean	Standard deviation	Standard error	Median	Maximum	Minimum	p
FR14	10	222,75	33,09	10,47	226,55	273,40	176,20	<0,001
FC14	10	803,7	79,48	25,13	793,15	944,40	664,60	

FR14, 14 day-old rat fetus; FC14, 14 day-old chicken fetus; N, sample size.

Table 3.3. Hepatic lipid droplet count results in 18-day-old rat fetus and 18-day-old chicken fetus.

Group	N	Arithmetic Mean	Standard deviation	Standard error	Median	Maximum	Minimum	p
FR18	10	282,59	15,05	4,76	283,90	297,60	244,00	<0,001
FC18	10	1147,72	75,42	23,85	1150,95	1237,60	1017,10	

FR18, 18 day-old rat fetus; FT18, 18 day-old chicken fetus; N, sample size.

Table 3.4. Hepatic lipid droplet count results in adult rat, 14-day-old rat fetus and 18-day-old rat fetus.

Group	N	Arithmetic Mean	Standard deviation	Standard error	Median	Maximum	Minimum	p
AR[a]	10	297,5	84,1	26,59	314,05	395,70	141,80	
FR14[b]	10	222,75	33,09	10,47	226,55	273,40	176,20	0,009
FR18[a]	10	282,59	15,05	4,76	283,90	297,60	244,00	

AR, adult rat; FR14, 14day-old rat; FR18, 18day-old rat; N, sample size.

Table 3.5. Hepatic lipid droplet count results in adult chicken, 14-day-old chicken fetus and 18-day-old chicken fetus.

Group	N	Arithmetic Mean	Standard deviation	Standard error	Median	Maximum	Minimum	p
AC[c]	10	357,41	84,89	26,84	340,95	503,10	256,10	
FC14[b]	10	803,7	79,48	25,13	793,15	944,40	664,60	<0,001
FC18[a]	10	1147,72	75,42	23,85	1150,95	1237,6	1017,1	

AC, adult chicken; FC14, 14day-old chicken; FC18, 18day-old chicken; N, sample size.

Table 3.6. Carbohydrate accumulation grading results in adult rat and adult chicken.

	Group		Total	p
	AR	AC		
1	1 (%10,0)	0 (%0,0)	1 (%5,0)	0,766
2	2 (%20,0)	1 (%10,0)	3 (%15,0)	
3	1 (%10,0)	1 (%10,0)	2 (%10,0)	
4	3 (%30,0)	3 (%30,0)	6 (%30,0)	
5	3 (%30,0)	5 (%50,0)	8 (%40,0)	
Total	10 (%100)	10 (%100)	20 (%100)	

AR, adult rat; AC, adult chicken.

Table 3.7. Carbohydrate accumulation grading results in 14-day-old rat fetus and 14-day-old chicken fetus.

	Grup		Total	p
	FR14	FC14		
1	10 (%100,0)[a]	0 (%0,0)[b]	10 (%100,0)	<0,001
2	0 (%0,0)[a]	5 (%50,0)[b]	5 (%25,0)	
3	0 (%0,0)[a]	3 (%30,0)[a]	3 (%15,0)	
4	0 (%0,0)[a]	1 (%10,0)[a]	1 (%5,0)	
5	0 (%0,0)[a]	1 (%100,0)[a]	1 (%5,0)	
Total	10 (%100)	10 (%100)	20 (%100)	

FR14, 14 day-old rat fetus; FC14, 14 day-old chicken fetüs.

Table 3.8. Carbohydrate accumulation grading results in 18-day-old rat fetus and 18-day-old chicken fetus.

	Grup		Total	p
	FR18	FC18		
2	8 (%80,0)[a]	1 (%10,0)[b]	9 (%45,0)	
3	2 (%20,0)[a]	5 (%50,0)[a]	7 (%35,0)	
4	0 (%0,0)[a]	3 (%30,0)[a]	3 (%15,0)	0,013
5	0 (%0,0)[a]	1 (%10,0)[a]	1 (%5,0)	
Total	10 (%100)	10 (%100)	20 (%100)	

FR18, 18 day-old rat fetus; FT18, 18 day-old chicken fetüs.

Table 3.9. Carbohydrate accumulation grading results in adult rat, 14-day-old rat fetus and 18-day-old rat fetus.

	Grup			Total	p
	AR	FR14	FR18		
1	1 (%10,0)[a]	10 (%100,0)[b]	0 (%0,0)[a]	11 (%36,7)	
2	2 (%20,0)[a]	0 (%0,0)[a]	8 (%80,0)[b]	10 (%33,3)	
3	1 (%10,0)[a]	0 (%0,0)[a]	2 (%20,0)[a]	3 (%10,0)	
4	3 (%10,0)[a]	0 (%0,0)[a]	0 (%0,0)[a]	3 (%10,0)	<0,001
5	3 (%10,0)[a]	0 (%0,0)[a]	0 (%0,0)[a]	3 (%10,0)	
Total	10 (%100)	10 (%100)	10 (%100)	30 (%100)	

AR, adult rat; FR14, 14day-old rat; FR18, 18day-old rat.

Table 3.10. Carbohydrate accumulation grading results in adult chicken, 14-day-old chicken fetus and 18-day-old chicken fetus.

	Grup			Total	p
	EC	FC14	FC18		
2	1 (%10,0)[b]	5 (%50,0)[a]	1 (%10,0)[b]	7 (%23,3)	
3	1 (%10,0)[b]	3 (%30,0)[b]	5 (%50,0)[a]	8 (%30,0)	
4	3 (%30,0)[b]	1 (%10,0)[b]	3 (%30,0)[b]	7 (%23,3)	0,044
5	5 (%50,0)[a]	1 (%10,0)[b]	1 (%10,0)[b]	7 (%23,3)	
Total	10 (%100)	10 (%100)	10 (%100)	30 (%100)	

AC, adult chicken; FC14, 14day-old chicken; FC18, 18day-old chicken.

DISCUSSION

The liver is metabolically the most active organ in the fetus. It is the only organ in which all metabolic pathways and metabolic enzymes are active (Krebs 1972). Glycogen and lipids are large forms of energy storage, which are tightly controlled by enzymes, hormones, and metabolic signaling pathways. For this reason, various studies have been conducted on enzymes that play an active role in liver carbohydrate and lipid metabolisms in both fetal period and adulthood using various animal species (Yeung et al. 1967, Leskes et al. 1971, Rideau et al. 2008). In the study conducted by Leskes et al. (1971), liver glucose-6-phosphatase activity of rats was determined in adult and fetal periods. The glucose-6-phosphatase activity in 18-day-old rat fetuses was found to be as frequent as 5% of the activity in adults. Therefore, glycogen granules were not found in all hepatocytes in the liver. In another study conducted by Dvorak (1971) with rats, it was reported that glycogen granules were found in the liver on the 18th day of fetal life. In the study we conducted, while large amounts of glycogen granules were found in most adult hepatocytes, less glycogen granules were found in the fetal rats. When these results were compared with chickens, it was noticed that there was a higher amount of glycogen accumulation in the 18-day-old chicken fetuses compared with that in the rat fetuses. In most of the adult rat and chicken hepatocytes, similar proportions and dense amounts of glycogen granules were observed. On the 18th day of fetal rat development, more glycogen granules were found in many hepatocyte cytoplasms, albeit not very dense, compared with the 14th day. This difference was found to be statistically significant. This situation can be explained by the ability of the animal to meet its increasing energy needs during the fetal rat development period, to complete its development in a healthy manner, and to guarantee the glucose reserves of the liver for postnatal metabolic transition. Thus, an increase in glycogenesis and gluconeogenesis metabolisms is observed in the fetal liver (Trefts et al. 2017). In the study conducted by Luzzatto (1981) with rat fetuses, he reported that glycogen granules in hepatocytes could be identified on the 18th day and lipid droplets on the 12th day of the fetal development. In this study, hepatic glycogen granules were observed in the light microscopic examinations of the 18-day-old rat fetuses. In addition, it was observed that glycogen granules in the 18-day-old rat fetuses were much less compared to those in the chicken fetuses. Machida et al. (1990) evaluated lipoprotein lipase (LPL) activity in the liver as an indicator of hepatic lipid metabolism and measured lipoprotein lipase activity in the fetal liver (15th, 17th, 19th, and 20th days). They found that LPL activity in the liver increased in the fetal period. The biochemical findings of Machida et al. (1990) are consistent with the light microscopic examinations of our liver tissue samples because the amount of hepatic lipid droplets in the 14- and 18-day-old rat fetuses also increased in our cross-sections. The increased lipolytic activity in the mother during the fetal period in order to meet the increasing energy need and the effect of maternal energy metabolism on the fetal energy metabolism may be the reason for pronounced hepatic fat accumulation in both periods (Hendrickse et al. 1985, Herrera et al. 2006, Rao et al. 2013). In rats, the fetus constantly receives substrate support from the placenta. With birth, this support suddenly stops and the baby begins to use endogenous substrates for glucose homeostasis. In order to prepare for a switch from exogenous support to endogenous substrate support, the fetus begins to store more glycogen and lipids at the end of pregnancy (Kimura 1991). This information clarifies the increase in the hepatic lipid and glycogen content in the fetal period in our study. In a study by Borrebaek et al. (2007) who measured the fetal liver enzyme activities of 14- and 19-day-old chickens, hexokinase enzyme activity and glycogen content was reported to have increased in the liver. In the study conducted by Willemsen et al. (2010) with chicken fetuses, it was reported that the amount of glycogen in the liver was higher on the 18th day compared with that on the 16th day in the control groups. In the light microscopic examinations of this study, it was seen that the amount of glycogen stored in the liver was higher on the 18th day compared with that on the 14th day. The reason for the difference in liver glycogen accumulation in rats and chickens is that fetal rats have maternal support during the embryonal development process, whereas fetal chickens do not have any external support.

Metabolic pathways related to lipids are very active in chicken embryos from mid-incubation until 2–3 days before hatching. At this stage, the embryo uses egg yolk fatty acids as its main source of energy. Therefore, fatty acid synthesis and beta-oxidation must be highly active at this stage of embryonic development (De Oliveira et al. 2008). In the study conducted by Zhao et al. (2007) with broiler chickens, tissue samples were taken from the liver and the hepatic triglyceride amount was measured. It was observed that the amount of liver triglyceride was higher on the 19th day of incubation compared with that on the 14th day. Wong and Cavey (1992) found that on the 14th day of incubation, all hepatocytes contained both lipid and glycogen. When these results were compared with the amount of hepatic lipid in the rats in our study, fewer lipid droplets were observed compared with the 14- and 18-day-old chicken fetuses and it was determined that there was a statistically significant difference between the 14- and 18-day-old rat and chicken fetuses in terms of

average fat droplet count. In this study, it was noticed that the amount of hepatic lipid increased significantly on the 18th day of chicken fetal development compared with that on the 14th day. Lipid droplets are observed more intensely with the development of fetal chicken liver and it can be explained by the fact that lipid metabolism is very active until a few days before hatching. Chickens need a greater amount of energy to survive and grow, both during and after the process of hatching. However, because the development of embryos takes place within the egg surrounded by certain limits, there is no external nutritional support. For this reason, the embryo stores a greater amount of glycogen and fat in order to meet the energy it will need in the last period of its development. This may be the reason why, in light microscopic examinations of our study, we observed that more glycogen and fat were stored in hepatocytes on the 18th day of incubation compared with that observed on the 14th day Yang et al. (2010) measured the amount of hepatic triglyceride and cholesterol in their study with broiler chickens. In the control group of the study, it was found that the amount of hepatic triglyceride and cholesterol in 14-day-old chickens was higher than that in adult chickens (63 days old). It was also reported that total cholesterol and triglycerides in the blood decreased in adulthood. The results of our study also support this situation histologically because it was determined that the amount of fat droplets in the adult chicken liver was lower compared with that in the fetal periods.

In chickens, the only source of lipid for the embryo during the incubation period is the egg yolk, and increased absorption of lipids in the egg yolk in the last week of the incubation period causes lipid concentration in the fetal liver to increase up to eight times (Uni et al. 2012). Providing substrate support to rats during the fetal period, the mother undergoes two metabolically differentiated periods in order to meet the increasing energy needs of the fetus. First, because of the limited fetal development, the excess food taken by the mother is stored as fat, the second is that the transfer of dietary lipids to the fetus increases with the mother's storage fat (lipolytic activity increases) during the period when fetal growth is much faster (López-Luna et al. 1986, Lopez et al. 1991, Chaves ans Herrera 1978, Knopp et al. 1970, Martin et al. 1994). These reasons suggest that the amount of hepatic lipid in the 18-day-old rat and chicken fetuses is higher than that of the 14-day-old rat and chicken fetuses.

When histological and statistical results were evaluated, significant differences were found in carbohydrate and fat metabolisms of the liver in rats and chickens because in addition to an endogenous source, rats also receive exogenous support during fetal development, whereas chickens only have an endogenous source. When it comes to adult rats and chickens, there was no difference between fat and carbohydrate storage. When the yield characteristics of animals are taken into consideration, it is understood how important liver carbohydrate and fat metabolisms are. In order to improve information about these metabolisms, the fetal and adult periods were compared and it is believed that the findings obtained may provide new clues at basic knowledge level on liver metabolism and may be a guide to the improvement of yield characteristics.

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RESEARCH ARTICLE

An Investigation of the History of the Chamber of Veterinarians in Van Region

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ABSTRACT

The chambers of veterinarian which are one of the organs of the Turkish Veterinary Medical Association are established in accordance with the Law no 6343. There are chambers of veterinarian in all regions of Turkey. With this research, it was aimed to reveal about the 30 years history of the Van Region Chamber of Veterinarians, which is located in the Eastern Anatolia Region, within the scope of the available material. The study was created by evaluating the data obtained as a result of the analysis of a limited number of first-hand documents from 1987-2016 obtained from the archives on the subject. In the study, it was found that the chamber was started operating in 1987 and the activity scope of the chamber included the provinces of Bitlis, Hakkâri and Van in 2016. After the chamber had provided service at different locations, it achieved a permanent labor office in 2014. It was concluded that the Van Region Chamber of Veterinarians provided benefits to the veterinary profession and therefore public health within the scope of the training programs, professional and scientific activities carried out from 1987 to 2016.

Key words: Chamber of Veterinarians, History of Veterinary Medicine, Van Region Chamber of Veterinarians

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Van Bölgesi Veteriner Hekimler Odasının Geçmişine Dair Bir İnceleme

ÖZ

Türk Veteriner Hekimleri Birliğinin organlarından biri olan veteriner hekim odaları 6343 sayılı Kanuna dayanılarak kurulmaktadır. Türkiye'nin tüm bölgelerinde veteriner hekim odaları bulunmaktadır. Bu araştırma ile mevcut materyal kapsamında Doğu Anadolu Bölgesinde yer alan Van Bölgesi Veteriner Hekimler Odasının yaklaşık 30 yıllık tarihinin ortaya çıkarılması amaçlandı. Çalışma, konuyla ilgili arşivlerden sağlanan 1987-2016 yıllarına ait ilk elden sınırlı sayıdaki dokümanın analizi sonucu elde edilen verilerin değerlendirilmesi ile oluşturuldu. Araştırmada, odanın 1987 yılında faaliyete başladığı ve 2016 yılında hizmet alanı içinde Bitlis, Hakkâri ve Van illerinin yer aldığı tespit edildi. Kuruluşun çeşitli adreslerde faaliyet gösterdikten sonra 2014 yılında kalıcı bir çalışma ofisine sahip olduğu belirlendi. Van Bölgesi Veteriner Hekimler Odasının 1987'den 2016 yılına kadar gerçekleştirdiği eğitim programları, mesleki ve bilimsel faaliyetler kapsamında veteriner hekimliği mesleğine ve dolayısı ile toplum sağlığına fayda sağladığı sonucuna varıldı.

Anahtar kelimeler: Van Bölgesi Veteriner Hekimler Odası, Veteriner Hekimler Odası, Veteriner Hekimliği Tarihi.

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INTRODUCTION

The chambers of veterinarian which are in service in Turkey are the professional organizations as public institutions established based on the Law No. 6343 which came into force in 1954, on the 'Execution of the Veterinary Profession, the Formation of the Turkish Veterinary Medical Association (TVMA) and its Chambers and the Works to be Performed'. By to the provisional fifth article of the law abovementioned, the central board of the Turkish Veterinarians Society has taken on the task of selecting the places where the first chambers of veterinarian will be established[1].

A chamber of veterinarians (CV) is established in every city center with a minimum of 30 veterinarians within its borders, or in regions with a minimum of 30 veterinarians, which are formed by merging neighboring provinces. Veterinarians who perform their profession freely have to be members of the chamber, while the membership of others is voluntary. Chambers of veterinarian; it consist of four boards namely the board of directors, the general assembly, the board of discipline and the board of auditors. Among them, the board of directors; ensures that the legislation on the performance of art is duly implemented; the general assembly elects veterinarians who will work in the chamber organs; the board of discipline; carries out the disciplinary proceedings of the members; on the other hand, the board of auditors has obligations such as supervising the compliance of the chamber activities with the legislation. Veterinarians who will work in chamber organs are elected for two years[2,3].

There are a total of 56 chambers of veterinarian in Turkey, 13 of which are regional chambers of veterinarian (Anon 2021). One of the regional chambers is the Van Region CV[4,5]. Since there is no scientific publication about the history of Van Region CV, which is located in the Eastern Anatolia Region of Turkey, it was decided to conduct this study. Within the scope of the document obtained, it was aimed to reveal about the 30 years history of the chamber since its establishment.

[1]The law on the Execution of the Veterinary Profession, the Formation of the Turkish Veterinary Medical Association and its Chambers and the Works to be Performed, The Official Gazette dated 18.03.1954 and numbered 8661.
[2]Footnote 1.
[3]Implementing Regulation on the Conduct of the Services of the Turkish Veterinary Medical Association, the Official Gazette 13. 06. 2006 and numbered 26288.
[4]Documents of the TVMA Archive (1954-2016).
[5]Documents of the Van Region CV Archive (1987-2016).

MATERIAL AND METHOD

The first contact was made with the Van Region CV in June 2017 to research its archive. Research permission was obtained from the Van Region CV in October 2017 and from the TVMA in December 2017. Since these organizations do not have a regular and complete archive, a limited number of documents could be accessed. In this framework, board of directors decision books (January 1987-December 2016), board of directors activity reports (2012-2014, 2014-2016), supervisory board reports (2012-2014, 2014-2016), general assembly council minute (2000), member logbooks and few other documents could be provided first-hand. The study also benefited from the relevant legislation and literature. Tags of the original documents were shown in the footnotes. The years 1987-2016 were included in the research within the scope of the first hand documents accessible. Existing material was evaluated by document analysis method.

RESULTS

It was determined that the founding board of the chamber had its first meeting on January 30 1987, upon the documents (dated 08.01.1987 and number 41319) received from the central council of the Turkish Veterinary Association. It was detected that Prof. Dr. Ataman Güre (Figure 1) was appointed as head, Associate Professor Hüseyin Timurkan as secretary, veterinarian Salih Kayabaşı as an accountant, and veterinarian Necmettin Harman and Associate Professor Nihat Mert as members at this meeting (Figure 2)[6]. It was also determined that a total of 39 veterinarians, 2 of whom were women, registered as members of the chamber[7] and the general assembly of establishment was held on July 18, 1987[8]. In the resolutions of the founding board of directors, it was determined that the name of the chamber name which is written as 'Van-Hakkari Region Chamber of Veterinarians' was written as 'Van Region Chamber of Veterinarians' in official documents since the date of general assembly of establishment (Figure 2)[9].

[6]Board decision dated 30.01.1987 number 1, the archive of Van Region CV.
[7]Member record notebooks, the archive of Van Region CV.
[8]Board decision dated 21.07.1987 and number 9, the archive of Van Region CV.
[9]Footnote 4, 5.

Figure 1: Prof. Dr. Ataman Güre.

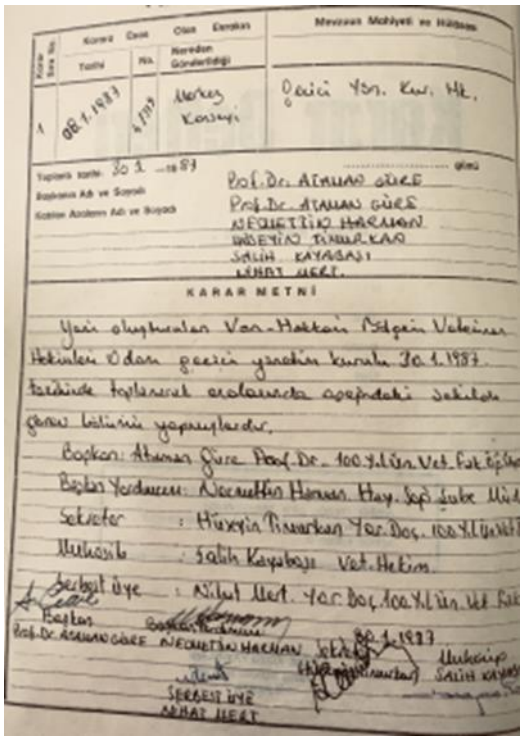

Figure 2: Board decision dated 30.01.1987, numbered one.

The founding board decided to open its first chamber office on Cumhuriyet Street (2nd Street, No: 23/1, Van) in March 1987[10]. In November 1988, the chamber was moved to the office building belonging to the Special Provincial Directorate of Administration on Sıhke Street (No: 1, Van)[11,12]. Upon the sale of this office building, the chamber was transferred to Yüzüncü Yıl University, Faculty of Veterinary Medicine, Head of Food Hygiene and Technology Department, which was the business address of the chamber head during that period at the beginning of 2007[13]. Eventually, Van Region CV acquired its own office, purchased on Milli Egemenlik Street (No: 11/20) in 2014[14]. It was determined that the second head of TVHB also attended the opening ceremony of the new office (Anon 2015).

It was determined that the three freelance veterinarians who spoke at the general assembly of Van Region CV in 2000 stated that the activities of the chamber were inadequate, that the chamber did not communicate adequately with the members for the election, that the academics were unfamiliar with the problems of the freelance veterinarians and therefore that the freelance veterinarians could solve their own problems only themselves, and that the academic and public employee members should withdraw from the elections and that the chamber head should be a freelance veterinarian member. It was detected that an academic member also agreed with these speeches. It was determined that a female member of the public employee who spoke at the above meeting stated that female veterinarians were not sufficiently connected to the chamber and that members of the public employee did not cause any difficulties to the freelance veterinarians but instead helped them. It was detected that the two academic members who spoke at the same meeting have stated that the people who came to the administration made sacrifices on behalf of the profession, the members should first provide financial and moral support instead of criticizing the chamber's work, all members (public employees, academics, and freelance veterinarians) were indifferent to the chamber, all segments of the chamber should work together in accordance with the relevant legislation[15].

In the research, it was determined that all of the heads of the Van Region CV since 2000 were academicians. In addition, it was determined that a large number of academicians have been elected to the organs of the Van Region CV at the general assemblies (29 of the 83 members of the board of directors, 27 of the 33 members of the board of auditors, 59 of the 69 members of the board of discipline, and 58 of the 75 major congress delegates are academics.). It was determined that there were no freelance veterinarians and female veterinarians among the 17 heads elected throughout Van CV's history.

[10]Board decision dated 10.03.1987 and number 6, the archive of Van Region CV.
[11]Board decision dated 15.11.1988 and number 17, the archive of Van Region CV.
[12]Board decision dated 20.11.1988 and number 19, the archive of Van Region CV.
[13]Board of decision dated 04.01.2007 and number 1, the archive of Van Region CV.
[14]Board work report dated 2012-2014, the archive of Van Region CV.
[15]General assembly council minutes dated 30.09.2000, the archive of Van Region CV.

The veterinarians elected to the chamber organs at the general assemblies of the Van Region CV are shown in Table 1-4[16,17]. It was determined that Bitlis and Hakkâri provinces were also in the responsibility area of the chamber in 2016[18,19].

In general, the decisions are taken routinely at Van Region CV; immediately after the election of the board members, the board of directors visit the supervisors of the public institutions in the province for the meeting and having professional meetings; determining the minimum wage tariff was approved by the central council, organizing celebration ceremonies and night to be held on the beginning anniversaries of veterinary medicine teaching in Turkey and on the day of the world veterinary medicine, and participation to chamber heads evaluation meetings and Turkish veterinary medicine congresses[20]

It was determined that the Van Region CV decided to give stethoscopes to the first three students who took the degree in the 1997-1998 academic year in the veterinary faculty[21], provide financial support to veterinary student congresses held on various dates[22,23,24] and to the First National Wildlife Congress[25].

Some activities that Van Region CV has decided to regulate are as follows; In-Service Training Seminar[26], twice Accredited Veterinary Training and HACCP Course[27,28], four times Artificial Insemination Course by Recto-Vaginal Method in Cattle[29,30]. It was detected that the Van Region CV organized the Cattle Reproduction Ultrasonography Course (17-18/05/2012)[31]; sent a representative to the Bursa Veterinary Student Congress (16-18/03/2013) and organized the Animal Welfare Control and Danish Practices Conference (20/03/2013)[32].

Within the framework of TVMA's protocol with the Red Crescent, it was determined that a veterinarian was assigned from the chamber during the sacrifices, and in 2011[33,34], in the sacrifices made by citizens to support the earthquake victims in Van[35].

It was also determined that the chamber management visited veterinary clinics at various times, trying to find solutions to existing problems and participated in clinical inspections with officials at the Ministry of Food, Agriculture and Livestock[36,37].

It was determined that Van Region CV did not carry out any publication activities (1987-2016)[38] and there was only one disciplinary case in which the chamber members are involved (1987-2016)[39].

DISCUSSION AND CONCLUSION

The archives of TVMA and its chambers are important in terms of illuminating the history of the chambers of veterinarians. In previous studies, it is mentioned that TVMA and Afyonkarahisar CV archives are insufficient (Melikoğlu and Kızıltepe 2008, Türkmenoğlu 2019). Similarly, in this study, the Van Region CV archive and TVHB's Van Region CV archive were found insufficient in terms of documents related to the researched subject.

[16]Footnote 7.
[17]Board decision books (30.01.1987-13.12.2016), the archive of Van Region CV.
[18]Footnote 14.
[19]2014-2016 period board work report, the archive of Van Region CV.
[20]Footnote 17.
[21]Board decision dated 30.06.1998 and number 98-1, the archive of Van Region CV.
[22]Board decision dated 28.04.2008 and number 2008-3, the archive of Van Region CV.
[23]Board decision dated 17.03.2014 and number 2014-1, the archive of Van Region CV.
[24]Board decision dated 10.04.2015 and number 2015-4, the archive of Van Region CV.
[25]Board decision dated 15.04.2015 and number 2015-5, the archive of Van Region CV
[26]Board decision dated 20.01.1990 and number 34, the archive of Van Region CV.
[27]Board decision dated 20.02.2007 and number 2007-3, the archive of Van Region CV.
[28]Board decision dated 11.03.2008 and number 2008-1, the archive of Van Region CV.
[29]Board decision dated 02.05.2015 and number 2015-6, the archive of Van Region CV.
[30]Board decision dated 13.02.2016 and number 2016-2, the archive of Van Region CV.
[31]Outgoing paper dated 07.05.2012 and number 2012-09, the archive of Van Region CV.
[32]Footnote 14.
[33]Board decision dated 01.11.2010 and number 2010-5, the archive of Van Region CV.
[34]Footnote 14.
[35]Outbound paperwork dated 31.10.2011 and number 365/1187, the archive of TVMA.
[36]Auditing board report dated 2012-2014, the archive of Van Region CV.
[37]Auditing board report dated 2014-2016, the archive of Van Region CV.
[38]Footnote 5.
[39]Outgoing documents dated 20.02.2012 and numbered 365/56, the archive of TVMA.

Table 1. The members of the board of directors elected at the General Assemblies of the Van Region CV.

Years	Head	Secretary	Accountant	Members	
1987	Nihat Mert	Alev Bitgel	Aziz Toker	Abdurrahman Kılıç	Necmettin Harman
1989	İsmet Şüküroğlu	B. Nadir Bildik	Aziz Toker	Abdurrahman Kılıç	Necmettin Harman
1992	Gürdal Dağoğlu	Şahabettin Yıldız	İsmail Çelik	Yakup Akgül	Erol Baytok
1994	B. Nadir Bildik	Nazmi Yıldız	Aziz Toker	Erol Baytok	Bekir Karaşan
1996	B. Nadir Bildik	Dide Kılıçalp	B. Emre Teke	Erol Baytok	Yavuz Görmen
1998	B. Nadir Bildik	Duran Bolat	Nazmi Atasoy	Ali Belge	Erol Baytok
2000	Y. Can Sancak	Ayşe Karaca	C. Tayyar Ateş	Şahabettin Yıldız	Volkan Dinler
2002	Y. Can Sancak	Birgül Güleryüz	H. Hüseyin Arı	Şahabettin Yıldız	Volkan Dinler
2004	Y. Can Sancak	Şahabettin Yıldız	Volkan Dinler	Şuheda Ceylan	Kani Yayla
2006	Y. Can Sancak	Şahabettin Yıldız	Kani Kaya	Şuheda Ceylan	Özay İlhan
2008	Y. Can Sancak	Şahabettin Yıldız	Kani Kaya	F. Ramazan İstanbullugil	Özay İlhan
2010	Y. Can Sancak	Şahabettin Yıldız	Özgür İşleyici	Şakir Ertaş	Musa Çetin
2012	Y. Can Sancak	Özay İlhan	Özgür İşleyici	Sami Yalçın	Musa Çetin
2014	Nazmi Atasoy	Loğman Aslan	Adem Düzgün	Musa Çetin	Ali Taşdemir
2016	Loğman Aslan	Cumali Özkan	Mahmut Ekinci	Mehmet Erkeç	Yasin Demirhan

Table 2. The grand congress delegates elected at the General Assemblies of the Van Region CV.

Years	Members
1987	Necmettin Harman, Nihat Mert
1989	İsmet Şüküroğlu, Necmettin Harman
1992	Fuat Odabaşıoğlu, Hüseyin Karadağ
1994	Ali Belge, Fuat Odabaşıoğlu, Hüseyin Karadağ, Hüseyin Timurkan, Gürdal Dağoğlu, Mehmet Cambazoğlu
1996	Bekir Karaşan, Duran Bolat, Gürdal Dağoğlu, Fuat Odabaşıoğlu, Hüseyin Karadağ, Y. Can Sancak
1998	Atanur Tezel, Birgül Güleryüz, Fikret Karaca, Fuat Odabaşıoğlu, Hüseyin Karadağ, Lokman Bayar
2000	Birgül Güleryüz, Fuat Odabaşıoğlu, H. Hüseyin Arı, H. Hüseyin Dönmez, Hüseyin Karadağ, Taylan Aksu
2002	Abdurrahman Aksoy, Ali Belge, Ayşe Karaca, C. Tayyar Ateş, Duran Bolat, İhsan Keleş
2004	Dide Kılıçalp, Duran Bolat, H. Hüseyin Arı, Hüseyin Karadağ, İbrahim Yurdakul, İhsan Keleş,
2006	Dide Kılıçalp, Duran Bolat, H. Hüseyin Arı, Hüseyin Karadağ, İhsan Keleş, Özgür İşleyici
2008	Abuzer Taş, Dide Kılıçalp, Duran Bolat, H. Hüseyin Arı, İhsan Keleş, Özgür İşleyici
2010	Abuzer Taş, Bahattin Çak, H. Hüseyin Arı, İhsan Keleş, Özay İlhan
2012	Abuzer Taş, Bahattin Çak, Duran Bolat, H. Hüseyin Arı, Tuncer Çakmak
2014	Abuzer Taş, Bahattin Çak, Özay İlhan, Taylan Aksu, Y. Can Sancak,
2016	Abdullah Kaya, Abuzer Taş, İ. Hakkı Behçet, Özay İlhan, Süleyman Kozat, Y. Can Sancak

Table 3. The members of the disciplinary committees elected at the General Assemblies of the Van Region CV.

Years	Members
1987*	
1989	Duran Bolat, Hayati Çamaş, Mehmet Gürkan
1992	Duran Bolat, Hayati Çamaş, Mehmet Gürkan, Necmettin Harman, Zulmet Kurunç
1994	Duran Bolat, İsmail Alkan, M. Serdar Değer, Suphi Deniz, Y. Can Sancak
1996	Ali Belge, Banur Boynukara, Cesim Akkol, Hüseyin Timurkan, M. Serdar Değer
1998	Ayşegül Bildik, Fatmagül Yur, Ferda Belge, İsmail Alkan, T. Zahit Ağaoğlu
2000	Bekir Karaşan, Duran Bolat, Fatmagül Yur, Hülya Sağmanlıgil, Yeter Değer
2002	E. Nurzen Bozkurt, Nihat Mert, Orhan Yılmaz, Suphi Deniz
2004	Ali Çınar, M. Akif Karslı, Nihat Mert, Orhan Yılmaz, Suphi Deniz
2006	İsmail Meral, Nihat Mert, Orhan Yılmaz, Reyhan Yıldız, Suphi Deniz
2008	Fatmagül Yur, Loğman Aslan, Mehmet Cambazoğlu, Orhan Yılmaz, Yakup Akgül
2010	Loğman Aslan, M. Serdar Değer, Mehmet Cambazoğlu, Yakup Akgül, Zafer Soygüder
2012	Ali Aslan, Fatmagül Yur, Fetih Gülyüz, Yakup Akgül, Zafer Soygüder
2014	Duran Bolat, Hüseyin Karadağ, İsmail Alkan, Suphi Deniz, Yakup Akgül
2016	Duran Bolat, İsmail Alkan, Mehmet Cambazoğlu, Nazmi Atasoy, Yakup Akgül

*Data for 1987 could not be found.

Table 4. The members of the auditors boards elected at the General Assemblies of the Van Region CV.

Years	Members
1987*	
1989	İsmail Çelik, Y. Eray Ulu, Zulmet Kurunç
1992	Birgül Güleryüz, Hülya Sağmanlıgil, Selim Gülbay
1994	Birgül Güleryüz, Ferda Belge, Hülya Sağmanlıgil
1996	Birgül Güleryüz, Nazmi Atasoy
1998	Bekir Karaşan, Gürdal Dağoğlu
2000	Gürdal Dağoğlu, M. Serdar Değer
2002	Fatmagül Yur, M. Serdar Değer
2004	Fatmagül Yur, M. Serdar Değer
2006	Fatmagül Yur, M. Serdar Değer
2008	Abdullah Kaya, M. Serdar Değer
2010	Abdullah Kaya, Fatmagül Yur,
2012	Abdullah Kaya, M. Serdar Değer
2014	M. Serdar Değer, Süleyman Kozat
2016	Abdullah Karasu, Özgür İşleyici

* Data for 1987 could not be found.

While Van Region CV had its own office from its establishment until 2007, from this year it was directed from the business address of the chamber head. It can be said that the chamber's having an independent office again in 2014[40] is a positive development in terms of carrying out activities more comfortably.

In a survey study published in 2003, on 94 veterinarians from 22 provinces in the Eastern and Southeastern Anatolian Regions, operating in different sectors, 63.8% (n:60) of the sample group "*does the chamber management work well?*" It was reported that they answered "*no*" to the question (Özen and Ateş 2003). The fact that various members stated that they found the chamber activities insufficient in the Van Region CV general assembly of 2000[41] also supports the above data. According to this data, it can be said that the activities carried out by the chamber and the expectations of its members do not exactly match each other.

Contrary to the freelance veterinarians wishes that a freelance head at the Van Region CV 2000 general assembly, the all of the chamber heads have been academicians since the said assembly (2000-2016)[42]. According to this result, it can be argued that members other than academics did not find sufficient support for the headship. However, there may be also other reasons for this situation.

[40]Footnote 10-14.

[41]Footnote 15.

[42]Footnote 7, 15, 17.

It has been reported that chambers in the provinces of Samsun (1969-2012), Kars (1989-2016), Afyonkarahisar (1969-2017) and Aydın (1994-2020) do not have any female head (Sanal and Melikoğlu Gölcü 2014, Kızıltepe 2017, Türkmenoğlu 2019, Koç 2020). Similarly, in this study, it was found that there was no female head of Van Region CV (1987-2006). This result may be a sign that it is not preferred for female veterinarian members to be the head of the chamber. However, other reasons may have also led to this result.

It can be said that social decisions taken in the chamber, such as visiting public institution chiefs after the elections and celebrating important days for veterinary medicine[43], contribute to the recognition and effectiveness of the veterinary profession in the society and the unity and solidarity of veterinarians. The events held on important days for the profession were also found in the rooms of Kars, Afyon and Aydın (Kızıltepe 2017, Türkmenoğlu 2019, Koç 2020).

It can be stated that Van Region CV's decisions to present a stethoscope to veterinary faculty students who are ranked (1998) and to provide financial support to veterinary student congresses held on various dates[44] are positive in terms of encouraging students. It has been reported that the Kars chamber also carries out various activities for students (Kızıltepe 2017).

Similar to other chambers (Sanal and Melikoğlu Gölcü 2014, Kızıltepe 2017, Türkmenoğlu 2019, Koç 2020), it can be argued that activities such as in-service training, vocational courses and conferences decided and organized by Van Region CV[45] contribute to the development of professional knowledge and skills of veterinarians.

It can be said that the participation of the Van Region CV in the inspections of veterinary clinics[46], as in the Kars chamber (Kızıltepe 2017), is important for animal and public health.

According to the relevant legislation, trying to publish professional publications is among the duties of the chambers[47]. Although the chambers of Kars (1989-2017) and Aydın (1994-2018) published chamber journal on various dates, it was determined that the their publication activities did not show continuity (Kızıltepe 2017, Koç 2020). It has been reported that the Afyonkarahisar Chamber has no publication activity (1968-2017) (Türkmenoğlu 2019). Similarly, in this study, it was found that Van Region CV had no publication activity (1987-2016)[48]. It can be said that this is a negative situation for the chamber.

Among the problems of the freelance veterinarians discussed at the 4th Veterinary Medicine Congress, there is also the sub-title of *'In the Fields Related to Professional Ethics, the Chamber Board of Directors and the Chamber Board of Disciplinary do not Work with, Sufficient Efficiency'* (Anon 2018). The disciplinary committee of Izmir CV, located in the Aegean Region, took 74 decisions (1992-2014) (Tong et al. 2014); Five disciplinary cases were found in Afyonkarahisar CV (1976-2017) (Türkmenoğlu 2019). It has been reported that the deontological-ethical rules on respect and competition for the veterinary profession in Turkey are mostly violated by self-employed veterinarians in the Eastern and Southeastern Anatolia Regions (Kızıltepe 2010). However, similar to the evaluation of one case in Kars Region CV (1989-2017) (Kızıltepe 2017), only one disciplinary case was found in Van Region CV[49]. This data may be indicate that the Van CV disciplinary committee has not been effectively operated for about the 30 years since its establishment. There may also be other reasons for this result.

As a result, beyond the shortcomings of Van Region CV such as the lack of an adequate and regular archive, the inactivity of the dignity council, and the lack of publication activity in its about the 30 years history (1987-2016); it can be claimed that it provides benefits to animal health, animal husbandry, vocational students, veterinarians and public health within the framework of the training programs, professional, scientific and social activities carried out on various dates.

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

Ethical Statement: This study is not subject to the permission of HADYEK in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees". In addition, the author declares that Research and Publication Ethics are observed.

[43]Footnote 17.
[44]Footnote 21-24.
[45]Footnote 26-32.
[46]Footnote 36,37.
[47]Footnote 1.
[48]Footnote 5.
[49]Footnote 39.

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RESEARCH ARTICLE

Protective Effect of Krill Oil Against Gentamicin Induced Oxidative Stress Mediated Nephrotoxicity in Rats

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ABSTRACT

This study aimed to evaluate the protective effect of krill oil against nephrotoxicity caused by gentamicin. Distilled water was given orally to the control and second groups (GI) for seven days while 500 mg/kg krill oil was given to the third (GII), fourth (GIII) groups. In addition, isotonic saline was administered subcutaneously to the control and GIII groups throughout the study, while 80 mg/kg gentamicin was administered to the GI, and GII groups. Alanine aminotransferase (ALT) and gamma glutamyltransferase (GGT) peptidase, total cholesterol, urea, and creatinine levels in plasma and, malondialdehyde (MDA) and total antioxidant status (TAS) levels in both plasma and kidney tissue supernatant were evaluated. Histopathological changes in tubules and glomeruli and vascular changes were evaluated by scoring. Urea level and ALT activity were found to be significantly lower in the GII and GIII groups compared to the GI group ($p<0.001$; $p\leq0.001$). As a result, it was observed that degenerative damage and glomerular changes in the tubule at the histological level mediated by oxidative stress were consistent with the increase in ALT, urea, and MDA levels. In this respect, it is suggested that krill oil can be used as a nephroprotective food supplement to contribute to treatment in cases of toxicity.
Keywords: Gentamicin, Kidney, Krill oil, Nephrotoxicity, Oxidative stress.

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Sıçanlarda Gentamisin ile İndüklenmiş Oksidatif Stres Aracılı Nefrotoksisiteye Karşı Krill Yağının Koruyucu Etkisi

ÖZ

Bu çalışmada, gentamisin'in neden olduğu nefrotoksisiteye karşı kril yağının koruyucu etkisinin değerlendirilmesi amaçlandı. Çalışmada yedi gün boyunca oral yolla kontrol ve ikinci grubuna (GI) distile su verilirken, üçüncü (GII) ve dördüncü (GIII) gruplarına 500mg/kg krill yağı verildi. Ayrıca çalışma boyunca subkutan yolla kontrol ve GIII gruplarına izotonik tuzlu su uygulanırken, GI ve GII gruplarına 80 mg/kg gentamisin uygulandı. Plazma alanin aminotransferaz (ALT) ve gama glutamiltransferaz (GGT), total kolesterol, üre ve kreatinin düzeylerine, hem plazma hem de böbrek doku süpernatından ise malondialdehit (MDA) ve total antioksidan kapasitesi (TAS) düzeylerine değerlendirildi. Histopatolojik olarak tubul ve glomeruluslardaki değişimler ile damarsal değişiklikler skorlanarak değerlendirildi. Üre düzeyi ve ALT aktivitesi GI gruba göre GII ve GIII verilen grupta anlamlı düzeyde düşük bulundu ($p<0.001$; $p\leq0.001$). Sonuç olarak, oksidatif stres aracılı olarak histolojik düzeyde tubulde dejeneratif hasar ve glomerular değişikliklerin özellikle ALT, üre ve MDA düzeyleri artışıyla uyumlu olduğu görüldü. Bu bakımdan, krill yağı nefroprotektif bir gıda takviyesi olarak toksisite durumlarında tedaviye katkı sağlamak için kullanılabileceği önerilmektedir.
Anahtar kelimeler: Böbrek, Gentamisin, Kril yağı, Nefrotoksisite, Oksidatif stres.

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INTRODUCTION

Aminoglycosides have been used for years in the treatment of diseases in veterinary and human medicine. These antibiotics are especially preferred to treat diseases caused by bacteria resistant to other antibiotics and gram-negative bacilli (Papich and Riviere 2018). Gentamicin, which is included in this class of antibiotics and is widely used in the treatment of important diseases, is one of the causes of drug-induced nephrotoxicity (Randjelovic et al. 2017). Oxidative stress has been recognized as an important contributing factor in some pathogenic processes that affect the kidneys. This leads to the possibility of using antioxidants to prevent nephrotoxicity (Sahnoun et al. 1997, Knight 1998, Acharya et al. 2013). It has been shown that gentamicin increases the formation of reactive oxygen species (ROS) such as hydroxyl radicals and hydrogen peroxides in the kidney cortex and reactive nitrogen species, which eventually lead to structural and functional deterioration in the kidney (Balakumar et al. 2010).

Krill are zooplankton-shaped crustaceans that are especially abundant in the North and South polar seas. Krill is a sustainable source of omega-3 polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) (Burri and Johnsen 2015). It also contains very effective antioxidants such as krill oil, vitamins (A and E), and astaxanthin (Cicero and Collett 2015). Krill oil causes an anti-inflammatory response due to omega-3 polyunsaturated fatty acids (EPA and DHA) that are found in its structure (Kwantes and Grundmann 2015). The consumption of these fatty acids has been reported to contribute to the treatment and prevention of diseases such as hypertension, diabetes, asthma, depression (Celebi et al. 2017).

Supplementary foods widely used in recent years; have been known to have a protective and preventive effect against most diseases (Halsted 2003, Özden et al. 2021). It is important to find the mechanism or substances that will reduce the nephrotoxicity caused by gentamicin. In this study; nephroprotective effects of krill oil against gentamicin-induced nephrotoxicity have been investigated by evaluating the damages caused by gentamicin after being metabolized and the protective effects of krill oil on the kidney damage were also determined.

MATERIAL AND METHOD

Animals

Twenty-four Sprague-Dawley (8 - 12 weeks old) male rats with an average weight of 180 - 220 grams were used in the study.

Animal Experimental Protocol

The animals used in this study were randomly selected and divided into 4 groups with 6 rats in each. The first group was the control group and was administered sterile distilled water orally and physiological isotonic saline subcutaneously for seven days. In the GI group, sterile distilled water was given orally and 80 mg/kg gentamicin (Gentavet %10®, Vetaş, Turkey) was administered subcutaneously for seven days (Matsushita et al. 2011, Dungca 2016).

The GII and GIII groups were given oral 500 mg/kg krill oil (Krilom® ultra krill oil, Tabilaç, Turkey) for seven days (Yeral et al. 2019). While 80 mg/kg gentamicin was administered subcutaneously to the GII for 7 days, physiological isotonic saline was administered subcutaneously to the GIII. 24 hours after the seventh-day applications; rats were anesthetized [10 mg/kg xylazine (Xylazinbio 2%®, Bioveta, Czech Republic), 90 mg/kg ketamine (Vetaketam®, Vetagro, Poland), intraperitoneal] blood was drawn from Vena cava caudalis. After blood collection, animals were sacrificed and kidney tissue was taken.

Sample Collection and Biochemical Analysis

Blood samples taken from rats were centrifuged at 3000 rpm for 10 minutes at 4 °C, and their plasma was separated. ALT and GGT activities, total cholesterol, urea, and creatinine (Abs, Turkey) levels in plasma were determined with commercial test kits in a spectrophotometer device (Shimadzu UV 1700, Japan).

Distilled water was used to remove the blood and similar residues from the kidney tissue and then washed with cold 0.9% NaCl and dried with gauze for the analysis of oxidative stress parameters. Dried tissues were wrapped in aluminum foils and stored at -80 °C. During preparation for the analysis, the kidney tissue was weighed approximately 0.5 g on a precision balance and 1/10 phosphate buffer (pH 7.4) was added. Firstly kidney tissues were divided into small pieces with a glass teflon homogenizer. Afterward, it was homogenized for 10 seconds on the ice, kept for 30 seconds, and homogenized using an ultrasonic homogenizer 5 times for a total of 50 seconds. Tubes containing homogenate were centrifuged at 13000 rpm for 10 minutes and the supernatants were obtained. MDA levels in plasma and tissue supernatant were determined by the method of Buege and Aust (1978) by measuring in a spectrophotometer device (Shimadzu UV 1700, Japan) at a wavelength of 536 nm. TAS levels in plasma and kidney tissue supernatant were determined with commercial test kits (Rel Assay Diagnostics, Turkey) with a microplate reader (Thermo Scientific™ Multiskan, UK) (Erel, 2005).

Pathological Examinations

Kidneys were examined according to macroscopic evaluation criteria dimension, shape, lesional

distribution, color, texture, and all tissue samples were fixed in 10% buffered formalin for 48 hours. After the fixation, the tissues were treated with graded ethanol and xylol series (Leica, TP1020, Germany) and blocked in paraffin (Leica 1150H, Germany). Five μm thickness sections were cut at rotary microtome (Shandon, AS320, UK). From paraffin blocks, sections were stained according to the hematoxylin-eosin (H&E) staining procedure (Luna 1968) and evaluated under a digital optical light microscope, and images were taken with a camera attachment (Olympus BX51digital microscope, DP25 camera attachment, Japan). For scoring histopathological findings, a number will be obtained by counting 10 fields at 400x magnification (10 HPFs). Counted fields were getting proportioned and stated as a percentage (%). According to density of findings, scores were semiquantitatively performed as (-): no finding 0-10%, (+): mild 10-30%, (++): moderate: 30-50%, (+++) strong: >50%.

Statistical Analysis

Statistical analyzes of the data were performed with the SPSS 25.0 package program (SPSS Inc., Chicago, USA). First of all, to determine the appropriate type of analysis, it was determined whether the data showed a normal distribution (Shapiro-Wilk test). Parametrically distributed parameters (parametric) were analyzed with a one-way ANOVA test, and Duncan's test was performed when F values were significant. In the statistical evaluation, the $p \leq 0.05$ level was accepted as an indicator of significant difference. Data were given as mean+standard error (X+SE).

RESULTS

Biochemical Parameters

Plasma biochemical parameters of the groups were given in Table 1. Plasma GGT activity was numerically decreased in the GII and GIII groups compared to the GI group, but there was no statistical difference ($p>0.05$). ALT activity was found to be significantly lower in the GII and GIII groups compared to the GI group ($p \leq 0.001$). Total cholesterol levels were found to be significantly higher in the GI and GII administered group compared to the control group ($p \leq 0.001$). Plasma urea levels were significantly decreased in the GII and GIII groups compared to the GI and control groups ($p<0.001$). Plasma creatinine levels were found to be increased in the GI and GII groups compared to the control group ($p<0.01$).

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Table 1. Some plasma biochemical parameter levels in rats applied with gentamicin and krill oil (n=6)

Parameters	Control group (C)	Gentamicin group (GI)	Gentamicin + Krill oil group (GII)	Krill oil group (GIII)	p
ALT (U/L)	62.37±4.97^{ab}	71.14±6.76^{a}	56.27±2.80^{b}	39.92±2.47^{c}	≤0.001
GGT (U/L)	6.87±1.35	8.22±0.70	7.65±0.67	7.43±0.58	>0.05
Total Cholesterol (mg/dl)	150.49±12.32^{b}	222.12±20.00^{a}	211.16±9.69^{a}	159.39±4.59^{b}	≤0.001
Urea (mg/dl)	41.29±0.54^{a}	42.01±0.43^{a}	38.94±0.57^{b}	36.62±0.65^{c}	<0.001
Creatinine (mg/dl)	0.51±0.12^{b}	1.03±0.11^{a}	0.99±0.021^{a}	0.76±0.05^{ab}	<0.01

^{a,b,c}: The difference between values with different letters on the same line is statistically significant ($p<0.01$; $p \leq 0.001$; $p<0.001$).

Oxidative Stress Parameters

It was determined that plasma MDA levels increased numerically in the GI group compared to the control group, but there was no statistically significant difference, while it decreased significantly in the GIII group ($p<0.05$). Plasma TAS levels decreased numerically in the GI group compared to the control group, but there was no statistically significant difference ($p>0.05$). Plasma MDA and TAS parameters of the groups are given in Table 2.

Table 2. Plasma MDA and TAS levels in rats applied with gentamicin and krill oil (n=6)

Parameters	Control group (C)	Gentamicin group (GI)	Gentamicin + Krill oil group (GII)	Krill oil group (GIII)	p
MDA (µmol/L)	1.20 ± 0.04[a]	1.27 ± 0.071[a]	1.13± 0.05[ab]	1.03 ±0.017[b]	<0.05
TAS (mmol/L)	0.74±0.08	0.45±0.11	0.61±0.07	0.69±0.07	>0.05

[a,b]: The difference between values with different letters on the same line is statistically significant (p<0.05).

The kidney tissue MDA and TAS parameters of the groups are given in Table 3. It was determined that kidney tissue MDA levels increased numerically in the GI and GII groups compared to the control group, but decreased numerically in the GIII group, but there was no statistically significant difference (p>0.05). There were numerical increases in renal tissue TAS levels in the other groups compared to the control group (p>0.05).

Macroscopical Findings

In GI group, kidneys were congested and swollen. In GII group, kidneys were mottled (integration of both partly hyperemic and pale areas) in appearance. In GIII group and control group, kidneys were normal in appearance at all animals.

Table 3. Renal tissue MDA and TAS levels in rats applied with gentamicin and krill oil (n=6)

Parameters	Control group (C)	Gentamicin group (GI)	Gentamicin + Krill oil group (GII)	Krill oil group (GIII)	p
MDA (µmol/g-wet tissue)	**6.02 ± 0.66**	**9.77 ± 3.45**	**7.06± 0.88**	**5.32 ±0.55**	**>0.05**
TAS (mmol/g wet tissue)	**1.11±0.21**	**1.25±0.09**	**1.36±0.07**	**1.44±0.18**	**>0.05**

Histopathological Findings

In the control (C) group, the only vasculature changes including hyperemic vessels were observed in a few areas in all cases. In GI group, degenerative and necrotic tubules were encountered in many fields. Tubule cells included cytoplasmic vacuolation, which was not stained. Some of the cells were karyopyknotic and/or karyolytic in appearance. Glomerular podocytes were hyperplastic and active. Bowmann capsules were filled and the glomerular bodies were enlarged in many fields in all cases of this group. There were a few macrophage and lymphocyte infiltration in a case. While there was no infiltration in other cases. Hyperemic vessels and vasculature changes including, the fullness of erythrocyte, vessel enlargement, and perivascular edema were common in every field in all cases. There were neither fibrotic changes nor inflammation apart from one case. The case was commented irrelatively from gentamicin toxicities.

In GII group, tubular degeneration was milder than GI group. There was no necrosis in tubule epitheliums apart from one case. The cases included necrosis in a few tubules in a restricted microscopical field. Glomerular reactions including podocyte activation, filling of Bowman capsule, etc. were milder. However, hyperemic vessels were common in every field as being in the previous GI group. Inflammation and fibrosis were not developed in any cases.

In GIII group, tubular degeneration was milder than being in the previous group. There were no necrotic changes in tubule epitheliums. Inflammation and fibrosis were not encountered in all cases. Glomerular reactions were almost the same as being GII group. Hyperemic vessels were common in five cases of this group. The vasculature reaction was milder when compared with previous groups. Semiquantitative scores in kidney histopathology according to experimental groups were illustrated in Table 4. Histopathological findings of experimental groups were illustrated in Figure 1.

Table 4. Semiquantitative scores in kidney histopathology according to experimental groups (n=6)

	Control group (C)	Gentamicin group (GI)	Gentamicin + Krill oil group (GII)	Krill oil group (GIII)
Glomerular reaction Podocytes	-(6/6)	+/++(1/6) ++(6/6)	-(2/6) -/+(2/6) +(2/6)	-(3/6) + (2/6) +/++(2/6)
Glomerular atrophic	-(6/6)	+(2/6) ++(4/6)	-(1/6) -/+(2/6) +(2/6) +/++(1/6)	-(3/6) -/+(3/6)
Glomerular Bowmann filling	-(6/6)	-(2/6) -/+(4/6)	-(4/6) -/+(1/6)	-(6/6)
Tubular degeneration	-(6/6)	+(1/6) ++(5/6)	-(1/6) -/+ (4/6) +/++(1/6)	-/+(6/6)
Tubulus necrosis	-(6/6)	-/+(1/6) +/++(1/6) ++(4/6)	-(5/6) -/+(1/6)	-(6/6)
Inflammatory cell infiltration	-(6/6)	-(5/6) -/+(1/6)	-(6/6)	-(6/6)
Hyperemia / Edema	+(6/6)	++(2/6) +++(4/6)	+/++(2/6) +++(4/6)	-(1/6) +(5/6)
Fibrosis	-(6/6)	-(6/6)	-(6/6)	-(6/6)

Mean for degeneration-necrosis and Podocyte activation. (-): no findings, (+): mild: in a few microscopic fields, (++): in many microscopic field, (+++): all microscopic fields for other findings including tubular and glomerular changes, hyperemia, inflammation, edema, fibrosis.

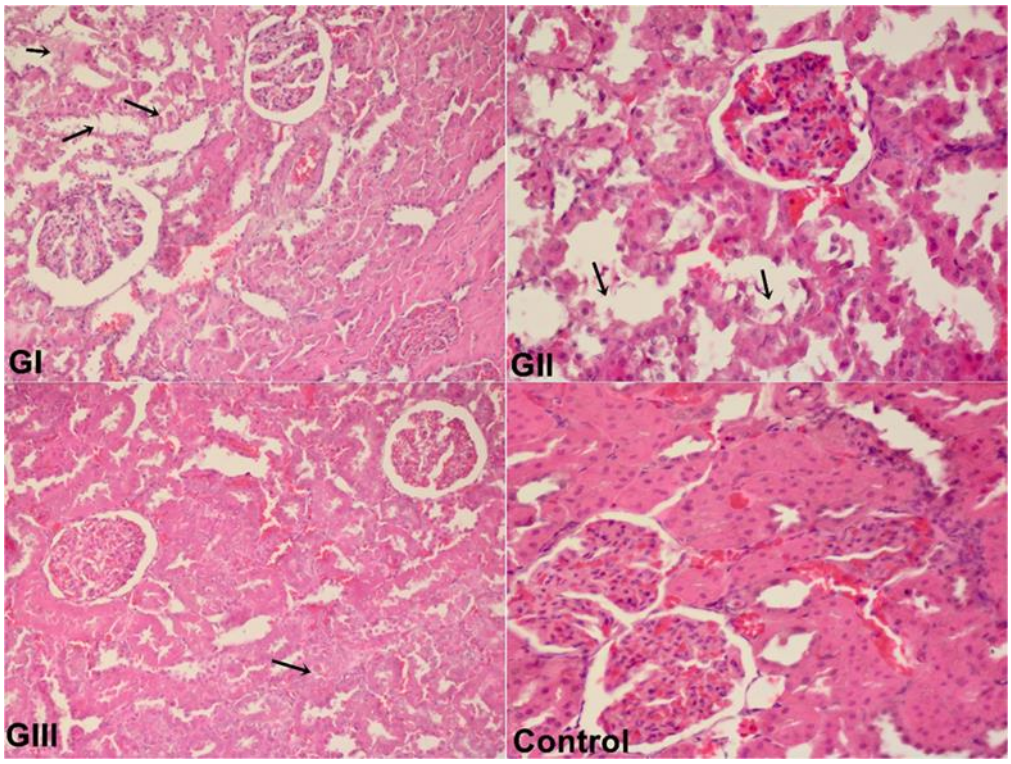

Figure 1: Histopathological findings of the experimental group. Degeneration in tubule epithelium (arrows). GI: Gentamicin group, GII: Gentamicin and krill oil group, GIII: Krill oil group, C: Control group, H&E, x400 magnification.

DISCUSSION

Gentamicin, an aminoglycoside antibiotic, is one of the most important antibiotics used to treat serious infections caused by gram-negative bacteria (Jiang et al. 217, Hayward et al. 2018). Especially due to the increase in the clinical use of these antibiotics, toxic conditions such as nephrotoxicity occur (Vargo and Edwards 2014). To significantly reduce this toxic effect of gentamicin, the use of various substances or agents is required (Cuzzocrea 2002, Khan et al. 2009, Quiros et al. 2011). Therefore the effect of krill oil, a food supplement, against kidney damage caused by gentamicin was investigated.

Many animal studies have been conducted with Krill oil in terms of these beneficial properties. Studies with krill oil have indicated therapeutic beneficial effects in cardiac infarction, obesity, depression, chronic low-grade, and ulcerative inflammation. It has been reported that LC n3 PUFA in krill oil regulates inflammatory C-reactive protein and cytokines levels, and decreases plasma triglycerides with phospholipids (Hung et al. 2001, Buang et al. 2005, Winther et al. 2011, Schneider et al. 2010, Leslie et al. 2015). It also contains astaxanthin, which is an antioxidant and a fat-soluble carotenoid (Kwantes and Grundmann 2015). In this study, it was observed that gentamicin regressed the damage in the kidney tissue, thus these results support the hypothesis of the presented study.

Ramasamy et al. (2017) investigated the protective effect of soybean oil against gentamicin (80 mg/kg) induced nephrotoxicity in a study on rats. In their study, they reported that urea and creatine levels increased in the gentamicin applied group, whereas urea and creatine levels decreased in the soybean oil and gentamicin given groups. They stated that the decrease in urea and creatine levels was due to the antioxidant effect of soybean oil. Similarly, in this study, it was found that krill oil reduced the increase in urea and creatine levels caused by gentamicin. The increase in urea and creatinine levels in the group given gentamicin is probably due to the decrease in glomerular filtration due to degenerations in the kidney (Hur et al. 2013).

Ataman et al. (2018) investigated the effect of fucoidan against gentamicin-induced nephrotoxicity on rats and reported that total cholesterol and ALT levels increased in the gentamicin given group, whereas total cholesterol and ALT levels decreased in the fucoidan and gentamicin given groups. Consistent with the aforementioned study, the presented study found that krill oil reduced the elevation in total cholesterol and ALT levels caused by gentamicin.

It is known that gentamicin plays a significant role in kidney damage by increasing ROS production (Said 2011, Tavafi and Ahmadvand 2011). Sandhya and Varalaxmi (1997) found a significant reduction in kidney GPx, GSH, SOD, and catalase activities in rats administered 100 mg/kg gentamicin. In addition, they also observed an increase in the production of MDA, the end product of lipid peroxidation, in the kidney. In the presented study, MDA levels increased in the kidney, but it was not found to be statistically significant.

In this study, the plasma TAS level was decreased consistently with the study of Acharya et al. (2013) who showed that serum TAS levels decreased in rats administered 70 mg/kg (i.m) gentamicin. TAS provides a guideline for an individual's ability to resist oxidative stress.

Although the mechanism of gentamicin-induced nephrotoxicity cannot be explained, several researchers have reported that damage directly caused by Gentamicin is a class of drugs that can cause the formation of ROS. MDA causes a decrease in the polyunsaturated fatty acids, which act as a substrate for free radicals.

Many studies have confirmed a link between oxidative stress and nephrotoxicity. It has been reported that deterioration in renal function is accompanied by either an increase in creatinine and urea levels or an increase in renal tissue MDA levels, which indicates lipid peroxidation (Cuzzocrea et al. 2002, Atessahin et al. 2003). Many mechanisms have been proposed to explain gentamicin toxicity, it has been suggested that nephrotoxicity causes oxidative stress and therefore antioxidant therapy is required to prevent it (Cuzzocrea et al. 2002, Atessahin et al. 2003, Du and Yang 1994, Walker et al. 1999). Ulutaş et al. (2006) investigated the effect of allopurinol on gentamicin-induced nephrotoxicity in rats. They found that gentamicin increased MDA levels in plasma. In this study, it was found that krill oil numerically reduced the increase in plasma MDA level caused by gentamicin.

Karakoyun et al. (2009) reported that gentamicin (80 mg/kg) increased the MDA level in rat kidney tissue and halofuginone reduced this increase. The presented study is consistent with the aforementioned study, and it was found that krill oil numerically reduced the increase in MDA level in kidney tissue. A study on rats investigated the antioxidant effect of date fruit extract against gentamicin-induced nephrotoxicity. In this study, they reported that the TAS level that gentamicin decreased in the kidney tissue, increased the date extract (Celik and Irak 2018).

Krill oil contains a very powerful natural antioxidant called astaxanthin, which gives it its red color. Studies on astaxanthin in previous years have been examined and it has been proven that astaxanthin has many

beneficial biological effects, including suppression of carcinogenesis in some cancer types such as bladder and colon cancer, prevention of cardiovascular diseases, protection against free radicals, strengthening and modulation of the immunological system (Pashkow et al. 2008, Higuera-Ciapara et al. 2006, Tripathi and Jena 2010).

The histopathological results of this study support the biochemical findings. Podocyte reactions and glomerular atrophy in the glomerulus against toxicity were evident in almost all areas in the gentamicin administered group alone, even if they were moderately severe. In some cases, it was observed that the glomerulus filled Bowman's space, albeit slightly. Another reflection of kidney damage is moderate degenerative and necrotic damage to the tubular epithelium in the cortical region in almost every case. It is thought that these develop after the severe vascular damage observed in each case in the study. Catabolic products and free radicals may cause stress as a result of the lack of oxygenation in the region. Because gentamicin causes cytotoxicity in the cells of the tissues where it is metabolized (Hung et al 2001, Schneider et al 2010).

Gentamicin is taken into the cytosol by endosomes and when it reaches a certain level, the structure of the cell membrane is deformed and gentamicin disperses into the cytoplasm and then goes to the mitochondria (Regec et al. 1989, Morales et al. 2010). It prevents energy production by disrupting oxidative respiration in mitochondria. Thus, free radicals gradually accumulate in the environment and initiate apoptotic mechanisms, leading the cell to degeneration and then to necrosis (Cuzzocrea et al. 2002, Peyrou et al. 2007).

High doses of gentamicin injection induce the reaction of the Bowman's capsule of the glomerulus (enlargement, necrosis of the epithelium lining the capsule, and lysis of the capsule) and cause a change in the level of glomerular filtration. Alterations are characterized by cell death, especially in the proximal tubular epithelium. As a result of these, absorption and secretion disorders begin in the filtration of urine (Regec et al. 1989, Peyrou et al. 2007, Li et al. 2009, Fauzi et al. 2020).

Fauzi et al. (2020) mentioned that inflammatory changes increase with the increase in the dose (20-50 mg/kg/CA), especially around the proximal tubules, in the kidneys of animals to which gentamicin is administered. In this study, glomerular reactions were noted, but no further changes such as lysis or necrosis in the glomeruli were encountered. The information that the proximal tubule epithelium in the cortex is affected by the tubules is overlapping with our study. However, inflammatory changes reported by Fauzi et al. (2020) were noted in a few foci in only one case. In our study, 80mg/kg dose of gentamicin was applied. In this regard, the effects of the reported doses and the gentamicin dose of our study on the inflammatory response seem to be independent of each other. According to the literature review, since the inhibitory effects of nephrotoxicity with Krill oil were not examined, it was concluded that this study was the first evaluation. In this study, it was observed that krill oil reduced the negative effects of glomerulus and tubules induced by gentamicin and it remained at a low level in scoring because the lesions were not seen in all areas. However, there is no literature on the subject that glomerular podocyte activation and mild atrophic changes may occur in half of the cases when krill oil is applied alone.

In our study, vascular changes related to hyperemia were noted in all three experimental groups except for the control group. In this context, it is seen that when krill oil is applied alone, it has a slightly hyperemic effect on the glomerular capillaries and interstitial capillaries. These hyperemic changes were observed to be more severe in combined applications with gentamicin, on the other hand, it was observed that in some cases it was milder than when gentamicin was administered alone. It is thought that krill oil reduces oxidative stress and increases a load of erythrocytes, possibly by triggering vascular mediators in the vessels to provide oxygenation to the region. This may be due to the possibility of stopping the damage in the oxidative respiration cascade formed in the mitochondria and protecting the tissue integrity in this way. It was concluded that more detailed research should be done specifically on the subject.

CONCLUSION

As a result, it was observed that damage and glomerular reactions in the tubule at the histological level mediated by oxidative stress were especially consistent with the increase in plasma MDA, ALT, creatinine, and urea levels. In this respect, krill oil is an effective biological product to prevent damage to kidney tissue and protect kidney tissue. In this, krill oil can be a nephroprotective food supplement that may contribute to the treatment of toxicities.

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

Ethical Approval: The study was carried out after the animal experiment approval of Kırıkkale University Local Ethics Committee (Decision number: 2020/06 - 43).

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RESEARCH ARTICLE

Structural and Technical Characteristics of Purebred Kıvırcık Sheep Enterprises in Kırklareli Province

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ABSTRACT

It was aimed to determine the current general structure of purebred Kıvırcık sheep enterprises, main issues of breeders, and their expectations from the authorities. A questionnaire was applied to farmers in 47 enterprises regarding sheep breeding and various measurements/observations were conducted in the sheepfolds. It has been determined that most of the breeders had a near-lifetime sheep breeding experience and many family members contribute to the breeding. The most commonly used roughage was dry pasture grass and straw, and factory feed and wheat were mostly preferred as concentrate feed. As a result of the study, the amount of area per mother sheep (0.704) and rootstock sheep + one lamb (1.260) was found to be insufficient. Most reported diseases were Bluetongue (45.65%), respiratory system diseases (30.43%) and enterotoxaemia (10.87%) for sheep, and diarrhea/digestive system (45.65%) and respiratory system diseases (36.96%) for lambs. While nearly half of the breeders stated that they were satisfied with sheep farming, the vast majority (81%) stated that they would continue to breed. Most important problems stated by the farmers were feed prices, low product prices, diseases/deaths, finding a shepherd, lack of organization. Most important expectations from authorities were stated as solutions for health issues, marketing problems, increasing product prices and financial support.

Keywords: Breeder expectations, Kırklareli, sheep breeding, structural characteristics

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Kırklareli'nde Saf Kıvırcık Irkı Koyun Yetiştiriciliği Yapan İşletmelerin Yapısal Ve Teknik Özellikleri

ÖZ

Yürütülen çalışmada saf Kıvırcık koyun işletmelerinin mevcut genel yapısının, yetiştiricilerin temel sorunlarının ve yetkililerden beklentilerinin belirlenmesi amaçlanmıştır. 47 çiftlikte koyun yetiştiriciliği ile ilgili anket uygulanmış ve ağıllarda çeşitli ölçümler/gözlemler yapılmıştır. Yetiştiricilerin çoğunun yaşam boyu koyun yetiştirme tecrübesine sahip olduğu ve birçok aile üyesinin koyun yetiştiriciliğine katkıda bulunduğu tespit edilmiştir. En sık kullanılan kaba yem kuru mera otu ve saman olup, kesif yem olarak daha çok fabrika yemi ve buğday tercih edildiği gözlenmiştir. Çalışma sonucunda belirlenen anaç koyun (0.704) ve anaç koyun + bir kuzu (1.260) başına düşen alanı miktarları yetersiz bulunmuştur. Yetiştiriciler tarafından en sık bildirilen hastalıklar koyunlar için Mavi dil (%45.65), solunum sistemi hastalıkları (%30.43) ve enterotoksemi (%10.87) ve kuzular için ishal/sindirim sistemi (%45.65) ve solunum sistemi hastalıkları (%36.96) olmuştur. Yetiştiricilerin yaklaşık yarısı koyun yetiştiriciliğinden memnun olduklarını belirtirken, büyük çoğunluğu (%81'i) yetiştiricilik yapmaya devam edeceklerini beyan etmiştir. Yetiştiriciler karşılaştıkları en önemli sorunları yem fiyatlarının yüksek, ürün fiyatlarının ise düşük olması, hastalıklar/ölümler, çoban bulamama ve örgütlenme eksikliği olarak belirtmişlerdir. Yetiştiriciler, yetkililerin çözüm getirmesine ihtiyaç duydukları en önemli konuların; sağlık sorunlarının çözümü, pazar sorununun çözümü, ürün fiyatlarının ve desteklerin artırılması şeklinde ifade etmişlerdir.

Anahtar kelimeler: Kırklareli, koyun yetiştiriciliği, yapısal özellikler, yetiştirici beklentileri

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INTRODUCTION

Sheep breeding in Turkey is generally conducted extensively by villagers, in the form of traditional small family businesses, and largely based on natural pastures in which the nutritional needs of animals are often not adequately met. Additionally, many diseases such as brucellosis, foot-and-mouth disease, ecthyma, enterotoxaemia also cause significant losses in sheep breeding. Therefore, the maintenance, feeding and housing conditions that are widely applied in the country result in low productivity and producers cannot earn a sufficient income (Anonymous 1986). Factors such as high feed costs, extreme fluctuations in lamb and milk prices, inadequacy of subsidies and supports, inability of breeders to market their products at affordable prices, and difficulties in obtaining shepherds/workers negatively affect the sustainability of sheep breeding, especially in recent years. In this context, many breeders gave up sheep breeding and migrated to big cities; and this reduced the potential for animal food production. However, in Turkey, which has a rapidly increasing population, the need for animal-based foods is increasing day by day and meat imports are preferred because of insufficient domestic production. This framework indicates that to prevent the transitioning of sheep breeders from a producer position to a consumer position, various socio-economic conditions should be provided to have a sustainable sheep breeding in their villages. Therefore, primarily, it is necessary to examine the existing structural features of sheep breeding deeply, identify problems and develop suggestions for the solution of these problems.

Kırklareli province offers animal breeders the opportunity to graze their animals in the pasture for most of the year with its rich forest and pasture opportunities. For this reason, sheep breeders prefer to use natural food sources for their animals as long as possible. In seasons when climate and pasture conditions are suitable, lambs are grazed together with their mothers in pasture and are tried reaching slaughter maturity without extra feeding. Kıvırcık breed, which is widely grown in Bulgaria, Greece, and in Turkey (mostly in Marmara Region (especially in Thrace, Bursa, Balıkesir and Çanakkale) and some provinces of the Aegean Region (Manisa, İzmir and Aydın)), stands out with its thin tail and tasty meat. It is a sheep breed that is well adapted to the climatic conditions of the aforementioned region (Ekiz et al. 2009, Ekiz et al. 2021).

The Istranca mountains, which cover a significant part of Kırklareli, and the rich plant diversity in the region contribute to the Kıvırcık breed having more delicious meat compared to other indigenous sheep breeds. Although the Kıvırcık breed is at the forefront with its superior meat quality, the milk obtained from the Kıvırcık sheep is marketed to many large milk and dairy products processors in Thrace and is converted into many products, especially white cheese and sheep yogurt. In the past years, it was aimed to increase milk and fertility of Kıvırcık and by crossing some culture breeds, Türkgeldi and Tahirova genotypes were created. The dissemination of these genotypes in public herds turned into uncontrolled crossbreeding over time and a great decrease was observed in the pure Kıvırcık population. However, the purest specimens of the Kıvırcık breed continue to exist in Kırklareli, because the animal circulation is lower compared to other provinces in the Marmara region and that breeders prefer to breed pure Kıvırcık instead of crossbreeding. The Kıvırcık breed has been taken under protection by the public, by the project of "Breeding of the Kıvırcık breed in the hands of the people" carried out by the Ministry of Agriculture and Forestry.

Many survey studies have been conducted to determine the socio-economic, structural, and technical characteristics of sheep breeding enterprises in different provinces of Turkey and to determine the problems of sheep breeding (Ayvazoğlu Demir et al. 2015, Bilginturan and Ayhan 2009, Bostancı 2006, Ceyhan et al. 2015, Dellal et al. 2002, Gezer 2010, Kandemir et al. 2015, Karakuş and Akkol 2013, Koyuncu et al. 2006, Tüfekçi and Oflaz 2015). However, no research has determined the structural characteristics and problems of sheep breeding in Kırklareli. In this study, it was aimed to reveal the general characteristics of the pure Kıvırcık sheep breeding enterprises in the villages of Kırklareli city centre, herd management practices, the current situation of the sheep shelters, the main problems of the breeders and their expectations from the authorities.

MATERIAL AND METHODS

The data used in the study were obtained from 47 purebred Kıvırcık farms in Kırklareli. Recently, because of uncontrolled crossing studies, the number of pure Kıvırcık farms has decreased considerably. In the study, all sheep farms raising pure Kıvırcık sheep in the villages of Kırklareli city centre were determined as the target population. In this context, all enterprises involved in the "Breeding of the Kıvırcık breed in the hands of the public" project conducted by the Ministry of Agriculture and Forestry, and one enterprise that took part in the "On-Site Protection and Development of Pet Genetic Resources National Project" was visited.

Additionally, Kırklareli Sheep and Goat Breeders' Association visited other businesses that stated to breed pure Kıvırcık, and 5 businesses outside the scope of the above-mentioned Ministry of Agriculture and Forestry projects were included in the research. The study was conducted in April-May 2015. The questions asked by the researchers in the

face-to-face interviews with the sheep breeders and the data gathered during the observations and measurements made in the shelters are given below:

A. Demographic information of breeders: Age of the breeder, number of households, experience in sheep farming, education level, source and number of the shepherds

B. General characteristics of the businesses: Business structure, barn ownership, agricultural and livestock activities performed in the enterprise

C. Herd composition and size: Number of sheep and rams in the flock, number of yearlings, number of sheep per ram

D. General feeding schedule and pasture usage: Which months of the year the sheep and the rams are taken to pasture, which months of the year the sheep and the rams are fed in the barn, which forages and concentrates are used, where the forages and concentrates are obtained from.

E. Shelter characteristics and conditions: Shelter structure type, shelter age, shelter location, wall, roof and floor materials, ventilation type, shelter dimensions, presence of sections inside the barn (birth chambers, lamb compartments, breeding herd section, yard usage), floor area per animal and per sheep in the barn.

F. Health/liveability problems, herd health management and shelter hygiene practices: Diseases seen in the enterprise last year, frequency and causes of aborts, number of lambs died until weaning, lamb death causes, usage of internal/external parasitic pesticides, body and foot bath use, barn disinfection, lameness frequency in the herd, frequency of hoof care and control, frequency of mastitis in the herd, management of placenta and umbilical cord care, wild animal attack status, records kept by breeders.

G. Opinions of breeders about sheep breeding: Reasons for breeders to continue sheep breeding, Change in the amount of sheep in the last five years, level of satisfaction with sheep farming, the most important problems of the breeders and their expectations from the authorities, personal future predictions about sheep farming.

Statistical analysis

Data collected from Kıvırcık sheep breeders in Kırklareli through a questionnaire were arranged in the Microsoft Excel program, and SPSS 13.0 program was used for descriptive statistical analysis of the gathered data. The findings obtained in the study were presented as "the frequency and rate of observation" or "mean value and standard deviation".

RESULTS

The general characteristics of the breeders and the sheep farms are presented in Tables 1 and 2. It was observed that the majority of breeders (53.19%) are between the ages of 41-60, the average age of the breeders was 45, the average experience in sheep breeding was 39.18 years, and more than half of them were primary school graduates. While most of the breeders in the study stated that family members (44.68%) were working as shepherds, also, it was seen that the second most common shepherd practice was permanent workers (21.28%) employed in addition to family members. It has been reported that mostly 2 to 4 shepherds are used in the farms investigated in the study.

Table 1. General characteristics of the surveyed breeders.

Traits	N	%
Age of the breeder	47	
20-40	15	31.92
41-60	25	53.19
60+	7	14.89
Number of households	47	
2	4	8.51
3	5	10.64
4	10	21.28
5	11	23.40
6	7	14.89
7+	10	21.28
Years of experience in sheep farming	47	
<10	7	14.89
11-20	4	8.51
21-30	11	23.40
31-40	13	27.66
41+	12	25.53
Level of education	47	
Literate	1	2.13
Primary school	28	59.57
Secondary school	5	10.64
High school	9	19.15
Bachelor and above	4	8.51
Shepherd source	47	
Family only	21	44.68
Family + permanent worker	10	21.28
Family + hired / seasonal worker	6	12.77
External permanent worker only	9	19.15
Permanent worker + seasonal worker	1	2.13
Number of shepherds	47	
1	4	8.51
2	17	36.17
3	9	19.15
4	10	21.28
5	2	4.26
6+	5	10.64

Table 2. General characteristics of the surveyed sheep farms

Shelter Features	N	%
Enterprise structure	47	
Small family business in/near the village	29	61.70
Small family business in the forest	13	27.66
Large-scale enterprises	5	10.64
Barn property	47	
Owned	42	89.36
In partnership	0	0.00
Rented	5	10.64
Plant production status	47	
No	8	17.02
Yes	39	82.98
Grown plants*	47	
Wheat	35	39.74
Barley	20	51.28
Triticale	18	46.15
Corn	15	38.46
Oat	13	33.33
Sunflower	8	20.51
Rye	7	17.95
Vetch	7	17.95
Clover	1	2.56
Other livestock activities	47	
Does not breed other animal species	8	17.02
Breeds other animal species	39	82.98
Other animal species *,#	39	
Beef cattle	3	7.69
Dairy cattle	27	69.23
Water Buffalo	2	5.13
Goat	37	94.87

* The surveyed breeders were given the opportunity to specify more than one choice.
# It refers to other animal species bred by 39 enterprises that breeds other animals.

61.70 % of the 47 businesses in the project were small family businesses in/around the village, 27.66% were small family businesses in the forest, and 10.64% were large-scale enterprises. The average number of Kıvırcık sheep in the farms included in the study was 287.94, the number of Kıvırcık rams in the stock herd was 10.26, the number of rams used for the first time in breeding was 2.38, and the number of sheep per ram was 31.75 (not presented in the tables). It has been stated that 89.36% of the barn ownership belongs to the breeders themselves, most the breeders also produce crops in addition to sheep breeding, and the most planted products were wheat, barley, corn, triticale, sunflower and oats. It has been observed that approximately 83% of the breeders raise other animals besides sheep. It was seen that goats (94.87%) were the most common species bred as a secondary animal in Kıvırcık herds in Kırklareli, followed by dairy cattle farming (69.23%).

Table 3. The duration of pasture usage in the surveyed farms.

Pasture usage features	Mean	SD
The number of months in which the ewe sheep are taken to pasture	11.36	1.39
Number of months in which ewe sheep are fed only based on pasture	7.22	1.69
Number of months in which the rams were taken to pasture	10.05	2.72
Time spent on pasture in winter, hours/day	6.59	1.25
Time spent on pasture in summer, hours/day	12.03	1.88

Table 4. The roughage and concentrate feeds used in the farms and their supply types.

Feeds	N	%
Forages*	47	
Straw	42	89.36
Dry clover grass	10	21.28
Dry pasture grass	44	93.62
Corn silage	19	40.43
Beet pulp	19	40.43
Vetch	6	12.77
Concentrates*	47	
Factory feed	41	87.23
Barley	38	80.85
Wheat	40	85.11
Sunflower	1	2.13
Rye	12	25.53
Oat	7	14.89
Corn	5	10.64
Triticale	21	44.68
Roughage supply method	47	
From the farm itself	16	34.04
Bought	2	4.26
Both	29	61.70
Concentrate feed supply method	47	
From the farm itself	4	8.51
Bought	21	44.68
Both	22	46.81

*Surveyed breeders were given the opportunity to specify more than one choice

It was observed that the stock sheep herd was taken to pasture almost throughout the year. While sheep are fed only on pasture for an average of 7.22 months, rams are taken to pasture for 10-11 months. From the statements of the breeders, it was concluded that sheep graze in the pasture for about 6 hours a day during the winter months, and about 12 hours in the summer months (Table 3).

The most commonly used roughage sources in the enterprises were determined as dry pasture grass (93.62%) and straw (89.36%). It has been observed that dry alfalfa, corn silage, beet pulp and vetch are other roughage sources used in the region. The most preferred concentrate feed source was found as commercial factory feed (87.23%), which followed by wheat and barley. In 34.04% of the enterprises, the roughage is obtained entirely from the internal resources of the enterprise; while approximately 62% of them provided it both from internal sources and purchased from outside. In 44.68% of the enterprises, concentrate feed is supplied only from feed factories; while in 46.81%, it is provided from both the internal resources of the enterprise and the feed factories (Table 4).

Table 5. Shelter characteristics of the surveyed sheep farms

Barn features	n**	%
Barn type	46	
Closed barn	40	86.96
Shed	1	2.17
Closed barn + shed	5	10.87
Barn age	46	
0-5 years	3	6.52
6-10 years	15	32.61
11-20 years	12	26.09
21-30 years	8	17.39
31 years and above	8	17.39
Barn Location	46	
Underneath the house	1	2.17
Adjacent to the house	10	21.74
In a separate area within the village	11	23.91
Outside the village	11	23.91
In the forest	13	28.26
Shelter Wall Material *	46	
Brick	13	28.26
Briquette	18	39.13
Concrete	5	10.87
Stone	4	8.70
Wood or wood + nylon	14	30.43
Shelter Roof Material *	46	
Tile	16	34.78
Clay	2	4.35
Concrete	2	4.35
Tin	6	13.04
Fibrous cement (Eternit) / shingle	12	26.09
Wood or wood + nylon	14	30.43
Shelter Floor Material	46	
Soil	42	91.30
Concrete	2	4.35
Some parts are soil, some are concrete.	2	4.35
Ventilation type	46	
No ventilation	10	21.74
Only windows	3	6.52
Only chimney	15	32.61
Windows and chimneys	18	39.13
Barn with a maternity pen	6	13.04
Barn with a lamb growing pen	23	50.00
Floor area per animal (ewe and lamb) in the barn, (m^2/animal)	0.704	0.323
Floor area per ewe in the barn, (m^2/ewe)	1.260	0.534

* *Surveyed breeders were given the opportunity to specify more than one choice
** In a farm where the survey was applied, this parts of the survey could not be done.

It was determined that 86.96% of the examined enterprises were closed-type barns, while 10.87% consisted of covered barn + shed. The most common shelter age group was determined as "6-10 years" (32.61 %). Additionally, it was observed that a significant part of the enterprises preferred the shelters (28.26%) built in the forest. Notably, 21.74% of the enterprises do not have any ventilation system.

It was observed that about half of the examined farms had a separate lamb rearing section, but only 13.04% of the farms had a maternity pen. The floor area per animal (for ewe + lamb) in the barn was calculated as 0.704 m² and per ewe is determined as 1,260 m² (Table 5).

Table 6. Health parameters of Kivircik farms

Parameters	**n****	**%**
Diseases seen in the last 1 year *	46	
Respiratory system diseases	14	30.43
Enterotoxaemia	5	10.87
Brucellosis	3	6.52
Blue tongue	21	45.65
Ecthyma	4	8.70
Parasitic diseases	2	4.35
None	13	28.26
Amount of abort observed farms	44	95.65
	Mean	**SD**
Number of aborted sheep	16.13	38.16
Percentage of sheep that abort within the farm, %	4.59	5.20
Number of lambs died until weaning	21.80	41.05
Percentage of lambs died until weaning, %	9.46	7.45
Number of lambs died after weaning	0.28	1.03
Percentage of lambs died after weaning, %	0.28	1.03
	n	**%**
Cause of lamb deaths according to breeders *	46	
Diarrhoea / digestive system diseases	21	45.65
Respiratory system diseases	17	36.96
High twinning	7	15.22
Crush / jamming	2	4.35
Bad motherhood / Yearling ewes not taking care of their offspring	1	2.17
Dystocia	1	2.17
Feeding / feeding error	2	4.35
Negligence / Poor management	1	2.17

* Surveyed breeders were given the opportunity to specify more than one choice.
** In a farm where the survey was applied, parts of the survey related to health parameters could not be done.

The diseases, abort rates and causes of lamb deaths observed in the last 1 year, according to the breeder statements are presented in Table 6. It was stated that the most common disease in the herds was "blue tongue", (45.65 %) which was followed by respiratory system diseases (30.43%) and enterotoxaemia (10.87%), and abort was observed in almost all the enterprises (95.65%). Because of the calculations made according to the breeder statements, it was estimated that 4.59% of the sheep in the farms aborted. It has been reported that an average of 21.80 lambs (approximately 9.46% of lambs born) died per farm until weaning. It was determined that lamb deaths after weaning were quite low. The breeders stated i) diarrhea/digestive system diseases (45.65%) and ii) respiratory system diseases (36.96%) as the two most important causes of lamb death. Additionally, high twinning and crushing/squeezing of lambs were also listed as other important causes of lamb death.

Table 7. Some biosecurity practices in the surveyed sheep farms

Traits	n**	%
Number of businesses applying parasitic pesticides	45	97.83
Number of parasitic pesticides applied per year	45	
1	9	20.00
2	32	71.11
3-4	4	8.89
Percentage of businesses applying a bath	5	10.87
Percentage of businesses applying a foot bath	4	8.70
Percentage of businesses performs hoof control	9	19.57
Percentage of businesses applying disinfection inside the barn	43	93.48
Substance used for disinfection	43	
Caustic lime	35	81.40
Chemical disinfectants	0	0.00
Caustic lime + Chemical disinfectants	8	18.60
The frequency of disinfection	43	
Monthly or more frequent	17	39.53
Every 3-4 months	8	18.60
Every 6 months	14	32.56
Once in a year	4	9.30
Method of intervention in the placentas*	46	
Buried	3	6.52
Given to dogs	40	86.96
Thrown away to garbage	8	17.39
Does any umbilical cord care performed?	46	
Yes	28	60.87
No	18	39.13
Have there been any wild animal attacks in the last 1 year?	46	
Yes	35	76.09
No	11	23.91

* Surveyed breeders were given the opportunity to specify more than one choice.
** In a farm where the survey was applied, parts of the survey related to health parameters could not be done.

Almost all the breeders reported that they applied parasitic pesticides to sheep (97.83 %). It has been declared that 71.11% of the enterprises is applied twice a year with parasitic pesticides. It was determined that the rate of enterprises that bathed the sheep (10.87%) and applied footbath (8.70%) were quite low. Similarly, it was determined that the hoof control of sheep was conducted in few enterprises. It was stated that 93.48% of the enterprises disinfect inside the barn at varying times during the year (Table 7).

It is seen that in approximately 90% of the surveyed enterprises, placentas are fed to dogs. Throwing into garbage or burying the neonatal membranes are less common methods. It has been reported that umbilical cord care of lambs after birth is performed in 60.87% of the enterprises. Respectively, 76.09% of these enterprises stated that they have faced a wild animal attack at least once in the last year (Table 7).

Table 8. The records kept in the farms and the lameness and mastitis status of the farms according to the breeders

Traits	n**	%
Records kept in the farms*	47	
None	5	10.64
Only the records for Ministry of Agriculture and Forestry projects	35	74.47
Disease, treatment, drugs, vaccinations etc.	4	8.51
Death records of animals and cause of deaths	6	12.77
Feed consumption	3	6.38
Date of rams joining to the ewe herd	4	8.51
Percentage of breeders indicating lameness in their flock	38	82.61
Percentage of breeders indicating mastitis in their flock	35	76.09
Percentage of businesses performing mastitis treatment ***	31	88.57
	Mean	**SD**
Percentage of lame animals in their flock according to the breeders	6.67	10.67
Percentage of animals with mastitis in their flock according to the breeders	1.31	1.44

** Surveyed breeders were given the opportunity to specify more than one choice.
** In a farm where the survey was applied, parts of the survey related to health parameters could not be done.
*** Calculated on the basis of businesses stating that mastitis is observed.

Table 9. Evaluations of the sheep breeders who were surveyed about their own businesses.

Reviews of sheep breeders	N	%
Reason for sheep farming	47	
Main occupation	44	93.62
Additional income	3	6.38
Satisfaction level with sheep breeding	47	
Satisfied	20	42.55
Partly satisfied	14	29.79
Not satisfied	13	27.66
Change in the number of sheep in the last 5 years	47	
İncreased	22	46.81
Decreased	7	14.89
Unchanged	18	38.30
Personal vision of future for sheep farming*	47	
Planning to continue sheep breeding	38	80.85
Thinking his/her children will not continue to sheep breeding	16	34.04
Thinking that he/she will quit sheep breeding in a short time	9	19.15

* Surveyed breeders were given the opportunity to specify more than one choice.

It is seen that most of the breeders do not keep records, except for the records kept within the scope of the "Breeding of the Kıvırcık breed in the hands of the public" project in most of the enterprises. The 82.61% of the breeders stated that lameness was observed in their herds; additionally, 76.09% of them stated that mastitis was observed. In light of the information given by the breeders, it is understood that 6.67% of the animals in their herds are lame and 1.31% have mastitis (Table 8).

It was stated that sheep breeding is the main occupation in 93.62% of the surveyed enterprises. 42.55% of the farmers reported that they were satisfied with the sheep breeding, while 27.66% of them reported that they were not. 46.81% of the breeders stated that the number of sheep in their herd has increased in the last five years and 80.85% of the farmers declared that they will continue to breed sheep. However, 34.04% of the breeders stated that they thought that their children would not breed sheep (Table 9).

The most important 5 problems stated by the farmers who bred Kıvırcık are listed as feed prices > low marketing/product prices > animal diseases, deaths > not being able to find a shepherd > organization. However, 31.91% of the breeders stated that they did not have any expectations from the authorities.

The most important expectations of the breeders; i. solution of health problems, ii. solution of the market problem, iii. increase in product prices, and iv. was expressed as the establishment of an animal market (Table 10).

Table 10. The most important problems of sheep breeding according to the surveyed breeders and the expectations of the breeders from the authorities.

Traits	N	%
Problems according to breeders*		
Marketing issues / Low product prices	36	76.60
High feed prices	37	78.72
Labour expenses	11	23.40
Insufficiency of pasture	3	6.38
Insufficiency of shepherd	19	40.43
Finding quality breeding sheep	2	4.26
Diseases / Deaths of animals	24	51.06
Organizing with other breeders	14	29.79
Old barn-shelters	1	2.13
Low number of veterinarians specialised about sheep breeding**	1	2.13
The social status of the sheep breeder / The problem of finding a spouse	2	4.26
Price instability	3	6.38
High broker profits / Determination of the product price by brokers	3	6.38
Have no problem	3	6.38
Solutions expected from the authorities *		
Solving the marketing problem	13	27.66
Solving health problems	24	51.06
Solving the credit problem	1	2.13
Solving the supplying the breeding animal problem	1	2.13
Increasing product prices	8	17.02
Increasing supports	1	2.13
Ensuring stability in the market	1	2.13
Establishment of animal stock market	3	6.38
There is no expectation	15	31.91

* Surveyed breeders were given the opportunity to specify more than one choice.
** Insufficient number of veterinarians who are experts in sheep breeding and will provide information on feeding issues

DISCUSSION

Considering the socio-cultural structure of the breeders; it is seen that the average age of the breeders is 45, and the experience period in sheep breeding is 39 years. These findings indicate that breeders have been dealing with sheep breeding since childhood and young people do not show much interest in sheep breeding. The decrease in young labour in sheep farming suggests that it may become an important threat to the sustainability of sheep farming soon. In many studies conducted in different regions of Turkey, it has been reported that sheep and goat breeding are mostly performed by middle-aged and older people (Acar and Ayhan 2012, Gezer 2010, Karakuş and Akkol 2013, Koyuncu et al. 2006,

Tüfekci and Olfaz 2015, Karadaş 2017, Kandemir et al. 2015, Kızıloğlu and Karayaka 2014).

When the educational status of the breeders is examined; approximately 27% of them are high school and above graduates, while 70-73% of them are primary school-secondary school graduates and there are no illiterate breeders. These results show that the education level of sheep breeders in Kırklareli is generally higher than that reported for sheep breeders in other regions of Turkey (Acar and Ayhan 2012, Bilginturan and Ayhan 2009, Bostancı 2006, Ceyhan et al. 2015, Karadaş 2017, Karakuş and Akkol 2013, Kızıloğlu and Karayaka 2014, Koyuncu et al. 2006, Tüfekçi and Olfaz 2015).

The study results showed that the shepherd's task is performed only by family members in approximately 45% of the sheep farms in Kırklareli. It is thought that the shepherding service is largely performed by individuals from within the family, because almost all the enterprises raising the Kıvırcık breed in the study were small family businesses. Considering that income from sheep breeding is very limited, it is seen that it is not an economical method for small family businesses to employ shepherds from outside the family. Similar to the results obtained in this study, Acar and Ayhan (2012) found 93.94% of goat farms in Isparta, Bilginturan and Ayhan (2009) reported 97.4% of sheep farms in Burdur, and Ceyhan et al. (2015) determined that in 63.5% of the sheep farms in Niğde province, the shepherd service is performed by family members.

Approximately 90% of the businesses visited within the scope of the project are small family businesses. This situation shows that the number of large-scale enterprises that also apply modern production techniques in Kırklareli is quite limited. The increase in the number of professionally managed large-scale enterprises in the region suggests that it can make a great contribution in terms of conducting more efficient and economical sheep breeding, improving the quantity and quality of the products obtained from sheep breeding and increasing the income of sheep breeders.

It has been observed that approximately 83% of sheep breeders also raise other animal species. It has been determined that approximately 95% of the Kıvırcık farms raise goats and 70% of them raise dairy cattle. Ceyhan et al. (2015) also reported that sheep farms in Niğde province reared the most goats and cattle beside sheep. Bilginturan and Ayhan (2009) determined that 46.9% of the sheep farms in Burdur also conduct other animal husbandry activities besides small ruminant breeding. Gezer (2010) reported that 46% of sheep breeders in Sivas also rear cattle. These results show that Kıvırcık sheep breeders in Kırklareli are more inclined to raise animals from other species compared to sheep breeders in other regions of Turkey.

To conduct sheep breeding activities economically, it is necessary to make use of natural resources, like pasture, as much as possible. In the study, it was observed that in Kırklareli, the Kıvırcık ewes were taken to pasture almost throughout the year, and that in 7 months of the year, the ewes were fed only on pasture, and no additional feed was given to these sheep in the barn. In other studies conducted in Turkey, the pasture usage periods were determined by Bilginturan and Ayhan (2009) as 7.27 months, Gezer (2010) and Dellal et al. (2002) approximately 7 months, Karakuş and Akkol (2013) as 7-8 months, Kızıloğlu and Karayaka (2014) as 6-7 months, Tüfekci and Olfaz (2015) stated that 40% of breeders in Kastamonu keep their animals in the pasture for 7-8 months and 60% of them keep it in the pasture for 9-10 months. The results obtained regarding pasture use of Kıvırcık sheep breeders in Kırklareli indicate that Kırklareli is more advantageous than many other regions of Turkey in terms of grazing opportunities.

It is seen that in 34.04% of the Kıvırcık farms, the roughage is provided entirely from the farm's own internal resources. However, in approximately 45% of the Kıvırcık farms, concentrate feed is supplied only from the feed factories; while 46.81% of them was supplied from both the internal resources of the enterprise and feed factories. The fact that 65.96% of the enterprises had to buy roughage and 91.49% of them had to buy concentrated feed shows that the Kıvırcık enterprises in Kırklareli are quite inadequate in meeting their own feed needs. Bilginturan and Ayhan (2009) stated that 53% of the sheep farms in Burdur raised their own feed, Bostancı (2006) stated that 80% of the sheep farms in Kırıkkale purchased factory feed from outside, Gezer (2010) stated that 92% of the sheep farms in Sivas made the concentrate feed by themselves, Dellal et al. (2002) determined that 61% of sheep and goat farms purchased factory feed. Karakuş and Akkol (2013) reported that 12.3% of the small ruminant farms in Van can provide roughage and 5.7% of them use concentrate feed from their own internal resources. The literature summarized above point out that the inadequacy of sheep farms in the production of fodder crops is an important problem observed in many regions of the country. However, the current research results show that the inadequacy of forage crop production in sheep breeding enterprises in Kırklareli is much more evident.

It was determined that the sheep was fed only on pasture for about 7 months in the Kıvırcık farms; in other periods (usually from mid-November to the end of March), the most common roughage given in addition to pasture was the straw (89.36% of enterprises) and dry pasture grass (93.62% of enterprises). It has been reported that straw, which is a poor quality fodder, is preferred as the main source of roughage in the winter feeding of broodstock sheep in sheep farms in other regions of Turkey as well as in Kırklareli (Bilginturan and Ayhan 2009, Gezer 2010, Dellal et al. 2002, Kandemir et al. 2015). This indicates that to conduct a more efficient sheep

breeding, the awareness of the breeders on animal nutrition should be increased and there is a need for studies to produce and spread higher quality feed such as alfalfa.

It has been observed that most Kıvırcık farms are closed barns and are built on soil ground. Bricks and briquettes are mostly used as wall materials in businesses located in or near the village, and materials such as tile, fibrous cement (Eternit®) / shingle and tin are used as roofing materials. Building materials used in small family businesses in or near the village found to be consistent with the statement of Ceyhan et al. (2015) for Niğde province sheep pens. On the other hand, it is seen that materials such as wood, brushwood, nylon, burlap pieces are used as wall and roof materials in family businesses in the forest. It is seen that the forest family businesses in Kırklareli differ from many regions of Turkey in terms of shelter building materials.

One in five businesses (21.74%) included in the study does not have windows or chimneys to provide ventilation. Bostancı (2006) reported that 46.7% of sheep pens in Kırıkkale did not have chimneys. Dellal et al. (2002) determined that the percentage of barns without chimneys in small cattle farms in the provinces of the GAP region was 86.5%. Sheep pens in Kirklareli seem more ventilated compared to the previously reported studies, although, not providing any form of ventilation is an unacceptable situation for welfare.

To reduce neonatal lamb deaths due to crushing and to establish a stronger and quicker bond between mother and lamb, it is recommended that ewes whose birth is approaching should be taken to the maternity pen and if possible, they should be kept here with their lambs for three days after birth (Dwyer 2008). Although 38% of the Kıvırcık farms do not have fixed maternity pens, it is seen that the breeders have created a separate shed within the barn for the ewes that are about to give birth. In this regard, the breeders should be informed and advice should be given to breeders about the construction of fixed maternity pens as far as the barn sizes allow.

The ideal density in sheep pens is reported as 0.70-1.00 m^2 per ewe without a lamb, 1.20-1.50 m^2 per ewe with 1 lamb and 1.50-1.75 m^2 per ewe with 2 lambs (Akçapınar 1994). In Annex A of the European Union directive no. 86/609, it is recommended to provide 1.5 m^2/sheep floor area for sheep weighing 35-60 kg. However, Anonymous (2020), suggested providing 3 m^2 area per sheep with lambs less than 6 weeks old for an ideal welfare level. Considering the density levels in the enterprises individually; it is seen that the floor area per animal (sheep + lamb) in the barn was less than 0.70 m^2 in 59.6% of the Kıvırcık farms. The ratio of farms with a floor area of less than 1.20 m^2 per ewe was determined as 51.06% (Results are not presented in the tables). These findings show that there is a need to inform the breeders about decreasing stocking density in sheep pens.

Breeders reported that the most common disease observed in the last year was "blue tongue" (45.65%). However, it should be kept in mind that this result is a special case of the year in which the research was conducted. Additionally, respiratory system diseases (30.43%) and enterotoxaemia (10.87%) were also determined as frequently observed diseases. It has been reported that 6.52% of the Kıvırcık enterprises had brucellosis in the last 1 year. It was calculated that abort was observed in 95.65% of the farms and an average of 4.59% of the sheep within the farm had abort. Bilginturan and Ayhan (2009) reported that external parasitic diseases, respiratory diseases and enterotoxaemia were the most frequently observed diseases in sheep farms in Burdur. Bostancı (2006) listed the most common diseases in sheep breeding farms in Kırıkkale as foot and mouth disease, smallpox, brucellosis and hoof diseases. Dönmez (2008) stated that the most common diseases encountered by sheep breeders in Bursa were enterotoxaemia with 4.3%, footstool with 8.5%, brucellosis with 10.6%, and small ruminant plague with 17%. In addition, they reported that 59.6% of the breeders in their study were encountered all of these diseases.

Karakuş and Akkol (2013) reported that the most common diseases in small ruminant farms in Van were external parasites (65.36%) and respiratory diseases (52.19%); they also determined that the rates of smallpox (44.57%), brucellosis (48.96%) and foot and mouth disease (44.57%) were quite high. It is seen that there are fewer disease problems in the Kıvırcık enterprises in Kırklareli compared to other studies. It is thought that the problems related to diseases such as foot and mouth and smallpox are observed much less in the enterprises visited within the scope of the research is due to the regular and successful vaccination of almost all of these enterprises by the Provincial Directorate of Agriculture and Forestry.

Abort is a serious economic loss and was observed in 95.65% of the Kıvırcık farms and an average of 4.59% of the sheep in the farms had aborted. The average abort rate in the studies conducted in different regions of Turkey was reported as 16.62% by Bostancı (2006), 3.06% by Dönmez (2008), 7.97% by Acar and Ayhan (2012). It is seen that the rate of aborted sheep determined for the Kıvırcık farms is compatible with the results obtained in other studies.

The results of the survey showed that 9.46% of live-born lambs died during the period until weaning, and mortality of lambs after weaning was very low. The breeders stated that diarrhea/digestive system diseases > respiratory system diseases > high twinning as the most important causes of lamb death. Bilginturan and Ayhan (2009) calculated the lamb mortality rate as 7.57% in sheep farms in Burdur. Karakuş and Akkol (2013) determined the lamb

mortality rate as 9.50% in small ruminant farms in Van. Kandemir et al. (2015) reported that 80.1% of sheep and goat farms in İzmir had lamb death during the rearing period, and the most important causes of the deaths were cold shock (44.8%), hunger (19.2%) and diarrhea (12.3%). It is seen that the lamb mortality rates in Kıvırcık farms in Kırklareli are relatively high. For this purpose, it is seen that there is a need to pay attention to breeding hygiene practices, especially for digestive and respiratory system diseases and to inform breeders about health protection practices.

It was determined that almost all of the Kıvırcık sheep breeding farms in Kırklareli use parasitic pesticides as a routine practice. The rate of enterprises applying a bath (10.87%) was found to be very low. On the other hand, it has been reported that 93.48% of the Kıvırcık farms performs shelter disinfection inside the barn regularly. Similarly, Bostancı (2006) reported that external parasitic control was performed in 97.78% of sheep breeding farms in Kırıkkale, and shelter disinfection was applied in 88.89% of the farms. Ceyhan et al. (2015) also reported in their study in Niğde that rate of businesses that use baths is very low. However, the rate of enterprises applying disinfection was determined as 15.2% and 73.7%, respectively, in studies conducted in Bingöl (Kızıloğlu and Karayaka 2014) and Kastamonu (Tüfekçi and Olfaz 2015).

82.61% of the farmers in the study stated that lameness was observed in varying amounts of sheep in their farms. Breeders reported that an average of 6.67% of the sheep had lameness problems. The rate of enterprises applying foot bath was determined as 8.70%, which is significantly inadequate and considering that foot bath is applied so little, the lameness rates determined in the enterprises are quite expected. Bostancı (2006) reported that foot baths were not applied in any sheep farms in Kırıkkale and hoof diseases were among the frequently observed diseases. Kandemir et al. (2015) reported that 98.8% of the small ruminant farms in İzmir do not have a foot bath.

It was determined that the enterprises other than the enterprises within the scope of the projects conducted by the Ministry of Agriculture and Forestry did not keep any records. It is seen that the majority of the enterprises that keep records within the scope of the projects of the Ministry only keep the records requested within the scope of the project. It is seen that the records on selection and sorting processes, health and disease records, feed consumption and economic parameters are seldom kept. Similarly, Bilginturan and Ayhan (2009) reported that 86.6% of the sheep farms in Burdur, Karakuş and Akkol (2013) stated 61.95% of the small ruminant farms in Van, Kızıloğlu and Karayaka (2014) reported 92.7% of the sheep farms in Bingöl and Şahinli (2014) determined that 52% of the sheep farms in Karaman did not keep any records. The findings obtained in the study are similar to previous statements.

It was stated that sheep breeding is the main business in 93.62% of the enterprises. While 42.55% of the breeders stated that they were satisfied with the sheep breeding, the rate of those who were not satisfied was determined as 27.66%. It was indicated that the number of sheep increased in the last five years in 46.81% of the farms, while the decrease in the number of sheep was determined as 14.89%. 80.85% of the Kıvırcık breeders stated that they will continue to breed sheep, however, 19.15% stated that they will quit sheep farming soon. However, 34.04% of the breeders stated that they thought that their children would not breed sheep. Bilginturan and Ayhan (2009) determined that 64.4% of the sheep breeders in Burdur were the only source of livelihood, 63.9% were satisfied with sheep breeding, and 43.3% were considering increasing the capacity. Dellal et al. (2002) determined that 84.9% of the ovine breeders in the provinces of the GAP region (Diyarbakır, Şanlıurfa, Gaziantep, and Adıyaman) and Karakuş and Akkol (2013) stated that 60.84% of the ovine farms in Van province was determined this production branch was their only source of livelihood. The fact that approximately 30% of the sheep breeders in Kırklareli are not satisfied with this field of activity, 15% decreased the number of sheep in their herd, 20% think of quitting sheep breeding soon, and most importantly, 35% think that their children will not be a sheep breeder, rated major threats in the region. Because of the improvement in living standards, especially the younger population does not want/contempt sheep breeding, which is a laborious business line, and it is seen that the migration from the village to the city has accelerated. This situation causes the problem of the evacuation of villages as well as the decrease in animal food production.

The last part of the study was about the most important problems of sheep breeding according to the breeders and 78.72% of the farmers reported high feed prices, 76.60% low marketing/product prices, 51.06% animal diseases and deaths, 40.43% did not find a shepherd, 29.79% insufficient organization among the sheep breeders as the most important problems of sheep breeding. The rate of breeders who stated that they did not see any problems was determined as only 6.38%. Bilginturan and Ayhan (2009) listed the most important problems of breeders as a marketing problem (39.1%), high feed prices (23.1%), insufficient pasture land (21.8%), credibility problems (9.2%). Ceyhan et al. (2015) reported that 70.8% of sheep farms in Niğde stated that the most important problem was expensive feed prices and the inadequate and poor quality of the pastures. Ceyhan et al. (2015) reported that 70.8% of the sheep breeders in Niğde declared that the most serious problem was the expensive feed prices and the inadequate and poor quality of pastures. According to breeders from the study of Ayvazoglu

Demir et al. (2015), the most important problems were high feed prices (24.2%), lack of reliable shepherds (18.2%), low demand (15.2%), diseases (13.6%), low wool prices. (12.1%), the ineffectiveness of unions/cooperatives (10.6%), and low-quality of pastures (6.1%). These notifications show that problems such as high feed prices, marketing problems, lack of a shepherd, animal diseases are among the most important problems of sheep breeding in many regions of Turkey. However, fewer complaints (6.38%) regarding the inadequacy of pasture in the surveyed enterprises may be related to the fact that these enterprises are mostly located in the high villages of the Strandja mountains or forest areas.

The most important expectations of the breeders from the authorities were solution to health problems (51.06%), solution of marketing problems (27.66%) and an increase in product prices (17.02%). However, notably the expectations of breeders from authorities regarding the supply of pasture and breeding animals are very limited. The 51.4% of the sheep breeders in Burdur stated that they wanted the market problem, 14.7% the credit problem, and 10% the health problems of animals to be solved. (Bilginturan and Ayhan 2009). On the other hand, unlike the Kıvırcık breeders in Kırklareli, 15.1% of the sheep breeders in Burdur demanded that the pasture problem and 7.7% the breeding animal supply problem to be solved.

CONCLUSION

As a result, approximately 90% of the Kıvırcık sheep breeding enterprises visited within the scope of the project were small family businesses. It has been determined that the average herd size in the small family businesses was 197 heads. It is seen that approximately ¼ of these enterprises do not have chimneys or windows for barn ventilation. It is seen that very few of the small family businesses have a fixed maternity pen and the stocking density is generally high. It has been determined that most of these enterprises cannot meet their roughage and concentrate feed needs from internal sources and they buy feed from outside. Almost all of the investigated small family businesses were produced with traditional methods; herd management, selection-sorting, production planning, product marketing and income-expenditure follow-up are conducted amateurishly. It may be possible for the breeders in the region to continue sheep breeding if they have a satisfactory level of income. To increase the income of sheep breeding enterprises and for sustainable sheep breeding, "i. Continuing the pasture-based feeding of the stock sheep herd, ii. Increasing forage crop production, iii. Improving shelter conditions, iv. Increasing herd size (capacity), v. Performing herd management and marketing operations more professionally, vi. Developing product marketing strategies and acting jointly, if possible, under the coordination of the Sheep and Goat Breeders' Association, vii. It is recommended that training seminars and courses be organized by the Provincial Directorate of Agriculture and Forestry and the Sheep and Goat Breeders' Association on record keeping, health protection, shelter hygiene and care-feeding procedures for breeders.

Ethics Committee Information: This study is not subject to HADYEK's permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

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

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PDK, AY and BE designed the experiment. The data acquisition was performed by PDK, HY, NÖ, RC, ÖK, AY and BE. PDK and BE did the statistical analysis. PDK wrote the paper. BE supervised all the procedures. All authors reviewed and approved the submitted paper.

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RESEARCH ARTICLE

Effect of Ascending Doses of Pentoxifylline Administration on Biochemical and Hematological Parameters in Goats

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ABSTRACT

Pentoxifylline (PTX) is a non-selective phosphodiesterase inhibitor drug used in human and veterinary medicine to promote microcirculation. Although PTX has been used in experimental studies in goats, there is no information about its safety. The aim of this study is to determine the effect of PTX on hematological and biochemical parameters following intravenous (IV) administration of 10, 20 and 40 mg/kg doses to Hair goats. In the study were used clinically healthy 9 goats. PTX was administered IV to goats at doses of 10, 20 and 40 mg/kg. Blood samples were taken before drug administration (control, 0 hour) and 12 hours after administration, and hematological and biochemical parameters were evaluated. No local or systemic adverse effects were observed in goats after the application of PTX at different doses. After administration of PTX at 20 and 40 mg/kg doses, white blood cell (WBC) level increased, while hemoglobin (HGB) level decreased at 10 mg/kg dose ($p<0.05$). When compared with the 10 mg/kg dose group, WBC, red blood cell (except 40 mg/kg), HGB and hematocrit values were found to be increased in the 20 and 40 mg/kg dose groups ($p<0.05$). While the administration of PTX at different doses caused a general decrease in biochemical parameters ($p<0.05$), no change was observed in troponin I level. In conclusion, it can be stated that the administration of PTX in goats at doses of 10, 20 and 40 mg/kg caused significant changes in hematological and biochemical parameters and caution should be exercised as these temporary changes in single-dose administrations may pose a risk in repeated administrations.

Keywords: Ascending dose, Biochemical, Goat, Hematological, Pentoxifylline

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Keçilere Artan Dozlarda Pentoksifilin Uygulamasının Biyokimyasal ve Hematolojik Parametrelere Etkisi

ÖZ

Pentoksifilin (PTX) beşerî ve veteriner hekimlikte mikrosirkülasyonun teşvik edilmesi amacıyla kullanılan non-selektif fosfodiesteraz inhibitörü bir ilaçtır. PTX keçilerde deneysel çalışmalarda kullanılmasına rağmen güvenilirliği hakkında herhangi bilgi bulunmamaktadır. Bu araştırmanın amacı Kıl keçilerine PTX'in 10, 20 ve 40 mg/kg dozlarda damar içi (IV) uygulamasını takiben hematolojik ve biyokimyasal parametreler üzerine etkisini belirlemektir. Araştırmada klinik olarak sağlıklı 9 adet keçi kullanıldı. PTX keçilere 10, 20 ve 40 mg/kg dozlarda IV yolla uygulandı. Kan örnekleri ilaç uygulaması öncesi (kontrol 0.saat) ve uygulamayı takiben 12.saatte alınarak hematolojik ve biyokimyasal parametreler değerlendirildi. PTX'in farklı dozlarda uygulaması sonrası keçilerde lokal ya da sistemik herhangi bir yan etki görülmedi. PTX'in 20 ve 40 mg/kg dozlarda uygulaması sonrası akyuvar (WBC) seviyesi artarken 10 mg/kg dozda hemoglobin (HGB) düzeyi azaldı ($p<0.05$). 10 mg/kg doz grubu ile karşılaştırıldığında, 20 ve 40 mg/kg doz gruplarında WBC, alyuvar (40 mg/kg hariç), HGB ve hematokrit değerlerinin arttığı belirlendi ($p<0.05$). PTX'in farklı dozlarda uygulaması, biyokimyasal parametrelerde genel olarak azalmaya neden olurken ($p<0.05$), troponin I düzeyinde herhangi bir değişiklik görülmedi. Sonuç olarak, keçilerde PTX'in 10, 20 ve 40 mg/kg dozlarda uygulamasının hematolojik ve biyokimyasal parametrelerde önemli değişikliklere neden olduğu ve tek doz uygulamalarda geçici şekillenen bu değişimlerin tekrarlı uygulamalarda risk oluşturabileceğinden dikkatli olunması gerektiği kanısına varıldı.

Anahtar kelimeler: Artan doz, Biyokimyasal, Hematolojik, Keçi, Pentoksifilin

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GİRİŞ

Pentoksifilin (PTX), 1-(5-oksoheksil)-3,7-dimetilksantin, teobrominden sentezlenen non-selektif bir fosfodiesteraz inhibitörüdür (Uney ve ark. 2019). PTX kanın viskozitesini azaltıp, periferik dokuların oksijenlenmesini sağladığından özellikle dolaşım bozukluklarının tedavisinde kullanılır. Ayrıca, tümör nekroz faktör-alfa (TNF-α) ve interferonlar gibi pro-inflamatuvar sitokinler ile oksidan enzimleri inhibe ettiği için anti-inflamatuvar ve antioksidan etkiye sahiptir (Corum ve ark. 2018, Sezik ve ark. 2020). PTX'in insanlarda topallık, vaskülit, kollajen bozuklukları, endotoksemi, septisemi, diyabetik bozukluklar ve kanser tedavisinde olumlu etkiler gösterdiği belirtilmiştir (Corum ve ark. 2019, Samlaska ve Winfield 1994). Veteriner sahada PTX'in köpeklerde vaskülit, atopik dermatit, kontakt alerji, dermatomiyozit ve sistemik lupus eritematozus, atlarda kutanöz vaskülit, laminit, endometrit-plasentit, taylarda septisemi ve sığırlarda vaskülit, laminit, endometrit-plasentit ve septisemi gibi durumlarda yararlı olabileceği ifade edilmiştir (Sykes ve Papich 2014, Uney ve ark. 2019). Keçilerde ise endotoksin kaynaklı akut faz cevabı baskılayabileceği (Van Miert ve ark. 1997) ve erken doğum öncesi intrauterin inflamasyonu azaltarak fetal beyin hasarını önleyebileceği bildirilmiştir (Sezik ve ark. 2020).

Keçilerde bruselloz, artritis-ensefalitis (caprine arthritis-encephalitis), mikoplazmozis, septisemi, endotoksemi, laminitis gibi inflamasyon ile seyreden birçok hastalık bulunur (Radostits ve ark. 2006). İnflamasyona bağlı salgılanan pro-inflamatuvar sitokinler özellikle beyin, böbrek ve karaciğer gibi hayati organlara giden kan akımında ve vasküler permabilitede bozulmalara neden olabilir (Yang ve Lee 2008). Ancak keçilerde mikrosirkülasyon ve periferik vasküler sistem bozukluklarında kullanılabilecek ruhsatlanmış bir ilaç yoktur. PTX, diğer türlerde olduğu gibi keçilerde de belirtilen amaçlar için etiket dışı olarak kullanılabilir. Etiket dışı kullanımda ilaçların etkili ve güvenli kullanımı için istenmeyen etkilerinin değerlendirilmesi gerekir. Bu değerlendirmede hematolojik ve biyokimyasal parametreler yararlı olabilir. Hematolojik parametreler kemik iliği fonksiyonlarının, biyokimyasal parametreler ise karaciğer, böbrek ve kalp fonksiyonlarının değerlendirilmesinde kullanılır (Corum ve ark. 2015, Coskun ve ark. 2018, Coskun ve ark. 2019).

PTX keçilerde bazı deneysel çalışmalarda kullanılmasına rağmen güvenilirliği hakkında herhangi bilgi bulunamamıştır. Bu araştırmanın amacı Kıl keçilerinde PTX'in 10, 20 ve 40 mg/kg dozlarda IV bolus şeklinde uygulamasını takiben hematolojik ve biyokimyasal parametreler üzerine etkisini belirlemektir.

MATERYAL ve METOT

Hayvanlar

Araştırma, genel klinik muayene ve hematolojik ölçümler sonucunda sağlıklı oldukları belirlenmiş, çalışma öncesi son iki aylık sürede herhangi bir ilaç uygulaması yapılmamış Kıl ırkı, dişi (17±2 aylık, 25.82±2.04 kg) 9 baş keçi üzerinde gerçekleştirildi. Hayvanlar, alışma periyodu için çalışma başlamadan 10 gün önce diğer hayvanlardan farklı ağıllara alındı ve çalışma süresince burada tutuldular. Hayvanlar yaş ve kilolarına uygun olarak günde 2 defa (sabah-akşam) konsantre yemle (Abalıoğlu Yem Sanayi A.Ş., Tarsus, Mersin) beslendi ve kuru ot ile suya ad-libitum olarak ulaşmaları sağlandı. Keçiler üzerindeki deneysel prosedür Siirt Üniversitesi Deney Hayvanları Yerel Etik Kurulu tarafından onaylandı (2021/21).

Deneysel Prosedür

Keçilere ilaç uygulaması için PTX'in analitik standardı (>%98, Tokyo Chemical Industry) serum fizyolojikte (50 mg/mL) çözdürüldükten sonra tüm doz gruplarına vena jugularisten IV bolus şeklinde uygulandı. Toplam 9 adet keçi farklı doz gruplarını alacak şekilde rastgele 3 eşit gruba ayrıldı. Araştırma, 15 günlük ilaç arınma süresini takiben 3 aşamalı çapraz dizayna göre gerçekleştirildi. PTX, çalışmanın ilk aşamasında 3 keçiye 10 mg/kg, 3 keçiye 20 mg/kg ve diğer 3 keçiye de 40 mg/kg dozunda uygulandı. Çalışmanın ikinci ve üçüncü aşaması, 15 günlük ilaç arınma süresini takiben keçiler farklı dozları alacak şekilde değiştirilerek gerçekleştirildi. Çalışma sonunda 9 keçininde tüm dozları alması sağlandı. Kan örnekleri 5 mL'lik enjektörler (21G 0.80x38mm, Genject A.Ş., Ankara, Türkiye) yardımıyla vena jugularisten PTX uygulamasından hemen önce (0.saat, kontrol) ve uygulama sonrası 12. saatte 2'şer mL olacak şekilde EDTA'lı ve anti-koagülantsız tüplere alındı. Hematolojik parametrelerin ölçümü kan örnekleri aldıktan kısa bir süre sonra gerçekleştirildi. Biyokimyasal analizler için toplanan kan örnekleri 4.000 g'de 10 dakika santrifüj edildikten sonra elde edilen serum örnekleri analiz zamanına kadar -80°C'de saklandı.

Hematolojik ve Biyokimyasal Parametrelerin Analizi

Hematolojik parametrelerden akyuvar (WBC), alyuvar (RBC), hemoglobin (HGB), hematokrit (HCT) ve trombosit düzeylerinin ölçümü kan hücresi sayım cihazında (BC-2800 Auto Hematology Analyzer, Mindray Bio-Medical Electronics, Shenzen, China) ve biyokimyasal parametrelerden aspartat transaminaz (AST), alanin transaminaz (ALT), albumin (ALB), gama-glutamil transpeptidaz (GGT), total protein (TP), kan üre nitrojen (BUN) ve kreatinin (KRE) düzeylerinin ölçümü ise otoanalizör cihazında (ILab-

300 plus, Instrumentation Laboratory, Milano, Italy) gerçekleştirildi. Troponin I (Tn-I) düzeyi ise kemilüminesans analiz yöntemi ile belirlendi (Abbott architect i2000SR, Abbott Core Laboratory, USA).

İstatistiksel Analiz

Araştırma verileri ortalama±standart sapma (SD) olarak sunuldu. İstatistiksel analiz için SPSS programı (SPSS 22.0 software, IBM) kullanıldı. Grup içi istatistiksel değerlendirme paried-t testi ile yapıldı. Doz grupları arasındaki değerlendirmede ise tek yönlü varyans analizi (ANOVA) ve postoch Tukey testi kullanıldı (SPSS 22.0). $p<0.05$ değeri istatistiksel önem derecesi olarak kabul edildi.

BULGULAR

PTX'in 10, 20 ve 40 mg/kg dozlarda IV uygulaması sonrası keçilerin klinik gözlemleri esnasında yeme ve içmelerinde, dışkılamalarında ve davranışlarında herhangi bir anormallik belirlenmedi. Keçilere PTX'in 10, 20 ve 40 mg/kg dozlarda IV uygulaması sonrası hematolojik parametrelere etkisi Tablo 1'de sunuldu.

Tablo 1. Keçilere pentoksifilinin 10, 20 ve 40 mg/kg dozlarda damar içi uygulamasının hematolojik parametrelere etkisi (ortalama ± SD).
Table 1. The effects on hematological parameters of intravenous administration of pentoxifylline at doses of 10, 20 and 40 mg/kg to goats (mean ± SD).

	10 mg/kg		**20 mg/kg**		**40 mg/kg**	
Parametre	**0.saat**	**12. saat**	**0.saat**	**12. saat**	**0.saat**	**12. saat**
WBC ($x10^9$/L)	15.54±4.82	16.46±5.01^{b}	15.58±4.75	21.52±2.97^{a*}	15.78±4.43	22.99±2.36^{a*}
RBC ($x10^{12}$/L)	16.25±2.84	14.59±2.38^{b}	16.73±2.77	18.10±2.00^{a}	16.43±2.50	16.60±1.23^{ab}
HGB (g/dL)	7.22±1.47	5.70±1.07^{b*}	7.39±1.36	8.02±0.61^{a}	7.36±1.19	7.61±0.78^{a}
HCT (%)	16.49±3.45	13.53±2.75^{b}	16.70±3.55	17.81±0.90^{a}	16.82±2.95	18.34±2.37^{a}

WBC; akyuvar, RBC; alyuvar, HBG; hemoglobin, HCT; hematokrit. *: Grup içinde 0.saate göre istatistiksel farkı gösterir ($p<0.05$).$^{a, b}$: Gruplar arasında 12. saatlerdeki istatistiksel farkı gösterir ($p<0.05$).
WBC; white blood cell, RBC; red blood cell, HBG; hemoglobin, HCT; hematocrit. *: Indicates statistical difference within the group according to the 0^{th} hour ($p<0.05$). $^{a, b}$: Shows the statistical difference between the groups at 12^{th} hours ($p<0.05$).

Grup içi değerlendirmede RBC ve HCT değerlerinde herhangi farklılık belirlenmedi ($p>0.05$). Ancak 20 ve 40 mg/kg dozlarda 12. saatte WBC seviyesi artarken 10 mg/kg dozda HGB azaldı ($p<0.05$). Gruplar arası değerlendirmede ise kontrol (0. saat) gruplarında herhangi bir farklılık tespit edilmedi ($p>0.05$). Ancak 12. saat verileri karşılaştırıldığında, 20 ve 40 mg/kg doz gruplarında WBC, RBC (40 mg/kg hariç), HGB ve HCT değerlerinin10 mg/kg doza göre arttığı belirlendi ($p<0.05$). Tüm örneklerde trombosit düzeyi ise cihaz kaynaklı sıkıntıdan dolayı ölçülemedi.

Keçilere PTX'in 10, 20 ve 40 mg/kg dozlarda IV uygulaması sonrası biyokimyasal parametrelere etkisi Tablo 2'de sunuldu. Grup içi değerlendirmede tüm doz gruplarında 12. saatte AST, TP, BUN ve KRE değerlerinin azaldığı belirlendi ($p<0.05$). Ayrıca 12. saatte 40 mg/kg dozda ALT, GGT ve ALB düzeyinde, 10 mg/kg dozda ALT ve ALB düzeyinde ve 20 mg/kg dozda GGT düzeyinde azalma görüldü ($p<0.05$). Gruplar arası değerlendirmede kontrol (0. saat) grupları arasında biyokimyasal değerlerde farklılık belirlenmedi ($p>0.05$). 10 ve 20 mg/kg doz grupları ile karşılaştırıldığında, 40 mg/kg doz grubunda ALB değeri 12. saatte azaldı ($p<0.05$). TP değeri 40 mg/kg dozunda en düşük düzeyde belirlenirken 20 mg/kg dozunda en yüksek düzeydeydi. 40 mg/kg uygulama sonrası, GGT ve KRE değerleri 10 mg/kg'a göre ve ALT değeri 20 mg/kg'a göre azaldı ($p<0.05$). Tn-I düzeyinde ise grup içi ve gruplar arası değerlendirmede herhangi bir farklılık görülmedi ($p>0.05$).

Tablo 2. Keçilere pentoksifilinin 10, 20 ve 40 mg/kg dozlarda damar içi uygulamasının biyokimyasal parametrelere etkisi (ortalama ± SD).
Table 2. The effects on biochemical parameters of intravenous administration of pentoxifylline at doses of 10, 20 and 40 mg/kg to goats (mean ± SD).

	10 mg/kg		20 mg/kg		40 mg/kg	
Parametre	**0.saat**	**12. saat**	**0.saat**	**12. saat**	**0.saat**	**12. saat**
AST (U/L)	53.00±9.68	37.78±4.21*	54.22±9.43	35.78±10.93*	55.44±11.22	28.44±7.49*
ALT (U/L)	11.22±2.72	8.33±2.35ab*	12.00±2.35	10.56±3.78a	12.44±2.19	5.44±1.59b*
GGT (U/L)	29.44±12.54	22.67±4.06a	31.89±13.54	18.00±6.63ab*	30.56±14.38	16.00±4.50b*
ALB (g/dL)	1.14±0.19	0.99±0.20a*	1.24±0.21	1.06±0.30a	1.21±0.20	0.66±0.09b*
TP (g/dL)	3.47±1.01	2.21±0.37ab*	3.64±1.01	2.39±0.82a*	3.67±1.07	1.68±0.45b*
BUN (mg/dL)	7.00±2.92	4.67±1.22*	8.44±2.88	4.22±2.05*	7.89±3.22	3.56±0.88*
KRE (mg/dL)	0.34±0.03	0.24±0.03a*	0.34±0.04	0.22±0.02ab*	0.34±0.03	0.20±0.00b*
Tn-I (ng/mL)	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00

AST; aspartat transaminaz, ALT; alanin transaminaz, GGT; gama-glutamil transpeptidaz, ALB; albumin, TP; total protein, BUN; kan üre nitrojen, KRE; kreatinin, Tn-I; troponin I.*: Grup içinde 0.saate göre istatistiksel farkı gösterir ($p<0.05$).a, b: Gruplar arasında 12. saatlerdeki istatistiksel farkı gösterir ($p<0.05$).
AST; aspartate transaminase, ALT; alanine transaminase, GGT; gamma-glutamyl transpeptidase, ALB; albumin, TP; total protein, BUN; blood urea nitrogen, CRE; creatinine, Tn-I; troponin I.*: Indicates statistical difference within the group according to the 0th hour ($p<0.05$). a, b: Shows the statistical difference between the groups at 12th hours ($p<0.05$).

TARTIŞMA

PTX, beşerî ve veteriner hekimlikte mikrosirkülasyonun teşvik edilmesi ve periferik oksijenlenmenin sağlanması amacıyla birçok patolojik bozuklukta kullanılmaktadır (Sezik ve ark. 2020). PTX'in etkisi doza bağlı olarak değiştiği için artan dozlarda uygulanabilir (Uney ve ark. 2019), ancak artan dozlarda kullanımda istenmeyen etkiler ortaya çıkabilir. Keçilerde yapılan deneysel çalışmalarda PTX 15-60 mg/kg dozda oral yolla ve 0.3-0.5 mg/kg (15-30 dakika) dozda infüzyon şeklinde kullanılmıştır. Bu araştırmada seçilen dozlar (10, 20 ve 40 mg/kg) ve örnek alım saatleri daha önce keçi ve koyunlarda yapılan çalışmalar dikkate alınarak belirlenmiştir (Corum ve ark. 2019, Sezik ve ark. 2020, Van Miert ve ark. 1997). Keçilere PTX'in 10, 20 ve 40 mg/kg dozda IV uygulaması sonrası herhangi bir yan etki görülmedi. Ancak koyunlarda 40 mg/kg (IV) dozda geçici taşikardi, hipersalivasyon ve huzursuzluk (Corum ve ark. 2019), sığırlarda 10 mg/kg (IV) dozda geçici hipersalivasyon ve huzursuzluk (Uney ve ark. 2019), atlarda 8.5 mg/kg dozda (IV) kalp atımında artış, terleme ve kaslarda kasılma (Liska ve ark. 2006) ve tavuklarda 100 mg/kg dozda (IV ve oral) derin solunum ve gözlerde kapanma (De Boever ve ark. 2005) gibi istenmeyen etkiler bildirilmiştir.

Mevcut çalışmada, 20 ve 40 mg/kg dozlarında WBC seviyesi artarken 10 mg/kg dozda HGB seviyesi azaldı. Ayrıca 10 mg/kg doz grubu ile karşılaştırıldığında, 20 ve 40 mg/kg doz gruplarında 12. saatte WBC, RBC (40 mg/kg hariç), HGB ve HCT değerlerinin arttığı belirlendi. Bu değişiklikler daha önce keçilerde belirtilen referans değerler içerisindedir (Jackson ve Cockcroft 2002, Mohammed ve ark. 2016, Njidda ve ark. 2013). PTX'in koyunlara 10, 20 ve 40 mg/kg dozda IV uygulamasının WBC, RBC ve HGB değerlerinde ve köpeklere 8 mg/kg dozda IV ve 30 mg/kg dozda oral uygulamasının hematolojik parametrelerde değişikliğe neden olmadığı rapor edilmiştir (Corum ve ark. 2016, Rees ve ark. 2003). PTX platelet adezyonunu önleyerek ve eritrositlerin flexibilite/deformabilitesini artırarak kan viskozitesini azaltır ve bu özelliklerinden dolayı mikrosirkülasyona bağlı hastalıklarda dokunun daha fazla kanlanmasını sağlar (Harris ve ark. 2010, Schroer 1985). PTX nötrofil solunum patlamasını engellemesi ve immun sistem üzerindeki olumlu etkilerinden dolayı WBC sayısını artırabilir (Carletto ve ark. 1997, Mohammadzadeh ve ark. 2008). PTX'in hemodiyalize bağlı gelişen anemide (Shahbazian ve ark. 2017) ve de eritropoietinin etkinliğini engelleyen pro-inflamatuvar sitokinlerin (TNF-α ve IFN-γ) üretimini baskılayarak HGB seviyesini artırdığı (Cooper ve ark. 2004) belirtilmiştir. Ayrıca insanlarda PTX tedavisinin HCT

değeri artırdığı rapor edilmiştir (Antunes ve ark. 2008, Golbasi ve ark. 2003).

Keçilere PTX'in 10, 20 ve 40 mg/kg dozlarda uygulaması AST, TP, BUN ve KRE değerlerinde azalmaya neden oldu. Ayrıca 40 mg/kg doz uygulaması ALT, GGT ve ALB düzeyinde, 20 mg/kg doz uygulaması GGT düzeyinde ve 10 mg/kg doz uygulaması ALT ve ALB düzeyinde azalmaya neden oldu. Diğer doz grupları ile karşılaştırıldığında, 40 mg/kg doz uygulaması ALB ve TP düzeylerini azalttı. Ayrıca 40 mg/kg uygulama sonrası, GGT ve KRE değerleri 10 mg/kg'a göre ve ALT değeri 20 mg/kg'a göre azaldığı belirlendi. PTX'in sığırlara 10 mg/kg, atlara 8.5 mg/kg ve koyunlara 10-40 mg/kg dozda IV uygulaması sonrası biyokimyasal parametrelerde herhangi bir farklılık görülmediği rapor edilmiştir (Corum ve ark. 2016, Liska ve ark. 2006, Uney ve ark. 2019). Ancak dislipidemik olmayan ve non-alkolik karaciğer yağlanması olan insanlara 800-1200 mg/gün ve 20 mg/kg/gün dozlarında PTX uygulamasının AST, ALT ve GGT düzeylerinde azalmaya neden olduğunu bildirilmiştir (Cioboată ve ark. 2017, El-Haggar ve Mostafa 2015, Tuncer ve ark. 2003). Non-alkolik karaciğer yağlanmasında PTX uygulamasının TNF-α, IL-6 ve IL-8 sentezini azaltarak karaciğer nekrozu ve hepatositlerin apoptozunu önlediği ve buna bağlı olarak karaciğer enzimlerinde azalmaya neden olduğu ifade edilmiştir (Genoves ve ark. 2014, Tuncer ve ark. 2003). Erkek ratlarda artan doz PTX uygulaması BUN ve KRE değerlerini azaltmıştır (Jalili ve ark. 2019). PTX, vazodilatatör prostaglandinlerin sentezini indükleyerek ve mikrosirkülasyonu teşvik ederek glomerüllere giden kan akımını artırır (Kim ve ark. 2003, Wadie ve ark. 2021). Böbreğe giden kan akımındaki artış sonucu glomerüler filtrasyon hızının artması plazmadaki BUN ve KRE miktarını azaltmış olabilir (Kim ve ark. 2003). Bu araştırmada, grup içi ve gruplar arası değerlendirmede Tn-I düzeyinde herhangi bir farklılık görülmedi. Koyunlara PTX'in 10, 20 ve 40 mg/kg dozda uygulamasının kalp belirteçlerinden birisi olan CK-MB düzeyinde değişikliğe neden olmadığı belirtilmiştir (Corum ve ark. 2016). Ayrıca doksorubisin ile indüklenen kardiyotoksisite modelinde PTX uygulaması Tn-I düzeyinde değişikliğe neden olmamıştır (Narin ve ark. 2004).

SONUÇ

Sonuç olarak keçilere PTX'in 10, 20 ve 40 mg/kg dozlarda IV yolla uygulamasının hematolojik parametreler ile karaciğer ve böbrek biyokimyasal parametrelerinde değişikliklere neden olduğu belirlendi. Bu nedenle, keçilere PTX'in IV yolla 10 ve 40 mg/kg doz aralığında uygulaması sonrası hematolojik ve biyokimyasal parametrelerin takip edilmesi gerekir. Ayrıca keçilerde PTX'in tekrarlı uygulaması sonrası güvenilirliğinin hematolojik, biyokimyasal, moleküler ve patolojik olarak ortaya konulmasına ihtiyaç vardır.

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

Etik İzin: Araştırma Siirt Üniversitesi Deney Hayvanları Yerel Etik Kurulu tarafından 29.06.2021 tarihinde ve 2021/01 numaralı oturumda görüşülmüş olup, 21 numaralı kararınca etik kurul izni alınmıştır.

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RESEARCH ARTICLE

Determination of Haematological and Some Biochemical Parameters in Lambs Born to Them, in The Late Pregnancy, Early and Late Lactation Periods of The Sheep C Vitamins at Different Doses Application

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ABSTRACT

This study was conducted to investigate the effects of vitamine C administration with different doses on some haematological and biochemical parameters in ewes of different pregnancy and lactation periods and in their lambs. A total of 48 sheep and 50 lambs born from them were used in the study. Ewes were divided into four groups. Group 1 (n:8) negative (-) control, Group 2 (n:13) positive (+) control were injected with phisiologic saline solution while Group 3 (n:13) and Group 4 (n:14) were injected with 2.5 ml and 5 ml vitamine C beginning from 4th month of pregnancy until delivery. While vitamin C administration in pregnant ewes had no significant effect on hemotological parameters and ALP, AST, TP, Creatinine, Urea, Fe, Na+ and Ca++ parameters; increase in glucose level during pregnancy and decrease in K+level during lactation were found to be statistically significant ($p<0.05$). Vitamin C was not found to be effective on the measured parameters in the comparisons between groups in lambs. In the intergroup comparisons, a statistically significant decreased in MCV and ALP levels (p:0.01) and an increased in Fe levels were detected in 4 week old lambs compared to 1 week old lambs (p:0.01). As a result, it is seen that the addition of vitamin C during pregnancy does not make much metabolic changes in pregnant sheep and lambs born from these sheep, however, further studies are needed to test different doses and administration routes of vitamin C.
Key words: Biochemical Parameters, Ewe, Lactation, Pregnancy, Vitamin C

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Farklı Dozlarda C Vitamini Uygulanan Koyunların İleri Gebelik, Erken ve Geç Laktasyon Dönemleri ile Bunlardan Doğacak Kuzularda Hematolojik ve Biyokimyasal Parametrelerin Tespiti

ÖZ

Çalışma; antioksidan vitaminlerden C vitamininin farklı dozda uygulamalarının, gebelik ve laktasyon dönemindeki koyunlarda ve bunlardan doğacak kuzularda hematoloji ve bazı biyokimyasal parametreler üzerindeki etkilerinin araştırılması amacıyla yapıldı. Çalışmada toplam 48 koyun ile bunlardan doğan 50 kuzu kullanıldı. Koyunlar dört gruba ayrıldı. Grup1 (negatif (-) kontrol, n:8) ve Grup2 (pozitif (+) kontrol, n:13)'deki koyunlara serum fizyolojik, Grup3 (n:13) ve Grup4 (n:14)'teki koyunlara ise gebeliğin 4. ayının başlamasıyla birlikte doğuma kadar her hafta sırasıyla 2.5 ml ve 5 ml C vitamini enjeksiyonu yapıldı. Gebe koyunlarda C vitamini uygulamasının hematolojik parametreler ile ALP, AST, ALT, TP, Kreatinin, Üre, Fe, Na+ ve Ca++ parametreleri üzerine önemli bir etkisi olmazken; gebelik döneminde glikoz düzeyinde artma, laktasyon döneminde ise K+ düzeyinde azalma istatistiksel açıdan önemli bulundu ($p<0.05$). Kuzularda yapılan gruplar arası karşılaştırmalarda C vitamini enjeksiyonu, ölçülen parametreler üzerine etkili bulunmadı. Grup içi kıyaslamalarda ise 4 haftalık kuzularda 1 haftalık kuzulara göre MCV ve ALP düzeylerinde istatistiksel açıdan önemli derecede azalma (p:0.01), Fe düzeyinde ise artma tespit edildi (p:0.01). Sonuç olarak; gebelik döneminde C vitamini ilavesinin gebe koyunlar ile bu koyunlardan doğan kuzularda metabolik olarak fazlaca değişiklik yapmadığı görülmekte, bununla birlikte C vitamininin farklı doz ve uygulama yollarının denenebileceği daha ileri çalışmalara ihtiyaç duyulmaktadır.
Anahtar Kelimeler: Biyokimyasal parametreler, C vitamini, Gebelik, Koyun, Laktasyon

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GİRİŞ

Üreme fonksiyonları ile vitaminler arasında bir ilişki olduğu uzun zamandır kabul edilmektedir (Parraguez ve ark. 2011). Askorbik asit; insanlar ve memelilerin küçük bir bölümü için diyetin esansiyel bir bileşenidir ve uzun yıllardır fertilite ile ilgili bulunmasına rağmen üreme sistemindeki rolü henüz tam olarak aydınlatılamamıştır. Askorbik asidin üreme prosesinde esansiyel bir biyokimyasal ve doğurganlıkta önemli bir faktör olduğu düşünülmektedir (Luck ve ark. 1995).

Ruminantlar için C vitamini; çoğunlukla glikozdan askorbik asit sentezine bağlıdır, çünkü diyetle alınan C vitamini rumenin alkali pH'sında mikrofloranın da etkisiyle yıkıma uğramaktadır (Nockels 1988). Askorbik asit sentezi bozulduğunda, ruminantlar C vitamini eksikliğinden diğer evcil hayvanlara oranla daha fazla etkilenirler (McDowell 1989). Stres durumunda adrenal bezlerde yüksek oranda bulunan C vitamini seviyesi hızlı bir şekilde düşmekte ve bu vitamini sentezleme yeteneğine sahip canlılarda bile yeterli olamamaktadır (Scott ve Woodman 1993). Gebelik periyodu ve takiben laktasyon dönemi hem anne hem de taşıdığı yavru için hassas dengelerin bir arada yürütüldüğü ve fizyolojik stresin, immun sistem üzerine etkisinin yoğun olduğu önemli bir süreçtir. Ruminantların gebelik periyodunda fetüsteki bağ doku artışı, C vitaminine duyulan ihtiyacın artmasına yol açarken, gebeliğin ilerlemesi bu gereksinimi daha da yükseltmektedir (Chattopadhyay ve ark. 1972). Ayrıca, organizmada yeteri kadar C vitamini sentezinin yapılmaması plazma kortizol seviyesini arttırmakta (Hodges ve Hotston 1970), sonuçta metabolizmada strese bağlı mekanizmalar devreye girmektedir. Gebeliğin devamını tehdit eden PGF2α'nın baskılanması ve gebeliğin devamını sağlayan progesteron hormonunun sentezi için C vitamininin gerekli olması, gebelik döneminde bu vitaminin ne kadar önemli bir rol oynadığını göstermektedir (Haliloğlu ve ark. 2008). Zhang ve ark. (2019) C vitamininin in vitro olarak üretilen koyun embriyolarında; embriyo gelişimini düzenlediğini ve embriyo kalitesini arttırdığını bildirmektedirler. Aynı zamanda maternal C vitamini ilavesinin sıcaklık stresi altındaki ineklerde gebelik oranını (Parraguez ve ark. 2011), koyunlarda ise fetal büyüme oranını artırdığını bildiren çalışmalar da mevcuttur (Sales ve ark. 2019). Ancak koyunlarda C vitamini uygulamalarının gebelik ve laktasyon periyodu ile bunlardan doğacak kuzularda biyokimyasal parametreler üzerine etkisini gösteren doyurucu sayıda çalışmaya rastlanılmamıştır. Bu nedenle çalışma; koyunlarda gebeliğin son döneminde uygulanan C vitamininin; geç gebelik, erken ve geç laktasyon dönemlerindeki bazı hematolojik ve biyokimyasal parametreler üzerine etkisini belirlemek ve bunlardan doğacak kuzularda aynı parametreleri değerlendirmek amacıyla planlanmıştır.

MATERYAL ve METOT

Bu çalışmaya Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü Hayvan Deneyleri Yerel Etik Kurulu Başkanlığının 27.09.2017 tarih ve 67 sayılı yerel etik kurul onayı alınarak başlandı.

Çalışma, Konya'nın Karatay ilçesinde bulunan Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü Müdürlüğü'nden sağlanan ortalama canlı ağırlığı 60±4,5 kg olan 2 yaşından büyük ve en az 1 kez doğum yapmış klinik olarak sağlıklı 48 adet Orta Anadolu (Konya) Merinosu ırkı dişi koyun üzerinde gerçekleştirildi.

Çalışmada kullanılan hayvanlarda herhangi bir hastalık, özellikle C vitamini eksikliğine neden olabilecek hastalıkların bulunmamasına dikkat edildi. Yarı kapalı beslenme modeli ile gebe olmayan, gebe ve laktasyondaki koyunların ve yeni doğan kuzuların ihtiyacı olan besin maddeleri dikkate alınarak NRC (National Research Council) standartlarına uygun olarak yemler hazırlandı (NRC 2007). Bu yönü ile tüm hayvanlar tek bir elden beslendi ve yemlemenin ölçüm parametrelerine olumsuz etkileri minimize edildi.

Çalışma öncesi tüm koyunlarda transrektal yolla 7.5 MHz rektal prob kullanılarak B-Mode Real Time ultrasound (Scanner 480 Vet, Esaote Pie Medical, Hollanda) cihazı ile incelemeleri yapılıp gebe olmadıkları tespit edildi. Ardından tüm koyunlara eşit muamele olması amacıyla Medroksiprogesteron Asetat (MPA) emdirilmiş süngerler (Esponjavet, Hipra, İspanya) 14 gün süre ile intravaginal olarak uygulandı ve süngerler çıkarıldıktan sonraki 24 saat içinde eCG (Chrono-gest, MSD, ABD) 1 ml. i.m. enjeksiyonu yapılarak koyunlar arasında östrüs senkronizasyonu sağlandı. Enjeksiyondan 12 saat sonra koyunların arasına arama koçu katılarak östrüse gelen koyunlar belirlenip ayrıldı, başka bir ortamda koç ile çiftleştirme işlemi yapıldı. Elde aşım adı verilen bu işlemle 5 gün boyunca kızgınlığa gelen koyunların çiftleştirilmesi gerçekleştirildi. Aşım sonrası 45. günde gebe kalan koyunların ultrasound muayenesi yapılarak üç gruba ayrıldı, gebe kalmayan koyunlara aşım sonrası 60. günde tekrar ultrasound muayenesi yapılarak, kesin gebe olmadıkları teşhisi konulup negatif kontrol grubu oluşturuldu.

Koyunlar G1 (n:8) negatif (-) kontrol grubu, G2 (n:13) pozitif (+) kontrol grubu, G3 (n:13) gebe 2.5 ml C vitamini grubu, G4 (n:14) gebe 5 ml C vitamini grubu olacak şekilde dört gruba ayrıldı. G1 ve G2 gruplarına enjeksiyon stresi oluşturmak amacıyla serum fizyolojik (%0.9 NaCl) enjeksiyonu yapıldı. Hayvanlara uygulanan tüm müdahaleler öncesinde su ve yem kısıtlamasına gidilmedi.

Gruplara Uygulanan Enjeksiyon Düzeni

-G1; gebe olanlarla aynı zamanda 2.5 ml serum fizyolojik kas içi (i.m. %0.9 NaCl) enjeksiyonu yapıldı.

-G2; gebeliğin 3. ayından sonra (+90 gün) doğuma kadar her hafta 2.5 ml i.m. serum fizyolojik (%0.9 NaCl) enjeksiyonu yapıldı.

-G3; gebeliğin 3. ayından sonra (+90 gün) doğuma kadar her hafta 2,5 ml i.m. vitamin C (Vetaş, Ascorvet, Türkiye) 250 mg/ml (625 mg/CA)) enjeksiyonu yapıldı.
- G4; gebeliğin 3. ayından sonra (+90 gün) doğuma kadar her hafta 5 ml i.m. vitamin C (Vetaş,
Ascorvet, Türkiye) 250 mg/ml (1250 mg/CA)) enjeksiyonu yapıldı.

Kan Örneklerinin Alınması

Kan örnekleri koyunlardan; çalışma başlamadan önce 0.gün (Eylül 2017), gebeliğin 105. (3.5 ay-Ocak 2018) ve 135. (4.5 ay-Şubat 2018) günlerinde, laktasyonun 15. (Erken laktasyon-Mart 2018) ve 75. (Geç laktasyon-Mayıs 2018) günlerinde, kuzulardan ise doğum sonrası 1. (Mart 2018) ve 4. haftalarda (Mart 2018) vena jugularisten alındı.

Hematolojik parametre ölçümleri vakumlu Lityum-Heparinli tüplere (Vacuette, ABD) alınan kan örneklerinde Veteriner Tam Otomatik Kan Analiz Cihazı kullanılarak (Mindray BC-2800Vet, Çin) yapıldı. Biyokimyasal parametreler için vakumlu jelli tüplere (Vacutest Kima, İtalya) kan örnekleri alındı ve 3000 devir/dk'da 10 dk santrifüj edildikten sonra serumları ayrıldı. Ayrılan serumlar analiz yapılıncaya kadar -80ºC'de muhafaza edildi. Biyokimyasal parametreler otoanalizör (ARCHITECT c8000, (ABBOTT, ABD) kullanılarak tespit edildi.

İstatistiksel Analiz

Verilerin normal dağılıma uygunlukları Kolmogrov-Smirnov Testi ile analiz edildi. Grupların karşılaştırılmasında tekrarlı örneklerde iki yönlü (Vitamin C uygulama grupları ve ölçüm zamanı) varyans analizi kullanıldı. Vitamin C uygulama grupları arasındaki çoklu karşılaştırmalar için Duncan testi, ölçüm zamanları arasındaki karşılaştırmalarda ise eşleştirilmiş örneklerde t-testi (bağımlı t-testi) kullanıldı. Sonuçlar ortalama ± standart hata (SEM) şeklinde sunuldu ve gruplar arasındaki farklılığın önemi $p<0.05$ olarak kabul edildi. Gruplar arası farklılıklar tablo ve şekiller üzerinde farklı harfler ile gösterildi. Verilerin analizinde SPSS istatistik programı kullanıldı (SPSS 17.0).

BULGULAR

Klinik Bulgular

Çalışmada, gebelik periyodunda koyunlarda herhangi bir olumsuz klinik bulguya rastlanmadı. Çalışmada gebe olan 40 koyundan (G2, G3 ve G4) toplam 66 adet kuzu dünyaya geldi (16 tanesi postnatal ve ishal sonucu öldü). Bu kuzulardan; doğum sonrasında (+) kontrol grubundaki koyunlardan doğan kuzulardan 7 tanesinin doğum komplikasyonu (postnatal kuzu ölümü) sonucu, 1 tanesinin de ilerleyen günlerde kuzu ishalleri sonucunda; gebe 2.5 ml C vitamini grubundaki koyunlardan doğan kuzulardan 2 tanesinin doğum komplikasyonu (postnatal kuzu ölümleri) sonucu, 3 tanesinin de ilerleyen günlerde kuzu ishalleri sonucunda; gebe 5 ml C vitamini grubundaki koyunlardan doğan kuzulardan 1 tanesinin doğum komplikasyonu (postnatal kuzu ölümleri) sonucu, 2 tanesinin de ilerleyen günlerde kuzu ishalleri sonucunda öldüğü görüldü.

Hematoloji

Hematolojik bulguların değerlendirilmesinde; WBC (Beyaz Küre Sayısı-m/mm3), RBC (Kırmızı Küre Sayısı-m/mm3), MCV (Ortalama Kırmızı Küre Hacmi-fl), MCH (Kırmızı Küredeki Ortalama Hemoglobin-pg), MCHC (Ortalama Eritrosit Hemoglobin Konsantrasyonu), RDW (Kırmızı Küre Dağılım Genişliği), PLT (Platelet), MPV (Ortalama Trombosit Hacmi-fl), PDW (Trombosit Dağılım Genişliği), PCT (Plateletkrit-%), HCT (Hematokrit-%) ve cHGB (Hemoglobin-g/dL) verileri çalışılarak sonuçlar Tablo 2'de verildi.

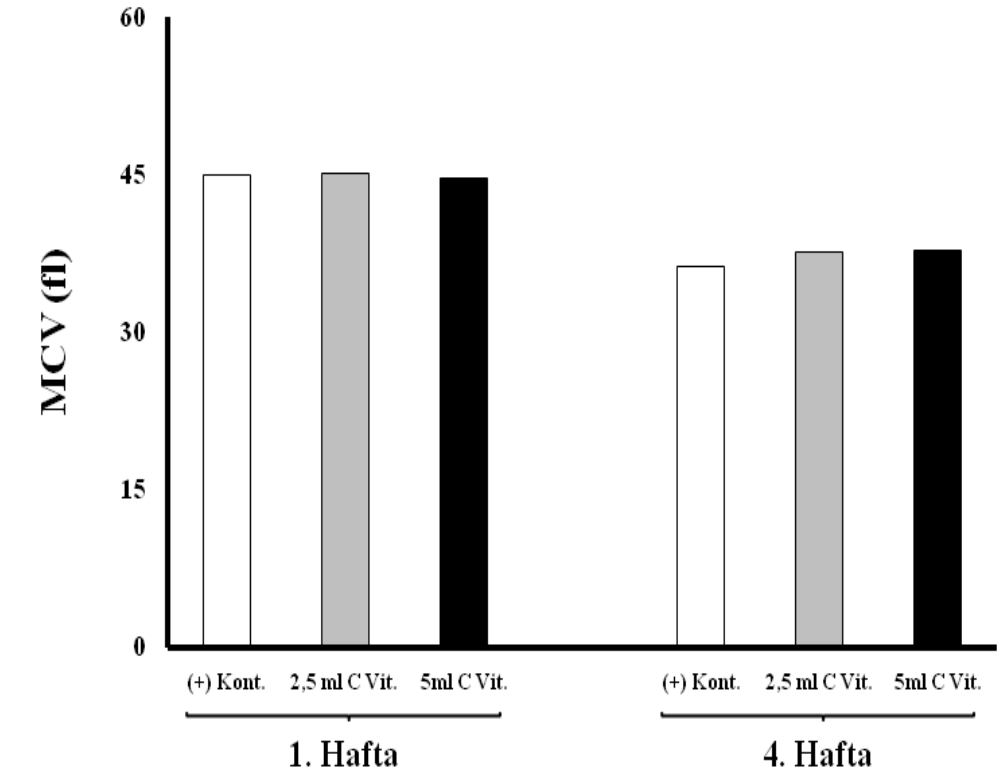
Koyunlarda çalışma grupları arasında grup içi ve gruplar arası kıyaslamalar yapıldığında hematolojik parametrelerde istatistiksel olarak önemli bir fark saptanmadı. Kuzularda ise grup içi kıyaslamalarda MCV parametresinde 1. haftaya göre, 4. haftada G2, G3 ve G4'teki azalma istatistiksel olarak önemli bulundu (p:0.01) (Şekil 1).

Rutin Biyokimya

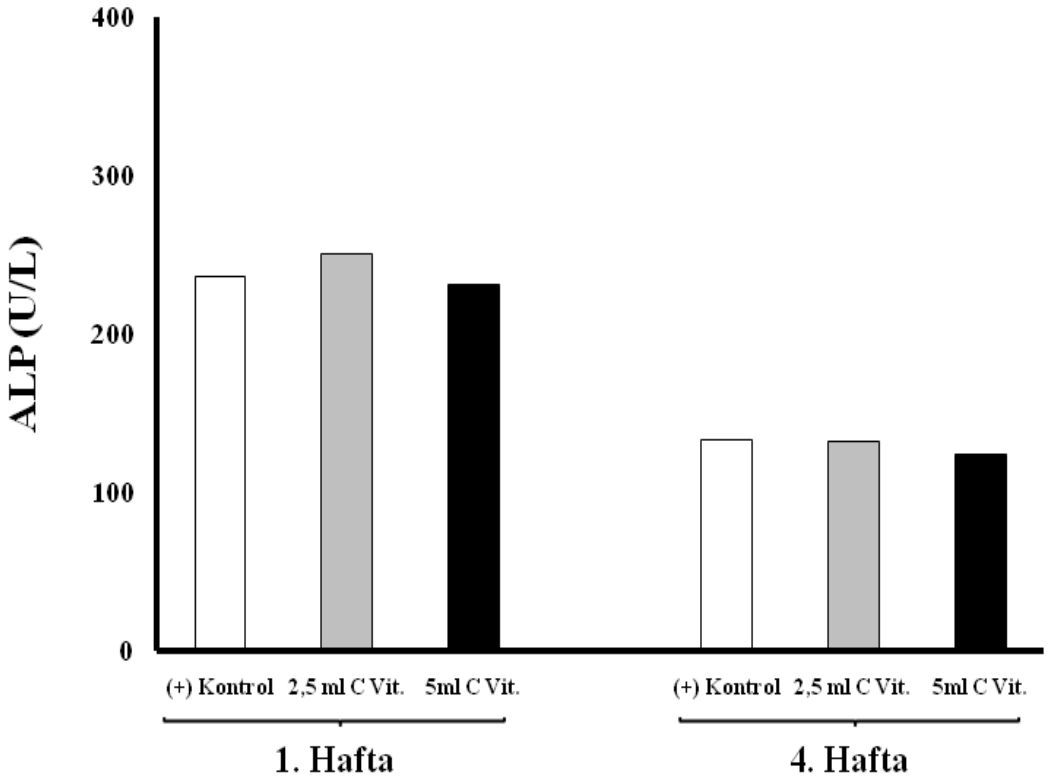
Rutin biyokimya bulgularının değerlendirilmesinde; ALP (Alkalen Fosfataz-IU/L), AST (Aspartat Aminotransferaz-IU/L), ALT (Alanin Aminotransferaz-IU/L), TP (Total Protein-g/dL), Kreatinin (mg/dL), Üre (mg/dL), Glikoz (mg/dL), Laktoz (mmol/L), Na+ (Sodyum-mmol/L), K+ (Potasyum-mmol/L), Ca++ (Kalsiyum-mmol/L) ve Fe (Demir-ug/dL) verileri çalışılarak sonuçlar Tablo. 3, 4 ve 5'te verildi.

Koyunlarda gruplar arası değerlendirmede ALP, AST, ALT, TP, Kreatinin, Üre, Fe, Na+ ve Ca++ parametrelerinde istatistiksel olarak önemli bir fark saptanmadı. Gebe grupların hepsinde gebelik periyodu süresince en yüksek glikoz düzeyine 4.5. ayda ulaşıldı ve bu hayvanlarda gebeliğin 3.5 ve 4.5. aylarında ölçülen glikoz düzeyi (-) kontrol grubu ile karşılaştırıldığında istatistiksel açıdan önemli derecede düşük saptandı. Laktasyonun 1. ayında glikoz düzeyi C vitamini ilavesi yapılmayan gebelerde (G2) daha düşük düzeyde idi. Laktoz düzeyi G3 grubunda diğer gruplara kıyasla laktasyonun 3. ayında gözle görülür derecede artmasına rağmen istatistiki açıdan önemsizdi. Doğum sonrası tüm gruplarda K+ düzeyi laktasyonun 3. ayında laktasyonun 1. ayına göre istatistiki açıdan önemli derecede azaldı (p:0.01).

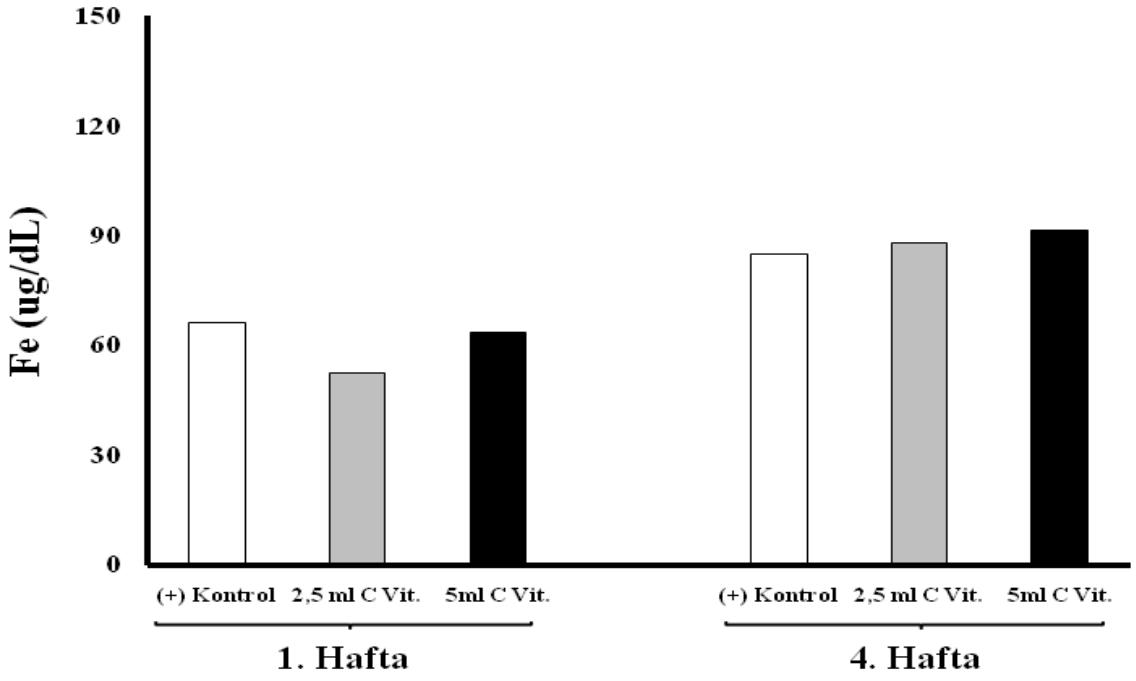
Kuzularda gruplar arası kıyaslamalarda, ALP, AST, ALT, TP, Kreatinin, Üre, Fe, Na+, K+, Ca++, Glikoz ve Laktoz, parametrelerinde istatistiksel olarak önemli bir fark saptanmadı. Grup içi kıyaslamalarda ise bütün gruplarda 4 haftalık kuzularda 1 haftalık kuzulara göre ALP düzeyinde istatistiksel açıdan önemli derecede azalma, Fe düzeyinde ise artma tespit edildi (p:0.01) (Şekil 2-3)



Şekil 1: Gebe gruplardaki koyunlardan (G2, G3 ve G4) doğan kuzularda MCV (fl) parametresinin 1. ve 4. haftadaki değerlerinin grup içi karşılaştırılması.
Figure 1: Intragroup comparison of the values of MCV (fl) parameter at 1st and 4th weeks in lambs born from ewes (G2, G3 and G4) in the pregnant groups.



Şekil 2: Gebe gruplardaki koyunlardan (G2, G3 ve G4) doğan kuzularda ALP (U/L) parametresinin 1. ve 4. haftadaki değerlerinin grup içi karşılaştırılması.
Figure 2: Intragroup comparison of the values of ALP (U/L) parameter at 1st and 4th weeks in lambs born from ewes (G2, G3 and G4) in the pregnant groups.



Şekil 3: Gebe gruplardaki koyunlardan (G2, G3 ve G4) doğan kuzularda Fe (ug/dL) parametresinin 1. ve 4. haftadaki değerlerinin grup içi karşılaştırılması.
Figure 3: Intragroup comparison of the values of Fe (ug/dL) parameter at 1st and 4th weeks in lambs born from ewes (G2, G3 and G4) in the pregnant groups.

Tablo 1. Koyunlarda gebeliğin ilk 100 ve son 50 günleri ile laktasyonun ilk 75, 75. günden sonraki rasyonları
Table 1. The rations of sheep in the first 100 and last 50 days of pregnancy and after the first 75 and 75 days of lactation

Yem Cinsi	Koyunların Gebeliklerinin İlk 100 Gününde Aldıkları Yem Miktarı	Koyunların Gebeliklerinin Son 50 Günü ve Laktasyonun İlk 75 Gününde Aldıkları Yem Miktarı	Koyunların Laktasyonun 75. Gününden Sonra Aldıkları Yem Miktarı
Kesif Yem	250 gr.	500 gr.	200 gr.
Buğday Sapı	250 gr.	250 gr.	250 gr.
Kuru Yonca	500 gr.	500 gr.	500 gr.
Mısır Silajı	1.5 kg.	1 kg.	1 kg.
Ad Libitum Su			

Kuzular İlk 10 gün; ad libitum anne sütü ile, 10. günden sonra ad libitum anne sütü, ad libitum kuzu başlangıç yemi, kuru yonca, ad libitum su ile beslendi. (TABLO 1 in altına gelmeli)

Tablo 2. Negatif kontrol (G1), pozitif kontrol (G2), 2.5 ml vitamin C (G3) ve 5ml vitamin C (G4) gruplarında çalışma başlangıcı (0. Gün), 3.5. Ay, 4.5. Ay, Laktasyonun 1. ve 3. Ayındaki hematolojik parametre değerleri (ORT±SEM) ile gebe koyunlardan doğan kuzuların 1 ve 4 haftalık yaşlarındaki hematolojik parametre değerlerinin istatistiksel karşılaştırması.

Table 2. Start of study (Day 0), 3.5 Months, 4.5 Months, 1st and 3rd Months of Lactation in negative control (G1), positive control (G2), 2.5ml vitamin C (G3) and 5ml vitamin C (G4) groups Statistical comparison of hematological parameter values (ORT±SEM) and hematological parameter values of lambs born from pregnant ewes at 1 and 4 weeks of age.

		Uygulama Grupları					**Uygulama Grupları**		
		Koyun					**Kuzu**		
Parametre	**Zaman**	G1 (n:8)	G2 (n:13)	G3 (n:13)	G4 (n:14)	**Zaman**	G2 (n:13)	G3 (n:17)	G4 (n:20)
WBC (m/mm^3)	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	7,82 ± 0,76 8,29 ± 0,53 8,10 ± 0,90 9,21 ± 0,85 8,42 ± 0,79	7,77 ± 0,58 8,78 ± 0,41 8,29 ± 0,69 9,24 ± 0,65 7,79 ± 0,60	8,05 ± 0,61 8,51 ± 0,43 8,60 ± 0,72 9,31 ± 0,68 7,83 ± 0,63	8,01 ± 0,56 8,11 ± 0,39 8,75 ± 0,66 9,54 ± 0,63 8,26 ± 0,58	1.hafta 4.hafta	8,73 ± 0,88 9,31 ± 0,64	7,70 ± 0,77 8,83 ± 0,56	8,31 ± 0,71 9,36 ± 0,52
RBC (M/mm^3)	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	11,54 ± 0,50 9,66 ± 0,30 9,64 ± 0,36 10,02 ± 0,51 11,12 ± 0,60	10,41 ± 0,38 8,31 ± 0,23 8,11 ± 0,28 8,31 ± 0,39 8,19 ± 0,46	11,24 ± 0,40 9,13 ± 0,24 8,41 ± 0,29 9,34 ± 0,41 10,08 ± 0,48	11,04 ± 0,35 8,96 ± 0,21 8,40 ± 0,26 8,89 ± 0,36 8,95 ± 0,42	1.hafta 4.hafta	9,23 ± 0,33 10,61 ± 0,32	8,77 ± 0,29 10,33 ± 0,28	9,17 ± 0,27 10,57 ± 0,26
MCV (fl)	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	35,69 ± 0,87 41,21 ± 0,93 41,83 ± 0,90 42,20 ± 1,05 41,88 ± 1,17	37,43 ± 0,66 42,56 ± 0,71 43,94 ± 0,69 44,61 ± 0,80 43,73 ± 0,90	34,34 ± 0,69 39,79 ± 0,74 40,84 ± 0,72 41,21 ± 0,83 39,28 ± 0,94	35,38 ± 0,61 40,99 ± 0,66 42,50 ± 0,64 43,35 ± 0,74 42,72 ± 0,83	1.hafta 4.hafta	$45,01 \pm 1,00^{A}$ $36,26 \pm 0,92^{B}$	$45,15 \pm 0,87^{A}$ $37,72 \pm 0,81^{B}$	$44,73 \pm 0,81^{A}$ $37,78 \pm 0,75^{B}$
MCH (pg)	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	8,99 ± 0,54 9,97 ± 0,23 10,97 ± 0,24 10,83 ± 0,31 10,79 ± 0,40	9,30 ± 0,36 10,42 ± 0,16 11,71 ± 0,16 11,54 ± 0,21 11,43 ± 0,27	8,96 ± 0,40 10,11 ± 0,17 11,44 ± 0,17 11,14 ± 0,23 10,71 ± 0,30	8,39 ± 0,35 10,24 ± 0,15 11,54 ± 0,15 11,36 ± 0,20 11,34 ± 0,26	1.hafta 4.hafta	12,45 ± 0,32 9,39 ± 0,24	12,27 ± 0,28 9,84 ± 0,21	12,29 ± 0,26 9,80 ± 0,20
MCHC	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	25,29 ± 0,42 24,51 ± 0,35 26,59 ± 0,35 26,00 ± 0,35 26,07 ± 0,36	25,12 ± 0,32 24,68 ± 0,27 26,87 ± 0,27 26,04 ± 0,27 25,76 ± 0,28	26,37 ± 0,33 25,61 ± 0,28 27,84 ± 0,28 27,17 ± 0,28 27,00 ± 0,29	25,72 ± 0,29 25,07 ± 0,25 27,27 ± 0,25 26,27 ± 0,25 26,63 ± 0,26	1.hafta 4.hafta	27,81 ± 0,28 25,95 ± 0,27	27,24 ± 0,24 26,22 ± 0,24	27,40 ± 0,22 26,06 ± 0,22
RDW	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	16,13 ± 0,36 13,33 ± 0,35 13,59 ± 0,34 13,59 ± 0,42 14,26 ± 0,44	15,77 ± 0,28 13,66 ± 0,27 13,25 ± 0,26 12,73 ± 0,32 13,55 ± 0,34	16,11 ± 0,29 14,50 ± 0,28 13,78 ± 0,27 13,34 ± 0,33 13,81 ± 0,35	16,29 ± 0,26 14,13 ± 0,25 13,86 ± 0,24 13,62 ± 0,29 13,75 ± 0,31	1.hafta 4.hafta	19,46 ± 0,89 18,53 ± 0,73	19,67 ± 0,78 18,14 ± 0,64	19,70 ± 0,72 17,34 ± 0,59

PLT	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	119,59 ± 23,96 120,86 ± 36,23 127,29 ± 39,60 126,86 ± 15,64 125,83 ± 19,99	130,00 ± 18,30 129,75 ± 27,67 129,33 ± 30,25 130,36 ± 11,95 131,25 ± 15,27	125,80 ± 20,05 124,73 ± 30,31 126,20 ± 33,13 124,26 ± 13,09 128,30 ± 16,72	127,50 ± 16,95 126,23 ± 25,62 132,86 ± 28,00 127,54 ± 11,06 126,71 ± 14,13	1.hafta 4.hafta	169,15 ± 24,12 174,46 ± 30,26	105,88 ± 21,10 217,65 ± 26,46	111,75 ± 19,45 200,85 ± 24,39
MPV (fl)	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	5,08 ± 0,11 5,53 ± 0,13 5,73 ± 0,20 5,69 ± 0,19 5,68 ± 0,15	5,13 ± 0,09 5,59 ± 0,10 6,07 ± 0,15 6,45 ± 0,14 5,86 ± 0,12	4,99 ± 0,09 5,40 ± 0,11 5,84 ± 0,16 5,94 ± 0,15 5,60 ± 0,12	4,94 ± 0,08 5,35 ± 0,09 5,91 ± 0,14 6,05 ± 0,13 6,01 ± 0,11	1.hafta 4.hafta	5,48 ± 0,16 4,98 ± 0,12	5,47 ± 0,14 5,29 ± 0,11	5,31 ± 0,13 5,29 ± 0,10
PDW	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	13,77 ± 0,19 14,56 ± 0,22 14,61 ± 0,25 14,41 ± 0,23 14,15 ± 0,19	14,04 ± 0,15 14,78 ± 0,17 15,08 ± 0,19 15,48 ± 0,17 14,68 ± 0,15	13,68 ± 0,15 14,21 ± 0,17 14,74 ± 0,20 14,83 ± 0,18 14,20 ± 0,15	13,68 ± 0,13 14,24 ± 0,15 14,69 ± 0,18 14,94 ± 0,16 14,78 ± 0,13	1.hafta 4.hafta	14,21 ± 0,24 13,64 ± 0,18	14,18 ± 0,21 14,05 ± 0,16	14,01 ± 0,19 13,89 ± 0,15
PCT (%)	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	0,08 ± 0,01 0,07 ± 0,02 0,11 ± 0,03 0,07 ± 0,01 0,05 ± 0,02	0,08 ± 0,01 0,07 ± 0,02 0,08 ± 0,02 0,10 ± 0,01 0,06 ± 0,02	0,07 ± 0,01 0,05 ± 0,02 0,08 ± 0,02 0,08 ± 0,01 0,08 ± 0,01	0,06 ± 0,01 0,07 ± 0,01 0,08 ± 0,02 0,08 ± 0,01 0,08 ± 0,02	1.hafta 4.hafta	0,10 ± 0,02 0,10 ± 0,02	0,06 ± 0,02 0,12 ± 0,02	0,08 ± 0,02 0,11 ± 0,01
HCT (%)	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	28,14 ± 1,00 26,57 ± 0,89 27,71 ± 1,11 30,43 ± 1,82 32,29 ± 1,65	27,25 ± 0,76 23,67 ± 0,68 25,00 ± 0,84 25,75 ± 1,39 25,58 ± 1,26	27,27 ± 0,80 23,55 ± 0,71 24,55 ± 0,88 26,82 ± 1,45 27,09 ± 1,31	26,93 ± 0,71 24,43 ± 0,63 24,86 ± 0,78 27,71 ± 1,29 26,86 ± 1,16	1.hafta 4.hafta	29,69 ± 1,25 26,23 ± 0,65	27,18 ± 1,10 25,29 ± 0,57	29,50 ± 1,01 26,10 ± 0,52
cHGB (g/dL)	0. gün 3.5. ay 4.5. ay Laktasyon 1. Ay Laktasyon 3. ay	9,73 ± 0,34 9,20 ± 0,31 9,50 ± 0,36 10,91 ± 0,52 10,93 ± 0,55	9,27 ± 0,26 8,07 ± 0,24 8,52 ± 0,28 8,69 ± 0,40 8,72 ± 0,42	9,32 ± 0,27 8,03 ± 0,25 8,32 ± 0,29 9,11 ± 0,41 9,23 ± 0,44	9,14 ± 0,25 8,32 ± 0,23 8,32 ± 0,27 8,90 ± 0,38 9,02 ± 0,40	1.hafta 4.hafta	10,11 ± 0,43 8,93 ± 0,23	9,21 ± 0,37 8,59 ± 0,20	10,02 ± 0,34 8,91 ± 0,19

Aynı sütundaki farklı harfler (A, B); gruplar (Ölçüm zamanları) arasındaki farkın istatistiksel olarak önemli olduğunu göstermektedir (p:0.01).
Different letters in the same column (A, B); shows that the difference between the groups (Measurement times) is statistically significant (p:0.01).

Tablo 3. Negatif kontrol (G1), pozitif kontrol (G2), 2.5 ml vitamin C (G3) ve 5ml vitamin C (G4) gruplarında çalışma başlangıcı (0. Gün), 3.5. Ay, 4.5. Ay, Laktasyonun 1. ve 3. Ayındaki biyokimyasal parametre değerleri (ORT±SEM) ile gebe koyunlardan doğan kuzuların 1 ve 4 haftalık yaşlarındaki biyokimyasal parametre değerlerinin istatistiksel karşılaştırması.

Table 3. Start of study (Day 0), 3.5 Months, 4.5 Months, 1st and 3rd Months of Lactation in negative control (G1), positive control (G2), 2.5ml vitamin C (G3) and 5ml vitamin C (G4) groups Statistical comparison of biochemical parameter values (ORT±SEM) and biochemical parameter values of lambs born from pregnant ewes at 1 and 4 weeks of age.

		Uygulama Grupları					**Uygulama Grupları**		
		Koyun					**Kuzu**		
Parametre	**Zaman**	G1 (n:8)	G2 (n:13)	G3 (n:13)	G4 (n:14)	**Zaman**	G2 (n:13)	G3 (n:17)	G4 (n:20)
ALP (IU/L)	0. gün	34,14 ± 10,62	32,17 ± 8,11	32,27 ± 8,47	32,92 ± 7,79	1.hafta 4.hafta	236,46 ± 38,82^{A} 133,08 ± 19,47^{B}	250,77 ± 33,95^{A} 131,71 ± 17,03^{B}	230,80 ± 31,30^{A} 124,45 ± 15,70^{B}
	3.5. ay	32,71 ± 8,10	35,00 ± 6,19	35,91 ± 6,46	30,23 ± 5,94				
	4.5. ay	30,86 ± 7,97	30,75 ± 6,08	29,91 ± 6,35	31,85 ± 5,85				
	Laktasyon 1. Ay	33,14 ± 6,24	35,83 ± 4,77	35,64 ± 4,98	36,08 ± 4,58				
	Laktasyon 3. ay	32,86 ± 4,58	37,50 ± 3,49	35,46 ± 3,65	33,69 ± 3,36				
AST (IU/L)	0. gün	38,57 ± 8,12	34,33 ± 6,20	39,40 ± 6,80	35,39 ± 5,96	1.hafta 4.hafta	27,54 ± 2,57 26,39 ± 12,44	24,82 ± 2,25 28,00 ± 10,88	26,00 ± 2,07 27,35 ± 10,03
	3.5. ay	38,00 ± 6,00	31,00 ± 4,58	36,10 ± 5,02	41,54 ± 4,40				
	4.5. ay	34,14 ± 7,16	37,42 ± 5,47	37,10 ± 5,99	33,46 ± 5,25				
	Laktasyon 1. Ay	33,43 ± 6,03	35,58 ± 4,61	35,20 ± 5,04	32,69 ± 4,42				
	Laktasyon 3. ay	39,14 ± 4,40	39,00 ± 3,36	42,10 ± 3,68	40,31 ± 3,23				
ALT (IU/L)	0. gün	9,56 ± 1,31	9,67 ± 1,00	9,10 ± 1,10	8,79 ± 0,93	1.hafta 4.hafta	5,08 ± 0,06 7,31 ± 1,54	5,00 ± 0,05 5,29 ± 1,34	5,05 ± 0,05 7,00 ± 1,24
	3.5. ay	9,43 ± 1,38	8,25 ± 1,06	8,10 ± 1,16	9,14 ± 0,98				
	4.5. ay	8,43 ± 1,25	9,00 ± 0,96	8,50 ± 1,05	9,64 ± 0,89				
	Laktasyon 1. Ay	8,43 ± 0,55	8,92 ± 0,42	8,80 ± 0,46	8,86 ± 0,39				
	Laktasyon 3. ay	8,14 ± 0,70	8,00 ± 0,53	8,30 ± 0,58	8,00 ± 0,49				

Aynı sütundaki farklı harfler (A, B); gruplar (Ölçüm zamanları) arasındaki farkın istatistiksel olarak önemli olduğunu göstermektedir (p:0.01).
Different letters in the same column (A, B); shows that the difference between the groups (Measurement times) is statistically significant (p:0.01).

Tablo 4. Negatif kontrol (G1), pozitif kontrol (G2), 2.5 ml vitamin C (G3) ve 5ml vitamin C (G4) gruplarında çalışma başlangıcı (0. Gün), 3.5. Ay, 4.5. Ay, Laktasyonun 1. ve 3. Ayındaki biyokimyasal parametre değerleri (ORT±SEM) ile gebe koyunlardan doğan kuzuların 1 ve 4 haftalık yaşlarındaki biyokimyasal parametre değerlerinin istatistiksel karşılaştırması.

Table 4. Start of study (Day 0), 3.5 Months, 4.5 Months, 1st and 3rd Months of Lactation in negative control (G1), positive control (G2), 2.5ml vitamin C (G3) and 5ml vitamin C (G4) groups Statistical comparison of biochemical parameter values (ORT±SEM) and biochemical parameter values of lambs born from pregnant ewes at 1 and 4 weeks of age.

		Uygulama Grupları					**Uygulama Grupları**		
		Koyun					**Kuzu**		
Parametre	**Zaman**	G1 (n:8)	G2 (n:13)	G3 (n:13)	G4 (n:14)	**Zaman**	G2 (n:13)	G3 (n:17)	G4 (n:20)
TP (g/dL)	0. gün	5,33 ± 0,69	5,10 ± 0,53	5,46 ± 0,55	6,09 ± 0,49	1.hafta	2,70 ± 0,34	2,19 ± 0,30	2,57 ± 0,28
	3.5. ay	3,21 ± 0,52	2,56 ± 0,40	2,60 ± 0,42	3,13 ± 0,37	4.hafta	2,31 ± 0,25	1,93 ± 0,22	1,94 ± 0,20
	4.5. ay	3,26 ± 0,60	3,33 ± 0,46	3,16 ± 0,48	3,41 ± 0,43				
	Laktasyon 1. Ay	2,79 ± 0,49	2,91 ± 0,37	2,77 ± 0,39	2,49 ± 0,35				
	Laktasyon 3. ay	3,11 ± 0,32	2,81 ± 0,25	2,98 ± 0,26	3,08 ± 0,23				
Kreatinin (mg/dL	0. gün	0,54 ± 0,07	0,59 ± 0,06	0,62 ± 0,06	0,62 ± 0,05	1.hafta	0,28 ± 0,02	0,27 ± 0,02	0,28 ± 0,02
	3.5. ay	0,36 ± 0,04	0,33 ± 0,03	0,33 ± 0,03	0,33 ± 0,03	4.hafta	0,30 ± 0,02	0,28 ± 0,02	0,27 ± 0,02
	4.5. ay	0,39 ± 0,06	0,44 ± 0,05	0,39 ± 0,05	0,42 ± 0,05				
	Laktasyon 1. Ay	0,36 ± 0,04	0,37 ± 0,03	0,34 ± 0,03	0,32 ± 0,03				
	Laktasyon 3. ay	0,41 ± 0,04	0,38 ± 0,03	0,38 ± 0,03	0,37 ± 0,03				
Üre (mg/dL)	0. gün	18,04 ± 3,58	16,39 ± 2,63	14,90 ± 3,00	15,23 ± 2,63	1.hafta	17,31 ± 3,64	14,00 ± 3,18	19,25 ± 2,94
	3.5. ay	17,29 ± 0,99	15,08 ± 0,73	16,50 ± 0,83	15,69 ± 0,73	4.hafta	17,23 ± 1,79	15,59 ± 1,57	15,30 ± 1,44
	4.5. ay	18,29 ± 2,38	17,39 ± 1,75	18,70 ± 1,99	19,85 ± 1,75				
	Laktasyon 1. Ay	17,57 ± 3,41	18,77 ± 2,50	15,90 ± 2,85	15,85 ± 2,50				
	Laktasyon 3. ay	17,86 ± 2,40	19,31 ± 1,76	17,60 ± 2,01	16,62 ± 1,76				
Glikoz (mg/dL)	0. gün	58,86 ± 2,23^{A}	59,58 ± 1,70^{AB}	59,27 ± 1,78^{A}	55,79 ± 1,58^{A}	1.hafta	111,85 ± 5,78	107,53 ± 5,06	106,10 ± 4,66
	3.5. ay	69,00 ± 2,15^{Ba}	56,92 ± 1,64^{Ab}	55,18 ± 1,71^{Ab}	57,50 ± 1,52^{Ab}	4.hafta	94,46 ± 3,05	91,29 ± 2,67	93,45 ± 2,46
	4.5. ay	82,71 ± 6,5^{C}	66,08 ± 4,97^{B}	70,18 ± 5,19^{B}	78,14 ± 4,60^{B}				
	Laktasyon 1. Ay	73,29 ± 3,82^{B}	70,25 ± 2,92^{C}	71,82 ± 3,05^{B}	70,29 ± 2,70^{BC}				
	Laktasyon 3. ay	66,86 ± 4,38^{AB}	70,42 ± 3,35^{C}	75,09 ± 3,50^{B}	66,43 ± 3,10^{B}				
Laktoz (mmol/L)	0. gün	1,65 ± 0,47^{A}	1,40 ± 0,38^{AC}	2,21 ± 0,38^{AB}	1,20 ± 0,34^{A}	1.hafta	1,93 ± 0,32	2,58 ± 0,28	2,50 ± 0,26
	3.5. ay	1,11 ± 0,21^{A}	1,02 ± 0,17^{A}	1,44 ± 0,17^{B}	1,20 ± 0,15^{A}	4.hafta	2,44 ± 0,29	2,49 ± 0,25	2,78 ± 0,23
	4.5. ay	2,95 ± 0,50^{Aa}	1,24 ± 0,40^{BCb}	2,43 ± 0,40^{ACab}	1,90 ± 0,35^{ABab}				
	Laktasyon 1. Ay	5,78 ± 0,72^{Ba}	3,40 ± 0,57^{Cb}	2,65 ± 0,57^{ACb}	2,48 ± 0,51^{Bb}				
	Laktasyon 3. ay	4,17 ± 0,86^{B}	3,13 ± 0,69^{B}	4,25 ± 0,69^{C}	2,10 ± 0,61^{B}				

Aynı sütundaki farklı harfler (A, B, C); gruplar (Ölçüm zamanları) arasındaki farkın istatistiksel olarak önemli olduğunu göstermektedir ($p<0.05$). Aynı satırdaki farklı harfler (a, b); gruplar (Uygulama grupları) arasındaki farkın istatistiksel olarak önemli olduğunu göstermektedir (p:0.01).

Different letters in the same column (A, B, C); shows that the difference between the groups (Measurement times) is statistically significant (p<0.05). Different letters on the same line (a, b); shows that the difference between the groups (Application groups) is statistically significant (p:0.01).

Tablo 5. Negatif kontrol (G1), pozitif kontrol (G2), 2.5 ml vitamin C (G3) ve 5ml vitamin C (G4) gruplarında çalışma başlangıcı (0. Gün), 3.5. Ay, 4.5. Ay, Laktasyonun 1. ve 3. Ayındaki biyokimyasal parametre değerleri (ORT±SEM) ile gebe koyunlardan doğan kuzuların 1 ve 4 haftalık yaşlarındaki biyokimyasal parametre değerlerinin istatistiksel karşılaştırması.

Table 5. Start of study (Day 0), 3.5 Months, 4.5 Months, 1st and 3rd Months of Lactation in negative control (G1), positive control (G2), 2.5ml vitamin C (G3) and 5ml vitamin C (G4) groups Statistical comparison of biochemical parameter values (ORT±SEM) and biochemical parameter values of lambs born from pregnant ewes at 1 and 4 weeks of age.

		Uygulama Grupları					**Uygulama Grupları**		
		Koyun					**Kuzu**		
Parametre	**Zaman**	G1 (n:8)	G2 (n:13)	G3 (n:13)	G4 (n:14)	**Zaman**	G2 (n:13)	G3 (n:17)	G4 (n:20)
Na^+ (mmol/L)	0. gün	144,86 ± 0,57	144,83 ± 0,43	144,55 ± 0,45	144,07 ± 0,40				
	3.5. ay	152,29 ± 1,38	149,42 ± 1,06	150,36 ± 1,10	149,93 ± 0,98				
	4.5. ay	152,57 ± 0,75	151,17 ± 0,58	151,73 ± 0,60	151,57 ± 0,53	1.hafta	142,62 ± 0,85	146,29 ± 0,74	143,45 ± 0,68
	Laktasyon 1. Ay	153,00 ± 0,76	153,08 ± 0,58	152,91 ± 0,61	152,36 ± 0,54	4.hafta	146,69 ± 0,65	148,59 ± 0,57	147,70 ± 0,52
	Laktasyon 3. ay	148,86 ± 0,62	147,33 ± 0,48	147,73 ± 0,50	146,29 ± 0,44				
K^+ (mmol/L)	0. gün	4,97 ± 0,18	4,85 ± 0,14^{A}	4,94 ± 0,14^{A}	4,89 ± 0,13^{AB}				
	3.5. ay	4,93 ± 0,14	4,66 ± 0,11^{AB}	4,71 ± 0,11^{A}	4,81 ± 0,10^{AB}				
	4.5. ay	4,87 ± 0,16	4,59 ± 0,12^{B}	4,71 ± 0,13^{A}	4,64 ± 0,11^{B}	1.hafta	4,48 ± 0,17	4,64 ± 0,15	4,34 ± 0,14
	Laktasyon 1. Ay	4,93 ± 0,22 ^{a}	5,92 ± 0,17^{Cb}	6,08 ± 0,18^{Bb}	5,71 ± 0,16^{Cb}	4.hafta	4,69 ± 0,12	4,83 ± 0,10	4,91 ± 0,10
	Laktasyon 3. ay	4,71 ± 0,20	4,75 ± 0,15^{AB}	5,06 ± 0,16^{A}	5,03 ± 0,14 ^{A}				
Ca^{++} (mmol/L)	0. gün	1,25 ± 0,03	1,23 ± 0,02	1,23 ± 0,02	1,24 ± 0,02				
	3.5. ay	1,28 ± 0,02	1,27 ± 0,02	1,25 ± 0,02	1,26 ± 0,02				
	4.5. ay	1,21 ± 0,04	1,17 ± 0,03	1,19 ± 0,03	1,18 ± 0,03	1.hafta	1,37 ± 0,03	1,39 ± 0,02	1,40 ± 0,02
	Laktasyon 1. Ay	1,25 ± 0,03	1,19 ± 0,02	1,18 ± 0,02	1,20 ± 0,02	4.hafta	1,38 ± 0,02	1,38 ± 0,02	1,38 ± 0,02
	Laktasyon 3. ay	1,30 ± 0,03	1,26 ± 0,02	1,21 ± 0,02	1,22 ± 0,02				
Fe (ug/dL)	0. gün	64,71 ± 11,93	64,00 ± 9,11	66,70 ± 9,98	66,14 ± 8,44				
	3.5. ay	65,43 ± 14,45	66,25 ± 11,03	68,00 ± 12,09	68,71 ± 10,22				
	4.5. ay	64,00 ± 14,65	61,50 ± 11,19	62,00 ± 12,26	63,00 ± 10,36	1.hafta	66,08 ± 11,76^{A}	52,53 ± 10,28^{A}	63,40 ± 9,48^{A}
	Laktasyon 1. Ay	60,86 ± 7,77	66,00 ± 5,94	61,30 ± 6,50	64,86 ± 5,50	4.hafta	84,85 ± 14,15^{B}	87,88 ± 12,37^{B}	91,55 ± 11,40^{B}
	Laktasyon 3. ay	60,86 ± 7,77	60,08 ± 6,31	67,00 ± 6,92	68,64 ± 5,85				

Aynı sütundaki farklı harfler (A, B, C); gruplar (Ölçüm zamanları) arasındaki farkın istatistiksel olarak önemli olduğunu göstermektedir (p<0.05). Aynı satırdaki farklı harfler (a, b); gruplar (Uygulama grupları) arasındaki farkın istatistiksel olarak önemli olduğunu göstermektedir (p:0.01).

Different letters in the same column (A, B, C); shows that the difference in the groups (measurement times) is statistically significant (p<0.05). Different letters on the same line (a, b); shows that the difference between the groups (Application groups) is statistically significant (p:0.01).

TARTIŞMA

Sunulan çalışmada, koyunlarda gebeliğin son aylarında enjeksiyon şeklinde yapılan C vitamini ilavesinin gebelik ve laktasyon döneminde hematoloji ve bazı biyokimya parametreleri üzerine olan etkisi değerlendirilerek bu annelerden doğan kuzularda da aynı parametreler incelendi. C vitamini rumen sindiriminden etkilendiği için oral yolla yapılan uygulamalara uygun değildir (Cummins ve ark. 1992). Bu sebeple sunulan çalışmada C vitamini enjeksiyonu gebe gruplardan ikisine i.m. olarak iki farklı dozda (2.5-5 ml dozlarında) ve gebeliğin 3. ayından sonra (+90) doğuma kadar uygulandı.

Bu çalışmada annelerde gebelik esnasında hiçbir grupta abort şekillenmedi. Ancak gebelikte C vitamini ilavesi yapılsın ya da yapılmasın gebe grupların hepsinde farklı oranlarda ölü doğumlar gerçekleşti. Bununla birlikte bu annelerden doğan toplam 6 adet kuzuda da ilerleyen günlerde ishal sonucu ölümler meydana geldi. Kuzu ölümleri incelendiğinde; C vitamini enjeksiyonu yapılan koyunlarda postnatal kuzu ölüm sayısının belirgin derecede düştüğü, ishal sonucu ölümlerin ise birbirine yakın olduğu görüldü. Bu durum farklı dozlarda uygulanan C vitamininin annelerde gebelik stresini azaltarak hem anne hem de yavru için daha iyi bir gebelik süreci oluşturduğunu, ayrıca C vitamini uygulanan gruplarda daha az postnatal kuzu ölümlerinin görülmesinde C vitamininin olumlu etkisi olduğunu düşündürmektedir.

Canlıların hayatlarında önemli bir yer tutan gebelik, doğum ve laktasyon gibi kritik dönemlerde metabolizmanın genel işleyişiyle birlikte, canlı vücudunda besin ve diğer maddelerin taşınmasında esasi görevi olan kan parametrelerinin takibi canlının daha optimal şartlarda hayatını sürdürmesine olanak sağlamaktadır. Bu amaçla hematoloji bulgularının değerlendirilmesi canlının içinde bulunduğu dönemi anlamak açısından birçok avantaj sunmaktadır.

Koyun ve keçilerde; WBC düzeyinin gebelikte yüksek, laktasyonda düşük olduğu, RBC, Hb ve PCV düzeylerinin gebelikte giderek azaldığı ve erken laktasyon döneminde de düşük düzeylerde kaldığı rapor edilmiştir (Mbassa ve Poulsen 1991, Jain 1993). Yapılan bazı çalışmalarda koyun ve keçilerde MCH, MCHC, RBC, PCV ve Hb düzeylerinde gebelikte artış, laktasyonda azalma olduğu bildirilirken (Mbassa ve Poulsen 1991, Sharma ve ark. 2015, Badawi ve AL-Hadithy 2014), aksine bazı literatürlerde PCV, Hb, RBC ve Hct düzeylerinde laktasyonda artış olduğu gösterilmiştir (El-Sherif 2001, Mohammed ve ark. 2014, de Oliveira ve ark. 2016). Bunun yanı sıra koyunlarda laktasyonda Hb, Hct, WBC, RBC, PCV, MCV, MCH ve MCHC düzeylerinde önemli bir farklılık olmadığını bildiren yayınlar da mevcuttur (Antunovic ve ark. 2011, Iriadam 2007, Manat ve ark. 2016). Durotoye (1987) gebe koyunlarda MCV düzeyinin, kuru döneme göre daha yüksek olduğunu, bu durumun gebelik dönemindeki RBC ozmotik direncinin kuru dönemden daha fazla olmasından kaynaklanabileceğini ileri sürmüştür. Sütçü ineklerde yapılan çeşitli çalışmalarda; Belic ve ark. (2010) Hb ve RBC düzeylerinin laktasyonda gebelik dönemine göre daha düşük olduğunu, Blum ve ark. (1983) erken ve geç laktasyon dönemlerinde Hb ve PCV seviyelerinde istatistiksel olarak önemli bir fark olmadığını, Koubkova ve ark. (2002) laktasyon döneminde Hct ve RBC seviyelerinde, Toharmat ve ark. (1998) ise periparturient dönemde Hb ve Hct düzeylerinde belirgin bir artış olduğunu rapor etmişlerdir. Çalışmamızda bazı literatürlerle uyumlu olarak C vitamini ilavesinin gebelik ve laktasyonda hematolojik değerlerde istatistiksel açıdan önemli bir farklılık oluşturmadığı gözlendi (Antunovic ve ark. 2011, Iriadam 2007, Manat ve ark.2016, Blum ve ark. 1983).

Antunovic ve ark. (2012) kuzularda doğum sonrası ilk ayda kan WBC, RBC, Hct ve cHGB seviyelerinde, Tennant ve ark. (1974) ise doğum sonrası ilk haftalarda RBC seviyesinde azalma olduğunu bildirmektedirler. Knowles ve ark. (2000) buzağılarda RBC, Hct, cHGB, MCV, MCH ve MCHC seviyelerinin doğum sonrası ilk 12 hafta boyunca yetişkinlere göre daha düşük olduğunu bildirirken, Jain (1986) buzağılarda WBC seviyesinin doğumda yüksek olduğunu ancak doğum sonrası 3. haftaya kadar yetişkinlerdeki seviyeye kadar azaldığını rapor etmiştir. Bazı yazarlar oğlaklarda düşük olan MCV seviyesinin, RBC düzeyindeki azalmayla birlikte yaş ilerledikçe arttığını bildirirken (Mbassa ve Poulsen 1993, Iriadam 2004), Elitok (2012);1 aylıktan küçük oğlaklarda MCV ve MCH seviyelerinin 1-4 aylık oğlaklara göre daha yüksek, PCV seviyesinin daha düşük, Hb seviyesinde ise bir değişiklik olmadığını rapor etmiştir. Çalışmamızda kuzularda elde edilen hematolojik parametrelerdeki (WBC, RBC, MCH, MCHC, RDW, PLT, MPV, PDW, PCT, HCT, cHGB) değişimler istatistiksel olarak önemli olmayıp referans değerler arasındaydı. Ancak bütün gruplarda 4 haftalık kuzularda bir haftalık kuzulara göre MCV parametresindeki azalma istatistiksel olarak önemliydi (p:0.01). MCV değerlerinde görülen bu azalma; kuzuların yaşamlarının ilk haftalarında kanlarında fötal eritrosit oranının yüksek ve fötal eritrosit volümlerinin yetişkin eritrositlerinden daha büyük olmasına bağlanabilir. Hematolojik parametrelerde literatürler arasında görülen farklılıklar; cinsiyet, mevsim, canlının fizyolojik durumu, ırk ve beslenme gibi faktörlere de atfedilebilir (Oramari ve ark. 2014).

Canlılar gebelik, doğum, laktasyon ve reprodüktif faaliyetler için gerekli olan metabolik enerji ve kaynakları vücutlarında yeterli miktarda bulundurmaya çalışırlar. Jawasreh ve ark. (2009) koyunlarda biyokimyasal parametrelerin bireysel beslenme ve metabolizma farklılıklarından etkilenebileceğini belirtmektedirler. Antunovic ve ark. (2017) keçilerde laktasyon döneminde serum laktoz düzeyinde istatistiksel olarak önemli bir değişiklik olmadığını rapor etmişlerdir. Çalışmamızda ise doğum sonrası laktoz düzeyi G3 grubunda diğer gruplara kıyasla

laktasyonun 3. ayında istatistiki açıdan önemsiz ancak gözle görülür derecede daha yüksek tespit edildi. Bu durum; laktasyonun 3. ayından önce yapılan rasyon değişikliğine bağlanabileceği gibi, laktozun gebeliğin son döneminde kolostrum yapımına katılmasına ve laktasyonun birinci ayında yoğun süt üretimi amacıyla kullanılıyor olmasına da atfedilebilir.

İneklerde yapılan bir çalışmada kuru döneme kıyasla laktasyon döneminde serum AST aktivitesinde artma, ALT aktivitesinde azalma (Stojevic ve ark. 2005), koyunlarda ise erken laktasyon döneminde TP ve glikoz düzeylerinde artma, AST düzeylerinde azalma olduğu bildirilmektedir (Antunovic ve ark. 2011). İnek ve koyunlarda laktasyonda gebelik dönemine göre TP ve üre seviyelerinde artma, glikoz seviyesinde ise azalma olduğunu söyleyen çalışmalar da mevcuttur (Koubkova ve ark. 2002, Williams ve Millar 1979, Juma 2010). Oddy ve ark. (1983) Merinos koyunlarında laktasyon döneminde serum üre düzeyinin düşük olduğunu, bunun vücuttaki üre döngüsü ve diyetteki düşük protein içeriği nedeniyle olabileceğini rapor etmişlerdir. Özyurtlu ve ark. (2007) koyunlarda yaptıkları çalışmada gebelik öncesi ve sonrası dönemler karşılaştırıldığında serum TP seviyelerinde bir değişiklik olmadığını saptamışlardır.

C vitamini her ne kadar ruminantların karaciğerinde glikozdan sentezleniyor olsa da, yarılanma ömrünün çok kısa olması, gebelik döneminde artan yavru büyüklüğü ve maternal glikozun fötal kan sirkülasyonuna mobilize olması nedeniyle, kan glikoz seviyesindeki azalmaya (Seidel ve ark. 2006) bağlı olarak C vitamini eksikliğinin ortaya çıkabileceği ifade edilmektedir (Weiss 2001). Prior ve Christenson (1978) gebe koyunlarda yaptıkları bir çalışmada maternal kan glikozunun yaklaşık %42.6'sının uterusta kullanıldığını bildirmişlerdir. Koyunlarda geç gebelik döneminde artan yavru büyüklüğü nedeniyle gerekli olan enerji ihtiyacını karşılaması ve hem koyun hem de fetüs tarafından kullanılan bir metabolit olması bakımından glikoz önemli bir yer tutmaktadır (Gürgöze ve ark. 2009). Koyunlarda serum glikoz seviyesi gebelikte laktasyon dönemine göre daha düşük olmakla birlikte (Balikci ve ark. 2007, Takarkhede ve ark. 1999, Henze ve ark. 1994, Jacob ve Vadodaria 2001), laktasyondaki ineklerde serum glikozunun büyük bir kısmı süt laktozunun sentezi için kullanılmaktadır (Bell 1995).

Kısraklarda organizmada meydana gelen hormonal değişikliklere bağlı olarak gebeliğin son dönemlerinde serum TP seviyesinin daha yüksek olduğu ileri sürülmektedir (Milinkovic-Tur ve ark. 2005). Aksine Jainudee ve Hafez (1994) geç gebelik döneminde TP düzeyinin azaldığını ve maternal serum TP seviyesindeki bu azalmanın; yavrunun gelişimi için gerekli olan proteinlerin anneye ait aminoasitlerden karşılanması nedeniyle olabileceğini rapor etmişlerdir. Bazı yazarlar koyunlarda ileri gebelikte, erken laktasyon dönemine kıyasla serum kreatinin seviyesinin daha yüksek olduğunu ve bu duruma fötal kas yapısının gelişimi için maternal mobilizasyondaki artışın ve fetüsün organik kalıntılarının eliminasyonunun neden olabileceğini ileri sürmüşlerdir (Antunovic ve ark. 2011, Santos ve ark. 2012). Yapılan çalışmada gebe hayvanlara C vitamini ilavesinin gerek gebelik gerekse laktasyon döneminde yukarıda belirtilen biyokimyasal parametreler üzerinde önemli bir değişiklik oluşturmadığı, metabolik süreçlere bağlı olarak dalgalanmalar meydana geldiyse de bunun istatistiki açıdan önemli olmadığı görülmüştür.

Evcil hayvanlarda gebelik döneminde, yavrunun giderek büyümesine, laktasyon periyodunda ise süt üretiminin artmasına bağlı olarak elektrolit dengesinde değişiklikler olabilmektedir (Kaneko ve ark. 2008). Antunovic ve ark. (2011) koyunların erken laktasyon döneminde Fe, Ca++, Na+ düzeylerinde azalma; P düzeylerinde ise artış olduğunu bildirmektedirler. Keçilerde yapılan bazı çalışmalarda ileri gebelik ve erken laktasyonda Fe, Ca++ ve Na+ seviyesinin azaldığı (Azab ve Abdel-Maksoud 1999, Krajnicakova ve ark. 2003, Ahmed ve ark. 2000), bunun ise mevcut minerallerin süte transfer olması nedeniyle olabileceği ileri sürülmüştür (Krajnicakova ve ark. 2003, Ahmed ve ark. 2000). Abdou (1995) keçilerde gebeliğin 4. ayına kadar Ca++ seviyesinde bir değişiklik olmadığını bildirirken, Özyurtlu ve ark. (2007) koyunlarda gebelik öncesi ve sonrası dönemler arasında serum Ca++ seviyelerinde fark olmadığını rapor etmişlerdir. İleri gebelikte koyunlarda K+ konsantrasyonunun yüksek olması, gebeliğin son döneminde meydana gelen metabolik değişikliklere atfedilmektedir (Antunovic ve ark. 2002). Goff (2006) sütçü ineklerde yüksek K+ seviyesinin metabolik asidozis nedeniyle, düşük K+ seviyesinin ise; K+'un hücre içine girişini arttıran yüksek insülin nedeniyle olabileceğini bildirmektedir. Çalışmamızda doğum sonrası tüm gruplarda K+ düzeyinin laktasyonun 3. ayında laktasyonun 1. ayına göre istatistiki açıdan önemli derecede azaldığı tespit edildi. Rasyon değişikliğine bağlı olarak glikoz üretimi belirli seviyelere ulaştığında salgılanan insülin hücre membranının hiperpolarizasyonuna neden olmakta bu durum K'un hücre içine geçişini kolaylaştırarak serum K+ seviyesinde azalmaya yol açabilmektedir. Bununla birlikte laktasyonun ilerlemesiyle süt üretimindeki azalmaya bağlı olarak K'un süt için kullanım miktarında düşme serumdaki sirkülasyonunda azalmaya sebep olmuş olabilir.

Gürdoğan ve ark. (2006) Akkaraman koyunlarında Fe konsantrasyonunun gebeliğin sonlarına doğru azaldığını, doğum sonrası ise arttığını ve bu durumun gebelikte Fe iyonun fetüs tarafından tüketimi veya adrenokortikal hormon artışı nedeniyle olabileceğini ileri sürmüşlerdir. Gebelik döneminde yavrunun hem anne hem de yavrunun ihtiyacının karşılanması için yoğun bir şekilde oksijen taşınması ve kullanılması nedeniyle, doğuma yakın dönemde ise hormonal değişikliklerin fazlaca görülmesi bakımından böyle bir değişikliğin olabileceği tahmin edilmektedir.

Çalışmamızda 4.5 aylık gebe koyun grupları arasında yapılan karşılaştırmalarda ise en yüksek glikoz düzeyi yüksek dozda C vitamini ilavesi yapılan G4 grubunda tespit edildi. Doğum sonrası en yüksek glikoz düzeyi

laktasyonun 3. ayında G3 grubundaki koyunlarda ölçüldü. Ruminantların gebelik periyodunda fetüsteki bağ doku artışı, C vitaminine duyulan ihtiyacın artmasına yol açarken, gebeliğin ilerlemesi bu gereksinimi daha da yükseltmektedir. Çalışmada ileri gebe koyunlarda özellikle de yüksek doz C vitamini ilavesi yapılan grupta glikoz düzeyi en yüksek düzeyde ölçülmüştür. Bu hayvanlarda C vitamini katkısının karaciğerde glikozun C vitamini sentezinde kullanımını azaltarak kan glikoz düzeyi üzerine olumlu etki yaptığını düşündürmektedir.

Sonuç olarak; istatistiki olarak farklılık gösteren bazı bulgularımız olmasına rağmen uyguladığımız farklı dozlardaki C vitamini enjeksiyonlarının anne ve yavruda önemli bir metabolik değişiklik meydana getirmediğini gördük. Bununla birlikte C vitamini uygulamasının glikoz seviyesini arttırdığı düşünüldüğünde; merinoslarda hipoglisemi ile seyreden hastalıklara karşı koruyucu olabileceği, ayrıca vücutta askorbik asit varlığıyla demir emiliminin daha fazla olduğu böylece de demir eksikliğine bağlı oluşabilecek hastalıklara karşı koruyucu etki oluşturduğu düşünülmektedir. Bu nedenlerden dolayı C vitamininin etkisini daha iyi anlayabilmek için hayvan sayısının fazla tutulduğu, farklı doz ve uygulama yollarının denenebileceği daha ileri çalışmalara ihtiyaç duyulmaktadır.

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RESEARCH ARTICLE

Comparison of Growth and Development Characteristics of Hair and Damascus Kids Reared under Extensive Conditions

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ABSTRACT

The present study aimed to compare the growth and the development of Hair and Damascus kids reared under extensive conditions. The body weights of Damascus goats were significantly higher than that of the Hair goats during the 360 days growth period ($P < 0.05$; $P<0.001$). Body weight was influenced by birth type only at birth and on the 360th day of the growth period, and single-birth kids had higher values than twin-born kids ($P < 0.05$). Exceptfor the 120th day of the growth period, the body weight of kids born in dam age group III was higher than that of kids born in the other dam age groups ($P < 0.05$; $P < 0.01$; $P < 0.001$). The breed effect was markedly observed after the 120th day of the growth period concerning body measurements especially chest depth, rump height, and body length. Damascus goats were significantly higher than that in the Hair goats for these traits ($P < 0.001$). It can be suggested that comparative studies containing the entire growth period should be conducted on other indigenous goat breeds (Angora, Honamlı, Kilis, and Norduz goat breeds) and to demonstrate the growth and development characteristics of these breeds.

Key Words: Damascus goat, development, growth, Hair goat, Turkey

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Ekstansif Şartlarda Yetiştirilen Kıl Ve Halep Keçilerinde Büyüme Ve Gelişim Özelliklerinin Karşılaştırılması

ÖZ

Bu araştırma ekstansif koşullarda yetiştirilen Kıl ve Halep oğlaklarında büyüme ve gelişim özelliklerinin karşılaştırılmasını amaçlamıştır. 360 günlük büyüme döneminde Halep keçilerinin canlı ağırlıkları Kıl keçilerinden belirgin düzeyde yüksek olmuştur ($P < 0.05$; $P < 0.001$). Canlı ağırlık sadece doğumda ve büyüme periyodunun 360. gününde doğum tipinden etkilenmiş ve tek doğan oğlaklar ikiz doğanlara göre daha yüksek değerlere sahip olmuştur ($P < 0.05$). Büyüme döneminin 120. günü hariç anayaşı III olan gruptan doğan oğlakların canlı ağırlıkları diğer anayaş gruplarında doğan oğlaklardan yüksek olmuştur ($P < 0.05$; $P < 0.01$; $P < 0.001$). Vücut ölçülerinde (özellikle göğüs derinliği, sağrı yüksekliği ve vücut uzunluğunda) ırk etkisi büyümenin 120. gününden sonra belirgin olarak gözlemlenmiştir. Bu özelliklerde Halep keçisi, Kıl keçisine göre belirgin derecede yüksek olmuştur ($P < 0.001$). Diğer yerli keçi ırkları (Ankara, Honamlı, Kilis ve Norduz keçi ırkları) üzerinde tüm büyüme dönemini içeren karşılaştırmalı çalışmaların yapılması ve bu ırkların büyüme ve gelişme özelliklerinin ortaya konulması önerilebilir.

Anahtar Kelimler: Büyüme, gelişme, Halep keçisi, Kıl keçisi, Türkiye

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INTRODUCTION

In Turkey, small family enterprises, which was practiced extensive goat breeding, prefer the Hair goat breed widely. However, Kilis and Damascus goat breeding is also carried out by breeders successfully. Hair goat is known as "Black goat" or "Anatolian black", an indigenous goat breed of Turkey, and it is a predominant breed and constitutes about 90% of the goat population in Turkey. Hair goats are specified by good resistance to diseases and parasites, the ability to withstand harsh climates and endure a poor quality of grazing. The purpose of hair goat breeding is to benefit from multiple yields (mainly meat and milk). But, the productivity of Hair goats isconsidered low. The color of the breed is generally black. However, brown, grey, white, or spottedanimals are also seen. It has generally large andpendulous ears and straight nose structure, but animals with medium-sized or short ears are also seen. The body size is large withan average withers height (70-75 cm), and a body weight of 40 - 45 kg in female goats at adult age. Damascus goat is known as "Shami goat" or "Aleppo goat" is raised in the southern provinces of Turkey insmall numbers. The most important advantage of this goat breeds raised in Turkey, especially in Southeast Anatolia, Central Anatolia, and the Mediterranean region, it makes better use of inefficient pastures than sheep in high-temperature conditions. In addition, it effectively utilizes the stubble areas after the harvest. The breed is well adapted to arid and semi-arid climatic conditions, also it has relatively high milk and fertility in these climatic conditions. It is a milk breed kept in the towns and aroundcities, in groups of 2-5 animals. The color of the animals variesfrom yellow to reddish-brown. Theblack coat color is extremely rare. However, there are varieties of Damascus goats with white, ash color, and red-white spots. It has long and pendulous ears and an arched nose structure. The body size is medium-large with an average withers height (65-70 cm) and a body weight of 40-50 kg in female goats. The udders well-developed. This breed is known for its high fertility and milk yield. (Akçapınar, 2015; Erdem et al., 2019; Yalçın, 1986; Barıtçı ve Adıgüzel, 2017). Body weight and body measurements are used to describe animals numerically, to determine breed characteristics, to compare breeds, and to monitor growth and development in animals. Body weight and body measurements are affected by environmental factors such as genotype, gender, age, birth type, year, and management (Akçapınar and Özbeyaz, 1999; Alızadehasl and Ünal, 2011).Although many studies have been conducted on the growth and development of Hair goats (Toplu and Altınel, 2008; Gökdal, 2013; Aktaş et al.,2015; Akbaş and Saatçi, 2016; Elmaz et al., 2016; Çelik and Olfaz, 2017; Alaşahan and Öztürk, 2019)the number of studies, which wasconducted on Damascus goats is so scarce(Güney et al., 2006; Khazaal, 2009; Mahmoud et al., 2012). Also, studies conducted on Damascus goats were limited to a certain period of growth (Abd-Allah et al., 2019; Tatar et al., 2019; Al-Dawood et al., 2020), and thus there is no research on body development in the entire growth period.In addition, there was no comparative study examining the growth and body characteristics of these two breeds in the same extensive conditions.Therefore,the present studyaimed to compare the growth and development of Hair and Damascus kids reared under extensive conditions.

MATERIALS AND METHODS

Location of the study, animals and their management

The study was carried out in a family enterprise, which was performed in extensive goat breeding in Yağbasan village. The Yağbasan villageis located at 40°05'39.0"N 33°37'32.7"E. It isapproximately 13 km from the Sulakyurt county district of Kırıkkale province in the Central Anatolia Region. The economic livelihood of the village is provided by agriculture and animal husbandry (Anonymous 2021-a; Anonymous 2021-b). The animal material of the study consisted of 51 Hair kids (24 males, 27 females) and 50 Damascus kids (25 males, 25 females) obtained from Hair and Damascus goat herds in the same enterprise. During the research, the animals were kept in the hands of the breeder, and no change was made in the management, care, and feeding conditions of the Hair and Damascus dams and kids. Goats were grazed by the breeder in open fields and pastures every day from morning to noon.At noon, the herds rested in the shaded area, and in the afternoon grazing continued until evening.In the evening the herd went back to their stockyard.To determine the growth and development characteristics individually, ear tags were applied to each kid separately at birth. Sex, type of birth, and dam age were recorded. Kids were suckling in the morning and at night everyday, and any extra feed wasn't given to kids. The weaning age of the kids was 105 days ofage. When kids were 105 d old age, they were started to go out to the graze with their dams.

Data Collection

Body weight and ten exterior traits were determined on each animal. The birth weight of the kids was taken within twelve hours after birth. Body weight of the kids were determined at regular intervals from birth to 12 months of age. The body weight of kids on the 30, 60, 90, 120, 180, 240, and 360 days of age were recorded. The body weights of the kids were taken with a precision digital scale sensitive to 20 g. The ages of dams were classified into three groups: 24-35 months of age (dam age group I), 36-47 months of age (dam age group II), and 48 months of age and older (dam age group III). Body measurements (head length, head width, ear length, chest depth, chest width, chest girth, withers height, rump height, body length, cannon bone

circumference) of kids on the 30, 90, 120, 180, and 360 days of age were measured. In body measurements, measuring type was used to determine head length, head width, ear length, chest girth, body length, and cannon bone circumference. Chest depth, chest width, withers height, and rump height were determined bya surveyor's stick. Head length (distance from Crista occipitalis to the tip of nose), head width (at the widest part of the head, the distance between two eye angles), ear length (the distance between auricula's junction to the cranium and the farthermost auricula's tip), body length (distance between tuber ischii and caput humeri), withers height (measured asthe distance between the most dorsal point of the withers and the ground), rump height (distance between the most dorsal point of the rump and the ground), chest depth (the highest point of the withers and the sternum vertical distance between the two caput humerus), chest girth (measurement of body circumference taken right after behind the scapulae), chest width (distance between left and right caput humeri),and cannon bone circumference (measure taken from the middle of the distance between the articulus carpi and the fetlock joint) were measuredin each animal with ear-tag. The circumference of the cannon bone was taken from the right hind leg. Throughout the research, individual body weight and body measurements were determined by the same researcher at regular one-month intervals from birth to one-year-old age on each goat (Toplu and Altınel, 2008; Yakubu, 2009; Alızadehasl and Ünal, 2011; Elmaz et al., 2012; Koncagül et al., 2012; Akbaş and Saatçi, 2016; Elmaz et al., 2016)

Statistical analysis

Effects of genotype, dam age, sex, and birth type on growth and development were analyzed by using the RepeatedMeasuresGeneral Linear Models.If GLM showed an acceptable level of significance ($P < 0.05$), Tukey's test was applied for post hoc comparison**.** Independent t-test was used to determine the significance of differences between breeds, sexes, and birth types. The statistical analyses were performed using the software package SPSS for Windows. Data were presented as means ± standard error (SE). A value of $P<0.05$ was considered statically significant (Dawson and Trapp, 2001).

RESULTS

Growth

Birth weight, 60th, 90th, 180th, 240th, and 360th days of age were significantly influenced by breed. Damascus kids were higher than Hair kids in terms of body weight at entire growth periods($P < 0.05$; $P <0.001$). Also, body weight was affected by sex, and the body weight of male kids was significantly higher than that of female kids during the entire growth period ($P < 0.05$; $P < 0.01$; $P < 0.001$). Birth type only affected the body weight at birth and360 days of age. Body weight of single-birth kids was higher than that of those twin-born kids ($P < 0.05$). Birth weight, 30th, 60th, 90th, 180th240thand 360th days of age were significantly affected by dam age. Except forthe 120th day of the growth period, the body weight of kids born in dam age group III was higher than that of kids born in the other dam age groups ($P < 0.05$; $P < 0.01$; $P < 0.001$) (Table 1).

Development

Although there was a difference between the two breeds in terms of head length was statistically significant on the 90th and 360th daysof the growth period, and on the 180th and 360th days in terms of head width, the differences in ear length were statistically significant in the entiregrowth period. Damascus kids were higher than the Hair kids in terms of head length on the 90th and 360th days of the growth period ($P <0.05$; $P < 0.01$). Likewise, the Halep kids got higher values in terms of head width on the 180th and 360th days of growth period ($P<0.05$; $P < 0.01$). Ear length was higher in Halep kids than those of hair goats during the entire growth period ($P < 0.001$). Head length and head width were influenced by sex in certain growth periods, however, ear length was not influenced by sex during the entire growth period. Male kids have higher values than female kids in head length and head width characteristics ($P < 0.05$; $P < 0.01$; $P < 0.001$). While chest depth and chest girth were affected by the breed on the 120th, 180th and 360th days of growth period, the effect of breed for chest width was statically significant on the 120th and 360th days of the growth period. Damascus kids were higher than the hair kids in terms of chest depth, chest width and chest girth characteristics in these days of growth period ($P < 0.01$; $P < 0.001$). Sex significantly affected chest depth and chest width especially on the 90th days of age and the later stages of the growth period. The chest depth, chest width and chest girth of male kids weresignificantly higher than that of female kids($P < 0.05$; $P < 0.01$; $P < 0.001$)(Table 2). The difference between breeds was statically significant for withers height and rump height on the 30th, 180th, and 360th days of the growth period. On the 30th, 180th, and 360th days of the growth period, in terms of withers height and rump height, Damascus kids were higher values than Hair kids ($P < 0.05$; $P < 0.001$). Withers height and rump heightwere influenced by sex with different statistical levelsentire growth period. Withers height and rump height of male kids were higher than those of female kids ($P < 0.05$; $P <0.001$). Body length and cannon bone circumference were affected by breed on the 120 th, 180 th and 360th days of the growth period. Damascus kids had higher body length and cannon bone circumference means than that of Hair kids ($P< 0.05$; $P <0.01$; $P <0.01$). Withers height and cannon circumference were affected by sex in entire stages of the growth period, which was higher in male kids than in female kids ($P < 0.05$; $P <0.001$) (Table 3)

Table 1. Body weights of Hair and Damascus goats from birth to 360 days of age (kg)($X \pm S_{\bar{x}}$).
Tablo 1. Kıl ve Halep keçilerinde doğumdan 360 günlük yaşa kadar canlı ağırlıklar(kg) ($X \pm S_{\bar{x}}$).

Factors		n	Birth	n	Day 30	n	Day 60	n	Day90	n	Day120	n	Day180	n	Day240	n	Day360
Hair	M	24	2.45 ± 0.05	22	6.17±0.35	21	10.65±0.39	21	15.39±0.56	21	19.28±0.59	21	20.16±0.49	21	20.97±0.41	21	22.64±0.44
	F	27	2.28±0.05	23	5.90±0.37	21	10.46±0.42	21	14.74±0.59	21	15.71±0.63	21	17.65±0.52	21	19.01±0.43	21	21.74±0.48
Damascus	M	25	2.49±0.04	23	7.13±0.31	22	12.77±0.36	22	18.41 ± 0.51	21	19.91±0.54	21	21.73±0.44	21	22.84±0.37	21	25.08±0.40
	F	25	2.54±0.05	24	5.62±0.32	22	10.68±0.39	22	13.75±0.55	21	15.50±0.58	21	20.39±0.48	21	21.58±0.38	21	23.98±0.44
TOTAL																	
Breed			*		-		*		*		-		***		***		***
Hair		51	2.36±0.04	45	6.05±0.26	42	10.54±0.29	42	15.04±0.41	42	17.33±0.43	42	18.79±0.36	42	19.90±0.30	42	22.15±0.33
Damascus		50	2.52±0.03	47	6.37±0.23	44	11.23±0.26	44	16.08±0.37	42	17.71±0.39	42	21.06±0.32	42	22.21±0.27	42	24.53±0.29
Sex			**		**		***		***		***		***		***		*
M		49	2.47±0.03	45	6.57±0.23	43	11.80±0.27	43	17.04±0.37	42	19.62± 0.37	42	21.11±0.39	42	21.99±0.27	42	23.97±0.29
F		52	2.41±0.04	47	5.89±0.25	43	10.07±0.29	43	14.24±0.40	42	15.61±0.43	42	19.02±0.35	42	20.30±0.29	42	22.86±0.32
Birth type			*		-		-		-		-		-		-		*
Single		67	2.48±0.03	58	6.36±0.28	56	10.93±0.23	56	15.78±0.36	56	17.95±0.35	56	20.16±0.28	56	21.17±0.24	56	23.56±0.26
Twinning		34	2.39±0.04	34	6.09±0.20	30	10.86±0.32	30	15.36±0.46	28	17.07±0.48	28	19.78±0.39	28	21.04±0.33	28	23.24±0.36
Dam age			***		***		***		*		-		***		***		**
2		31	2.31±0.05 ^{b}	29	6.15±0.32 ^{b}	27	10.86±0.36^{b}	27	15.57±0.51^{b}	26	17.41±0.54 ^{b}	26	19.81±0.44^{b}	26	21.01±0.37^{b}	26	23.40±0.41^{b}
3		41	2.51±0.04^{a}	35	6.44±0.28 ^{a}	33	10.99±0.32 ^{a}	33	15.54±0.45^{b}	32	17.67±0.48 ^{a}	32	20.44±0.39 ^{a}	32	21.45 ±0.33^{a}	32	23.48±0.36 ^{a}
4+		29	2.50±0.04 ^{a}	28	6.04±0.29 ^{a}	26	10.84±0.33 ^{b}	26	15.64±0.47^{a}	26	17.53±0.49 ^{b}	26	19.64±0.41 ^{b}	26	20.84±0.34 ^{b}	26	23.27±0.37^{b}

M: Male, **F:** Female; -: P> 0.05; *: P< 0.05; **: P< 0.01; ***: P< 0.001, $^{a\text{-}b}$: means within a column with different letters are significantly different ($p < 0.05$)
M: Erkek, **F:** Dişi; -: P > 0.05; *: P < 0.05; **: P < 0.01; ***: P < 0.001, $^{a\text{-}b}$: aynı sütunda farklı harfleri taşıyan ortalamalar arası farklılık önemlidir ($p < 0.05$)

Table 2. Head length, head width, ear length, chest depth, chest width and chest girth in Hair and Damascus kids(cm) ($X \pm S_{\bar{x}}$)

Tablo 2.Kıl ve Halep keçilerinde baş uzunluğu, baş genişliği, kulak uzunluğu, göğüs derinliği, göğüs genişliği ve göğüs çevresi ile ilgili değerler (cm) ($X \pm S_{\bar{x}}$)

Factors		Head length						Head width						Ear length					
Breed	**Sex**	**n**	**Day 30**	**Day 90**	**Day 120**	**Day 180**	**Day 360**	**n**	**Day 30**	**Day 90**	**Day 120**	**Day 180**	**Day 360**	n	**Day 30**	**Day 90**	**Day 120**	**Day 180**	**Day 360**
Hair	M	20	21.87 ± 0.21	22.84 ± 0.30	26.68 ± 0.25	26.99 ± 0.58	27.76 ± 0.32	19	10.06 ± 0.24	10.11 ± 0.16	11.71 ± 0.14	12.18 ± 0.17	12.47 ± 0.18	18	14.35 ± 0.29	17.16 ± 0.44	17.74 ± 0.19	18.02 ± 0.21	18.40 ± 0.22
	F	20	20.89 ± 0.21	22.01 ± 0.31	25.81 ± 0.24	26.33 ± 0.55	27.18 ± 0.30	18	9.14 ± 0.23	9.41 ± 0.15	11.67 ± 0.13	12.01 ± 0.16	12.18 ± 0.19	19	14.65 ± 0.28	16.66 ± 0.43	17.81 ± 0.18	18.10 ± 0.19	18.89 ± 0.21
Damascus	M	20	21.32 ± 0.20	23.91 ± 0.29	26.31 ± 0.27	27.18 ± 0.56	28.65 ± 0.33	19	9.45 ± 0.22	10.03 ± 0.15	11.68 ± 0.12	12.98 ± 0.16	13.03 ± 0.17	19	15.59 ± 0.27	18.89 ± 0.42	19.33 ± 0.19	19.82 ± 0.23	21.35 ± 0.19
	F	19	20.98 ± 0.15	22.63 ± 0.20	25.42 ± 0.24	25.79 ± 0.57	27.92±0.29	19	8.94±0.21	9.52±0.14	11.28±0.11	12.22±0.15	12.49±0.16	19	16.09±0.25	19.11±0.43	20.19±0.17	21.33±0.24	22.23±0.21
TOTAL																			
Hair		40	21.38 ± 0.15	22.42±0.20	26.24±0.17	26.66±0.41	27.47±0.19	37	9.61±0.16	9.77±0.11	11.69±0.09	12.10±0.12	12.33±0.13	37	14.51±0.20	16.91±0.29	17.78±0.134	18.06±0.23	18.65±0.15
Damascus		39	21.15 ± 0.14	23.28±0.25	25.87±0.20	26.49±0.42	28.28±0.22	38	9.19±0.15	9.78±0.12	11.48±0.09	12.60±0.11	12.76±0.12	38	15.84±0.23	19.00±0.32	19.76±0.132	20.57±0.20	21.79±0.15
	M	40	21.59 ± 0.15	23.37±0.23	26.49±0.16	27.08±0.44	28.21±0.20	38	9.75±0.14	10.07±0.11	11.69±0.01	12.58±0.11	12.75±0.11	37	14.99±0.22	18.05±0.31	18.56±0.134	18.94±0.19	19.92±0.16
	F	39	20.93 ± 0.14	22.32±0.25	25.62±0.17	26.06±0.39	27.55±0.18	37	9.04±0.16	9.47±0.12	11.48±0.01	12.12±0.12	12.34±0.13	38	15.37±0.18	17.88±0.29	19.01±0.132	19.71±0.17	20.56±0.14
Breed			-	*	-	-	**		-	-	-	**	*		***	***	***	***	***
Sex			***	***	***	-	*		**	***	-	**	*		-	-	-	-	-
Factors		**Chest depth**						**Chest width**						**Chest girth**					
Breed	**Sex**	**n**	**Day 30**	**Day 90**	**Day 120**	**Day 180**	**Day 360**	**n**	**Day 30**	**Day 90**	**Day 120**	**Day 180**	**Day 360**	**n**	**Day 30**	**Day 90**	**Day 120**	**Day 180**	**Day 360**
Hair	M	18	14.42 ± 0.20	20.17 ± 0.37	24.06 ± 0.16	24.22 ± 0.18	24.72 ± 0.23	18	7.22 ± 0.19	13.53 ± 0.31	15.16 ± 0.01	15.81 ± 0.12	16.23 ± 0.12	19	42.43 ± 0.62	51.46 ± 0.39	64.49 ± 0.24	67.09 ± 0.22	69.99 ± 0.27
	F	20	13.83 ± 0.28	18.62 ± 0.35	22.56 ± 0.15	23.19 ± 0.16	24.06 ± 0.22	20	6.55 ± 0.17	13.04 ± 0.29	13.47 ± 0.09	15.23 ± 0.11	15.51 ± 0.11	18	39.55 ± 0.63	50.73 ± 0.41	61.42 ± 0.25	63.48 ± 0.23	65.67 ± 0.28
Damascus	M	20	14.34 ± 0.29	20.91 ± 0.39	24.65 ± 0.17	26.07 ± 0.17	26.48 ± 0.23	19	7.04 ± 0.18	13.12 ± 0.28	15.40 ± 0.02	16.36 ± 0.13	17.52 ± 0.13	19	43.76 ± 0.61	52.93 ± 0.42	66.63 ± 0.23	72.80 ± 0.19	73.28 ± 0.27
	F	18	14.41 ± 0.20	18.92 ± 0.36	23.94 ± 0.14	24.08 ± 0.18	26.08 ± 0.24	19	7.37 ± 0.19	12.50 ± 0.30	14.35 ± 0.09	14.93 ± 0.12	15.99 ± 0.12	18	42.51 ± 0.63	52.66 ± 0.40	62.13 ± 0.25	63.74 ± 0.21	71.68 ± 0.30
TOTAL																			
Hair		38	14.11±0.20	19.35±0.30	23.27±0.12	23.68±0.12	24.37±0.16	38	6.87±0.14	13.27±0.21	14.27±0.07	15.50±0.08	15.85±0.08	37	41.03±0.44	51.11±0.29	63.01±0.18	65.33±0.15	68.33±0.19
Damascus		38	14.37±0.19	19.97±0.26	24.32±0.13	25.13±0.13	26.29±0.17	38	7.21±0.13	12.81±0.20	14.87±0.07	15.64±0.07	16.76±0.09	37	43.15±0.45	52.80±0.28	64.44±0.17	68.39±0.14	72.50±0.20
	M	38	14.37±0.22	20.56±0.25	24.37±0.15	25.19±0.14	25.64±0.18	37	7.13±0.13	13.32±0.21	15.28±0.06	16.09±0.07	16.89±0.08	38	43.09±0.43	52.20±0.27	65.56±0.18	69.94±0.16	71.63±0.18
	F	38	14.10±0.20	18.76±0.29	23.21±0.14	23.61±0.13	25.02±0.17	39	6.95±0.12	12.77±0.19	13.90±0.05	15.08±0.06	15.75±0.07	36	41.03±0.45	51.69±0.28	61.78±0.16	63.61±0.15	68.68±0.21
Breed			-	-	***	***	***		*	-	***	-	***		**	***	**	***	***
Sex			-	***	***	***	*		-	*	***	***	***		**	-	***	***	***

M: Male, **F:** Female; -: P> 0.05; *: P< 0.05; **: P< 0.01; ***: P <0.001; **M:** Erkek, **F:** Dişi; -: P > 0.05; *: P < 0.05; **: P < 0.01; ***: P < 0.001

Table 3. Withers height, rump height, body length, and cannon bone circumference in Hair and Damascus kids(cm) ($X \pm S_{\bar{x}}$)
Tablo 3. Kıl ve Halep oğlaklarında cidago yüksekliği, sağrı yüksekliği, vücut uzunluğu ve incik çevresi ile ilgili değerler (cm) ($X \pm S_{\bar{x}}$)

Factors		Withers height						Rump height					
Breed	**Sex**	**n**	**Day 30**	**Day 90**	**Day 120**	**Day 180**	**Day 360**	**n**	**Day 30**	**Day 90**	**Day 120**	**Day 180**	**Day 360**
Hair	M	18	36.31 ±0.51	48.44±0.57	49.25±0.75	51.84±0.24	53.22±0.29	20	37.95±0.59	49.56±0.65	51.25±0.43	52.20±0.19	54.61±0.26
	F	19	35.05 ±0.49	45.78±0.55	48.48±0.73	51.29±0.23	52.08±0.28	20	35.90±0.60	46.87±0.64	49.15±0.45	50.59±0.21	53.30±0.29
Damascus	M	18	38.96 ±0.51	48.38±0.56	49.88±0.76	52.89±0.21	56.47±0.30	20	39.75±0.57	49.90±0.65	51.68±0.42	56.83±0.17	57.98±0.28
	F	19	36.37 ±0.49	46.27±0.55	47.15±0.72	51.31±0.25	55.29±0.27	19	37.44±0.59	47.49±0.62	48.18±0.46	52.52±0.20	56.20±0.30
TOTAL													
Hair		37	35.66±0.36	47.08±0.40	48.85±0.52	51.56±0.17	52.64±0.20	40	36.93±0.40	48.21±0.46	49.97±0.32	51.39±0.18	53.95±0.20
Damascus		37	37.63±0.35	47.30±0.41	48.48±0.51	52.08±0.16	55.86±0.19	39	38.63±0.42	48.73±0.43	50.20±0.35	54.73±0.16	57.11±0.21
	M	36	37.63±0.37	48.41±0.41	49.56±0.53	52.36±0.17	54.84±0.20	40	38.85±0.40	49.73±0.45	51.46±0.33	54.51±0.20	56.30±0.20
	F	38	35.71±0.35	46.03±0.39	47.82±0.50	51.30±0.15	53.69±0.22	39	36.65±0.43	47.17±0.46	48.67±0.32	51.53±0.15	54.71±0.23
Breed			***	-	-	*	***		*	-	-	***	***
Sex			***	***	*	***	*		***	***	***	***	***
Factors		**Body length**						**Cannon bone circumference**					
Breed	**Sex**	**n**	**Day 30**	**Day 90**	**Day 120**	**Day 180**	**Day 360**	**n**	**Day 30**	**Day 90**	**Day 120**	**Day 180**	**Day 360**
Hair	M	19	33.53 ±0.59	47.93±0.64	53.72±0.24	56.48±0.24	59.05±0.08	19	6.01±0.14	7.66±0.14	8.03±0.04	8.15±0.08	8.22±0.08
	F	20	30.80 ±0.57	43.70±0.63	53.13±0.23	54.85±0.24	58.78±0.07	19	5.67±0.13	6.80±0.16	7.06±0.07	7.39±0.05	7.68±0.07
Damascus	M	19	34.00 ±0.55	48.21±0.61	57.58±0.24	59.55±0.19	61.07±0.04	19	6.33±0.10	7.41±0.15	8.20±0.03	8.67±0.08	8.80±0.06
	F	19	31.44 ±0.55	45.82±0.59	55.33±0.22	58.37±0.21	59.70±0.06	19	6.06±0.12	6.48±0.11	7.37±0.06	7.59±0.07	8.01±0.07
TOTAL													
Hair		39	32.13±0.409	45.76±0.453	53.42±0.171	55.64±0.16	58.91±0.06	38	5.84±0.09	7.23±0.10	7.55±0.04	7.77±0.05	7.95±0.06
Damascus		38	32.72±0.414	47.01±0.459	56.46±0.173	58.96±0.17	60.38±0.04	38	6.19±0.10	6.95±0.11	7.79±0.03	8.13±0.04	8.41±0.03
	M	38	33.76±0.414	48.07±0.459	55.65±0.173	58.02±0.15	60.06±0.06	38	6.17±0.08	7.53±0.08	8.12±0.06	8.41±0.03	8.51±0.02
	F	39	31.12±0.409	44.76±0.453	54.23±0.171	56.61±0.14	59.24±0.05	38	5.87±0.10	6.64±0.11	7.22±0.05	7.49±0.05	7.84±0.06
Breed			-	-	***	***	***		*	-	*	*	**
Sex			***	***	***	***	***		*	***	***	***	***

M: Male, **F:** Female; -: P> 0.05; *: P< 0.05; **: P< 0.01; ***: P<0.001; **M:** Erkek, **F:** Dişi; -: P > 0.05; *: P < 0.05; **: P < 0.01; ***: P < 0.001

DISCUSSION

Growth

In the present study, birth weight obtained for Hair kids (2.36 kg) was lower than those reported by Alaşahan and Öztürk (2019)for Hair kids (3.11 kg) raised undersemi-intensive conditions in the province of Van. The values obtained for male Hair kids at birth (2.45 kg) implicitly accordedwith the birth weight value reported for males (2.46 kg) raised under the extensive system in the province of Aydın, while the values obtained for female goats (2.28 kg) were higher than the value reported in the same study (1.92)(Toplu and Altınel, 2008). The body weight values obtained for male and female Hair goats on the 60th day of the growth period (10.65 and 10.46 kg) were higher than the values reported by Erten and Yılmaz (2014) for male and female hair goats (9.86 and 9.75 kg) raised under extensive conditions in the province of Van. In the study, the average body weight obtained hair goats on the 60th day (10.54 kg) was lower than the average body weight (11.80 kg) reported by Şimşek and Bayraktar (2006) for hair goats raised under the intensive conditions in the province of Elazığ. In this study, the average body weight value for Hair kids obtained for the 120th days of age (17.33 kg) was lower than the other study values reported for Hair goats (21.64 kg) at the same age(120th day) (Alaşahan and Öztürk, 2019). Body weights obtained for hair goats on the 180th and 360th days of the growth period (18.79 kg and 22.15 kg) were lower than the values specified on the 180th and 360th days for the hair goats raised under intensive (26.69 kg) and extensive (24.80 kg) conditions in provinces of Elazığ and Amasya (Gökdal et al., 2013; Çelik and Olfaz, 2018). Body weights of the male and female Damascus kids at birth, 60th and 90th days of the growth period (2.49 and 2.54 kg, 12.77 and 10.68 kg and 18.41 and 13.75 kg) lower than those of Taşkın et al. (2000)who reported that body weight values at birth, 60th and 90th day of male and female Damascus kids (4.35 and 4.01 kg,17.08 and 16.91 kg, 21.33 and 20.27 kg) raised under intensive conditions in the province of Tokat. In the present study, the body weights of the male and female Damascus kids obtained on the 60 th, 90 th, 120 th, 180 th, 240 th days of the growth period (12.77 and 10.68 kg, 18.41 and 13.75 kg, 19.91 and 15.50kg, 21.73 and 20.39 kg, 22.84 and 21.58 kg, respectively) were lower than the other studies, which was carried out in the same growth periods of Shami (Damascus) goats (14.98 and 14.41 kg, 23.25 and 22.00 kg, 23.60 and 19.50, 30.40 and 24.90 kg, 38.0 and 29.60 kg, respectively) reared under the intensive system in Lebanon and Sudancountry(Khazaal,2009; Mahmoud et al., 2012). Depending on the region where it is grown and the management conditions provided by the breeders, there was a wide variation in growth characteristics. This variation causes differences in growth-related values ofthe studies conducted in different regions and management conditions. Body weight of Damascus kids was higher than those of Hairkids throughout the growth period. Many other studies on other goatbreeds indicated that the differences in the body weight among different goatbreeds, i.e. the body weight is atop of Saanen X Hair crossbred F1 over Saanen breed kids (Akdağ et al., 2011), Hair goat is superior to Honamlı kids (Aktaş et al., 2015), Honamlı X Hair hybridsis superior to Honamlı breed kids (Akbaş and Saatçi, 2016), and Hamdani over Hair breed kids (Alaşahan and Öztürk, 2019). It is obvious that the differences in body weight between breeds, which are compared in terms of growth characteristics under the same management conditions, were due to genotype differences. In some studies mentioned above, this situation has occurred as hybrid-vigor in hybrids where two breeds are crossbreeding is implemented.Thus, it is possible to explain the reason for the differences in body weight between Hair goats and Damascus goats during the growth period with genetic differences.120th-day body weight of kids was only affected by sex. This situation was implicitly compatible with the other study conducted in Hair kids (Atay et al., 2010). The effect of birth type was statically significant on the birth and 360th day of the growth period in Hair and Damascus goats. Single-birth kids showed higher values in terms of body weight than those born twin-birth kids. This finding was incompatible with studiesconducted inother goat breeds,which reported that birth type affected only birth weight inHairkids (Toplu and Altınel, 2008), thebirth weight and 60thday body weight of Hamdani and Hair kids (Alaşahan and Öztürk, 2019), and 60th, 90th, and 120th day body weight of Saanen and Saanen X Hair kids (Akdağ et al., 2011; Akbaş et al., 2013).In other studies, it has been reported that the birth type affects the body weight in all periods of the 120-day growth period in Honamlı, Hair and Honamlı X Hair (F1) goats (Akbaş and Saatçi, 2016), and the 180-day growth period in Angora goats (Erol et al., 2014).In the present study, it was determined that the influence of dam age on body weight was statically significant with different levels at birth, 30th, 60th, 90th, 180th, 240th and 360th days of the growth period. The growth of kids born to dam age group III was higher than in other dam age groups.These findings coincided with the other studies emphasizing that dam age influences body weight of the Hair and Angora kids throughout the growth period (Toplu and Altınel, 2008; Erol et al.,2014). The birth weight of the dam age III group was higher than those other other age groups. This result utterly concordant to Tatar et al. (2019) which emphasizes that the birth weight of goats born from 4-year-old kids were higher than those born from other dam age groups (3 and 5 year-old-age groups) in Damascus goats.

Development

In this study, it was determined that the head length (27.76 cm) and head width (12.47 cm) values obtained from male Hair goats at the 360 days of age were higher in terms of head length (25.70 cm), and lower in terms of head width (13.30) than the values obtained from 1-4 years old male Hair kids raised in extensive conditions in Burdur, Antalya and Fethiye provinces.Ear length values determined for Hair male kids in the same research (18.0 cm) are exactly consistent with the present study (18.40 cm) (Elmaz et al., 2016).The values of chest depth, chest girth, withers height, and body length obtained on the 90th day (19.35, 51.11,47.08, and 45.76 cm) and 180th day (23.68, 65.33, 51.56, and 55.64 cm) of the growth period in the Hair goats, in general,were consistent with the values reported for the chest depth, chest girth, withers height, and body length on the 90th(20.10, 52.20, 44.70, and 45.10 cm) and 180th day (25.70, 65.70, 52.30, and 53.20 cm) of the growth period in Hair goats raised under semi-intensive conditions in the province of Van (Yılmaz et al., 2013).In the present study, chest girth, withers height and body length values obtained on the 30thday (41.03, 35.66, and 32.13 cm), 90th day (51.11, 47.08, and 45.76 cm),360th day (68.33, 52.64 and 58.91cm) of the growth period in Hair goats were lower than the valuesobtained for 30th day (44.65, 38.20 and 35.67 cm), 90th day (53.53, 45.17 and 43.06 cm), and 360th day (73.26, 59.42 and 60.15 cm) for Hair goats raised under intensive conditions inthe province of Elazığ for the same body characteristics (Şimşek and Bayraktar, 2006). We think that the disparity of these two studies in terms of body development is due to the difference between the rearing systems of the enterprises. Also, researchers emphasized that the kids were continuously fed with rearing ration, dried alfalfa, barley straw, and also sometimes fed with corn during the period when they separated from their dams. The number of studies on body development in Damascus goats is so scarce, and these studies are limited to some periods of growth. For this reason, this section was written within the framework of other published studies. The values obtained for chest depth, chest girth, withers height, body length values in male and female Damascus goats onthe 360th dayof the growth period (26.48 and 26.08 cm, 73.28 and 71.68 cm, 56.47 and 55.29 cm, 61.07 and 59.70 cm) were lower thanthe values reported 1.5 years old age male and female Shami (Damascus) goats (35.80 and 33.86 cm, 78.73 and 74.13 cm, 64.80 and 65.13 cm, 77.80 and 74.73 cm, respectively) reared under intensive conditions in the subtropical regions of Egypt (Abd-Allah, 2019). Chest depth(26.29 cm), chest width(16.76 cm), chestgirth (72.50 cm), withers height (55.86 cm), and body length (60.38 cm)values obtained from 1-year-old Damascus goats in the present study were lower than 3-year-old Damascus (32.11 cm, 19.56 cm, 90.44 cm, 71.89 cm, and 74.89 cm, respectively) and Kilis goats(31.92 cm, 19.50 cm, 89.92 cm, 68.75 cm, and 73.58 cm, respectively), in which some yield characteristics were compared in Damascus and Kilis goats raised under intensive conditions in the province of Diyarbakır (Tatar et al., 2019). The data obtained in these studies were taken from animals of different age groups, raised in different provinces, and under different management conditions. Therefore, we think that the differences with other studies in terms of body measurement values of Damascus goats were due to age, management, and region differences. The difference between the two breeds was statistically significant in terms of head length on the 90th and 360th days, and in terms of head width on the 180th and 360th days of the growth period. Ear length, on the other hand, was significantly different between breeds during the entire growth period. Head length and head width were influenced by sex, however, ear length was not influenced by sex in the entire growth period. This finding is partially similar to the study emphasizing that the effect of sex difference is statistically significant in terms of head length, head width and ear length in Hair goats reared under extensive conditionsin the provinces of Burdur, Antalya, and Muğla (Elmaz et al., 2016). Chest depth, chest width, and chest girth were affected by breed on the 120th day and 360th day of the growth period. Damascus goats had higher values than Hair goats in terms of chest depth, chest width, and chest girth. Sex significantly affected chest depth, chest width, and chest girth especially on the 90th day and later phases of the growth period. Abd-Allah et al. (2019) reported that sex affects chest width and chest girth, and male kids had higher values than females in a study conducted on Shami (Damascus) goats reared under intensive conditions in subtropical regions of Egypt. Males kids were higher values than females kids for these traits in the entire growth period. These findings were consistent with the other study conducted on Hair and Saanen X Hair goats reared under extensive conditions in terms of withers height and cannon circumference characteristics (Çelik and Olfaz, 2018), and it's also consistent with the research conducted on Honamlı, Hair and Honamlı X Hair goats reared under extensive conditions in terms of body length and rump height characteristics (Akbaş and Saatçi, 2016). Mavrogenis et al. (1984) reported that "There is evidence that considerable variation exists among and within animals concerning body weight and growth rate." About the findings of this study, our opinion is that it is possible to generalize this information in terms of body development in goats. According to many studies development in animals is regulated byan intricate relationship among physical factors, genetic-environmental interactions, as well as feeding and management practices, and combined effect of these factors may promote or retard the development process, which in turn can affect body development in adult age (Al-Dawood et

al., 2020; , Steinheim et al., 2008, Koritiaki et al., 2013; Teixeira et al., 2017).

When the research was evaluated in general, the values related to growth and development obtained in this study were lower than other studies. As we observed during the research, forage quality in the region decreases in both dry and cold seasons, which is insufficient for growth and development. Since the feeding of goats in the region is based only on grazing, malnutrition especially in terms of energy and protein may negatively affect growth and development. Fontaneli et al.(2005) stated that "During both the dry and cold seasons, forage and feedstuff quality is frequently decreased being inadequate for the high nutritional demands observed during growth, gestation, and lactation; all of themare physiological processes that demand high-quality supplements regarding protein and energy content"

CONCLUSIONS

Our results demonstrate that a comparison of body weights of Hair and Damascus goats, in terms of their sex, birth type, and dam age, approved that body weights of Damascus goats were significantly higher than those Hair goats. The body weight of kids born in dam age group III was higher than that of kids born in the other dam age groups (dam age II and IV). The growth and development traits of the kids were considerably affected by breed and sex diversity. In terms of their development characteristics, Damascus kids had higher values than the Hair kids. During the growth period in both breeds, body weight and development characteristics of male kidsgot higher values than female kids. It can be suggested that comparative studies containing the entire growth period should be conducted on other indigenous goat breeds (Angora, Honamlı, Kilis, and Norduz goat breeds) and to demonstrate the growth and development characteristics of these breeds.

Ethics Committee Information: This study is not subject to HADYEK's permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

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

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RESEARCH ARTICLE

Investigation of Aflatoxin Presence in Herbal Teas Used For Weight Loss

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ABSTRACT

In this study, the presence of Aflatoxin B1(AFB1) and Aflatoxin B2 (AFB2) were investigated in 10 green tea, 10 fennel, 10 rosemary and 10 senna samples from 14 different sales points in Konya Akşehir. (AFB2) was investigated. Analyses were made by HPLC method. While AFB2 was not detected in any of the 40 herbal tea samples, AFB1 was detected in 65,0% (n=26). According to the results of the analysis, the average amounts of AFB1 in green tea, rosemary, fennel and senna samples were found to be 0,0084, 0,0235, 0,0178, 0,0187 ng/g respectively. Although there was no regulation on herbal teas in the Turkish Food Codex Contaminants Regulation, the legal limit for AFB1 in dried fruits in the same regulation was 8 µg/kg, and in spices 5 µg/kg. It was observed that the aflatoxin levels of green tea, rosemary, fennel and senna samples examined in the study did not exceed the legal limits set for fruits and spices. While an average of 0,01085 ng/g AFB1 was detected in unpackaged a level of 0,02651 ng/g was detected in packaged teas. There was no significant difference between tea types in terms of amount ($p>0,05$). A significant difference was found in the presence of aflatoxin according to how the herbal teas were sold (packaged or unpackaged) ($p<0,05$). It was determined that the amount of AFB1 was higher in packaged herbal teas.

Keywords: Aflatoxin, Fennel, Green tea, Herbal tea, HPLC, Senna

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Zayıflama Amacıyla Kullanılan Bitkisel Çaylarda Aflatoksin Varlığının Araştırılması

ÖZ

Bu çalışmada, Konya Akşehir'de bulunan 14 farklı satış noktasından 10 adet yeşil çay, 10 adet rezene, 10 adet biberiye ve 10 adet sinameki örneğinde Aflatoksin B1(AFB1) ve Aflatoksin B2 (AFB2) varlığı araştırıldı. Analizler HPLC yöntemi ile yapıldı. Toplam 40 bitkisel çay örneğinin hiçbirinde AFB2 tespit edilmezken, %65,0'inde (n=26) AFB1 tespit edildi. Analiz sonuçlarına göre, AFB1 ortalama miktarları yeşil çay, biberiye, rezene ve sinameki örneklerinde sırasıyla; 0,0084, 0,0235, 0,0178, 0,0187 ng/g olarak bulundu. Türk Gıda Kodeksi Bulaşanlar Yönetmeliği'nde bitki çaylarına ilişkin bir düzenleme bulunmamakla birlikte aynı yönetmelikte yer alan kurutulmuş meyvelerde AFB1 için yasal sınır limit 8 µg/kg, baharatlarda ise 5 µg/kg olarak belirtilmiştir. Çalışmada incelenen yeşil çay, biberiye, rezene ve sinameki numunelerine ait aflatoksin düzeylerinin meyveler ve baharatlar için belirlenen yasal limitleri aşmadığı görülmektedir. Ambalajsız çaylarda ortalama 0,01085 ng/g AFB1 tespit edilirken, ambalajlı olanlarda 0,02651 ng/g düzeyinde tespit edildi. Miktar açısından çay türleri arasında anlamlı bir fark bulunmadı ($p>0,05$). Bitkisel çayların ne şekilde satıldığına göre (ambalajlı ya da ambalajsız) aflatoksin varlığına ilişkin anlamlı bir farklılık bulundu ($p<0,05$). Ambalajlı satılan bitkisel çaylarda aflatoksin miktarının daha yüksek olduğu tespit edildi.

Anahtar kelimeler: Aflatoksin, Bitkisel çay, HPLC, Rezene, Sinameki, Yeşil çay

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GİRİŞ

Bitkisel tedavi, sağlığı korumak ve bazı hastalıkları iyileştirmek amacıyla yüzyıllardır popülerliğini koruyan bir tedavi yöntemidir (Faydaoğlu ve Sürücüoğlu 2011). Yüzyıllar öncesinde Mezopotamya, Eski Yunan, Hitit, Roma, Selçuklu ve Osmanlı dönemlerinde bitkisel ilaçlar kullanılmış, Cumhuriyet döneminde ise çeşitli kullanım amaçları bilimsel araştırmalarla desteklenmiştir (Özbek 2005). Dünya'da tedavi amaçlı kullanılan bitkilerin sayısı 20,000 civarında olup (Faydaoğlu ve Sürücüoğlu 2011) dünya nüfusunun %70-80'i bu bitkilerden faydalanmaktadır (Ongan 2018).

Bitki ve bitki çaylarına olan ilgi, XIX. yüzyılda sanayileşmenin bir sonucu olarak ortaya çıkan ve yüzyıl hastalığı olarak bilinen obezitenin yaygınlaşması ile artmış ve kullanımı günümüze kadar devam etmiştir (Aslan ve Orhan 2010). Türkiye'de bitkisel ürün kullanım alışkanlığını belirlenmek amacıyla yapılan bir çalışmada, bireylerin %84,3'ü bitkileri bitkisel çay olarak kullandıklarını ifade etmiştir. Aynı çalışmada bitkisel ürünlerin en yaygın ikinci kullanım amacının %21,3 oranla vücut ağırlığı kaybı sağlamak olduğu belirtilmiştir. Yapılan çalışmalarda, bitkisel çayların en sık zayıflama amacıyla kullanılması (Ongan 2018), bitkisel karışımlarda en fazla kullanılan çayın sinameki (Saraçoğlu ve Ergun 2006) ve aktarlardan en çok satın alınan çayların ise yeşil çay, sinameki, rezene ve biberiye olması dikkat çekmektedir (Öner ve ark. 2017).

Bitkisel çaylar üretim, depolama ve taşıma aşamalarında ağır metal, mikroorganizma ve toksinler ile de kontamine olabilmektedir (Chan 2003; Van Breemen ve ark. 2008). Bitki çaylarının başlıca kontaminasyon kaynağı olan mikroorganizmalar, hasat öncesi, hasat, kurutma, sınıflandırma, öğütme, işleme, paketleme, depolama gibi üretim aşamalarının tamamında bulaşabilmektedir. Araştırmalarda bitkisel çayların çeşitli mikroorganizmaları barındırabildiği, bunlar içerisinde patojenler ve mikotoksijenik küflerin de yer aldığı gösterilmektedir (Scolari ve ark. 2001; Stević ve ark. 2012).

Mikotoksinler patojenik küfler tarafından üretilen ikincil metabolik ürünlerdir (Galvano ve ark. 2001). Küfler içerisinde *Aspergillus*, *Penicillium* ve *Fusarium* genuslarına ait olan türler insan ve hayvanlarda önemli sağlık problemlerine neden olan mikotoksinleri oluştururlar (Erzurum 2001). Aflatoksinler, *Aspergillus* cinsine ait birçok farklı tür tarafından oluşturulan toksisitesi yüksek ve oldukça sık karşılaşılan ve üzerinde en çok araştırma yapılan mikotoksin grubudur (Hussein ve Brasel 2001). Tanımlanmış mevcut aflatoksin sayısının 20'nin üzerinde olduğu bilinmekte, Aflatoksin B1 (AFB1), Aflatoksin B2 (AFB2), Aflatoksin G1 (AFG1) ve Aflatoksin G2 (AFG2)'nin toksik etkisi en yüksek üyeler olduğu bildirilmektedir (Hussein ve Brasel 2001; Yentür 2012). Besinlerde en sık görülen AFB1 ve AFB2'yi üreten iki küften biri *Aspergillus flavus*, diğeri ise *Aspergillus parasiticus* olup; AFB1, AFB2 ile birlikte AFG1 ve AFG2'yi de üretebilmektedirler (Heperkan 2006). İnsanlar üzerinde teratojenik, tremorjenik, kanserojenik, hemorajik, hepatotoksik, nefrotoksik ve nörotoksik etkileri olan (Steyn 1995) mikotoksinlerin oluşumunda en önemli faktörlerden biri depolama şartlarıdır (Demirel ve Yıldırım 2000). Depolanan ürünün nem oranı, sıcaklığı, bağıl nem oranı, depolama süresi, deponun nisbi nemi, üründeki küf yoğunluğu ve ürünün genetik potansiyeli, diğer mikroorganizmaların varlığı gibi faktörlerin yanı sıra bitkinin stresi, hava sıcaklığı, kurutma hızı, gibi bazı faktörler de toksin oluşumunda etkili olabilmektedir (Peraica ve ark. 1999). Toksin içeren besin veya besin maddesi sindirim yoluyla vücuda girdikten sonra göstereceği etkilerin şiddeti mikotoksinin türü, miktarı, canlının genel beslenme durumu ve yaşına bağlıdır (Omurtag ve Yazıcıoğlu 2004).

Sinameki, yeşil çay, rezene, biberiye ve diğer bitkisel çaylarda uçucu fenolik bileşenlerin belirlenmesi, antioksidan kapasiteleri, antimikrobiyel aktiviteleri ve mikrobiyel kalitelerine yönelik çalışmalar yapılmıştır (Dağdelen ve Aşyemez 2014; Arslan 2013). Ancak mikotoksin kontaminasyonunun incelendiği çalışma sayısı oldukça azdır. Bununla birlikte bazı çalışmalarda, siyah çay ve farklı bitki çaylarında aflatoksin varlığı araştırılmış, ancak bu çalışmalarda bitki çayları herhangi bir sınıflandırmaya dahil edilmemiştir (Asghar ve ark. 2018; Vial ve Jardy 1999).

Bu çalışmada baharatçı, aktar, market ve eczanelerden temin edilen ve zayıflama amacıyla yaygın şekilde tüketilen sinameki, yeşil çay, rezene, biberiye çaylarında insan sağlığı üzerine olumsuz etkileri olduğu bilinen Aflatoksin B1 ve Aflatoksin B2 varlığının araştırılması amaçlandı.

MATERYAL ve METOT

Numune Toplama

Çalışmada, 2020 yılı Mart ve Nisan aylarında Akşehir'de bulunan 14 farklı satış noktasından (aktar, baharatçı, market, eczane) ambalajlı (n=16) ve ambalajsız (n=24) 10 adet yeşil çay, 10 adet rezene, 10 adet biberiye ve 10 adet sinameki olmak üzere toplam 40 numune rastgele örnekleme yöntemiyle toplandı.

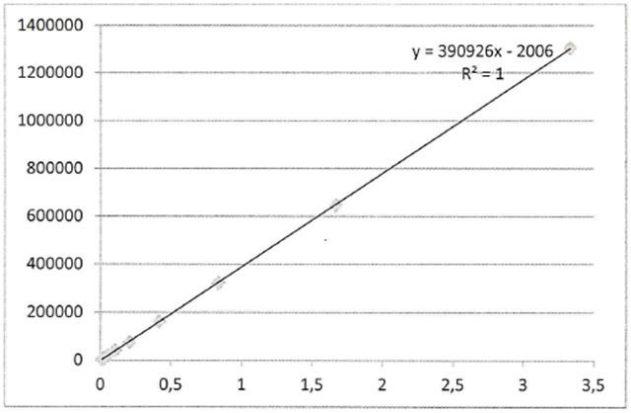
Aflatoksin B1 ve B2 Analizi

Her bir örnek hassas terazide (Precisa, XB 220A, Precisa Gravimetrics AG, İsviçre) 50 gram tartılarak blenderda öğütülerek homojenize edildi. Üzerine 300 mL %50 asetonitril %50 metanol içeren çözücü ve 4 gram NaCl eklenerek homojenize edildikten sonra karışım Whatman No:4 Filtre kağıdından süzülerek aflatoksinler ekstrakte edildi. Süzüntünün 3 mL'si

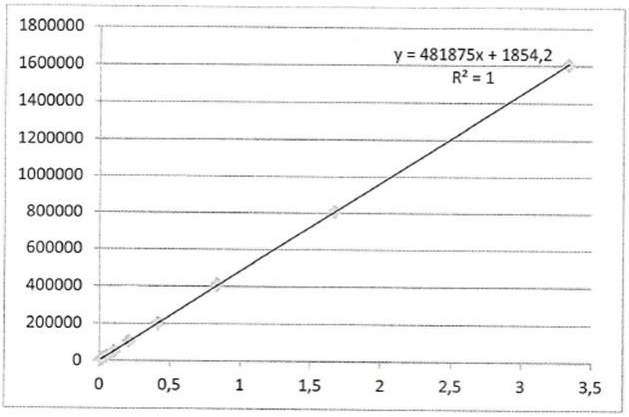
pipet yardımıyla bir behere alınarak üzerine 12 mL fosfat tamponlu salin (PBS) eklendi. İmmunoaffinite kolon, vakum manifoldu (Supelco Visiprep 57030-U, Sigma-Aldrich Chemie GmbH, Almanya) ve vakum pompası (Isolab GM-0.5, Interlab, Türkiye) kullanılarak 5 mL/dk hızla 10 mL PBS ve ardından 20 mL PBS çözeltisi geçirilerek kolon yıkandı. Aflatoksinler kolondan saniyede 1 damla geçecek şekilde 1 ml metanol ile elüe edildi. Kolondan 1 mL saf su geçirilerek 0,45 µm 25 mm çapında PTFE Syringe filtre kullanılarak süzüldükten sonra cam viallere alınarak HPLC yöntemi ile analiz edildi. Örnekler analiz ediline kadar 4ºC'de muhafaza edildi.

Örneklerdeki aflatoksin miktarı, izokratik koşullarda floresans dedektörlü HPLC sisteminde belirlendi. Aflatoksin analizi, izokratik koşullarda Shimadzu RF-20A model floresans dedektörlü (Shimadzu, Japonya) Shimadzu HPLC sistemi (LC-20 AT, Shimadzu, Japonya) kullanılarak yapıldı. Inertsil (GL Sciences, Inc., CA, ABD) ODS-3 C18 paslanmaz çelik kolon (150 x 4,6 mm, 5 µm) kullanıldı. Floresans dedektörü, 360 nm eksitasyon ve 433 nm emisyon dalga boylarına ayarlandı. Mobil faz, su/asetonitril/metanol (6:2:3, v:v:v) 350 µl, 4 M nitrik asit (HNO3), 120 mg potasyum bromür (KBr) ile hazırlanarak, filtre edildi ve ultrasonik banyoda (Wise Clean, Wisd Lab. Inst.) 10 dk bekletilerek gazı giderildi. Akış hızı 1,0 mL/dakika olarak ayarlandı. Enjeksiyon işleminden önce sisteme bağlı olan ve 30 dakika süreyle şartlandırılmış olan Kobra Cell (R-Biopharm Rhone Ltd., Glasgow, UK) ile aflatoksinlerin türevlendirilmesi sağlandı. Örnek direkt olarak 100 µL HPLC sistemine enjekte (SIL- 20ACHT oto enjeksiyon sistemi) edilerek analiz edildi. Örneklerdeki aflatoksin konsantrasyonlarına ilişkin hesaplamalar aflatoksin kalibrasyon grafiği kullanılarak LC Solutions (Shimadzu, Japonya) paket programı ile yapıldı.

Aflatoksin standardı kullanılarak hazırlanan stok çözelti ile farklı konsantrasyonda aflatoksin standart çözeltileri hazırlandı. Aflatoksin konsantrasyonuna karşılık, HPLC kromatogramında elde edilen pik alanlar grafiğe geçirilerek aflatoksin kalibrasyon grafiği oluşturuldu. Örneklerdeki aflatoksin konsantrasyonları da aflatoksin kalibrasyon grafiği kullanılarak LC Solutions paket programı ile oluşturuldu. Tespit limiti (Limit of Detection; LOD) ve tayin limiti (Limit of Quantification; LOQ), metodun standart sapma değerine dayanan hesaplama yöntemi ile hesaplandı (Can ve Velioğlu 2018). Geri alma oranlarını belirlemek amacıyla, birer adet yeşil çay, biberiye, rezene, sinameki örneği toplam 4 µg/kg aflatoksin içerecek şekilde çalışma çözeltisi ile kontamine edilmiş örnekler yukarıda ayrıntısıyla açıklanan aşamalardan geçirildi. Elde edilen süzüntüler, cam viallere alınarak HPLC'de analiz edildi ve geri alma oranları hesaplandı. Aflatoksin B1ve B2'nin kalibrasyon grafiği Şekil 1 ve Şekil 2'de gösterilmiştir.



Şekil 1: Aflatoksin B1'in kalibrasyon grafiği
Figure 1: Calibration diagram of Aflatoxin B1
x: AFB1 konsantrasyonu (ppb) y: Pik alanı



Şekil 2: Aflatoksin B2'in kalibrasyon grafiği
Figure 2: Calibration diagram of Aflatoxin B2
x: AFB2 konsantrasyonu (ppb) y: Pik alanı

Verilerin İstatistiksel Değerlendirmesi

Araştırmada elde edilen veriler SPSS 21,0 for Windows paket programı ile analiz edildi. Aflatoksin içeriğinin karşılaştırılması, bitkisel çay türleri ve ambalaj durumuna göre Ki-kare Testi ile yapıldı. Aflatoksin miktarları bakımından bitkisel çay türleri arasında anlamlı fark olup olmadığı ise tek yönlü varyans analizi ile çözümlendi. Satış şekline göre aflatoksin miktarının karşılaştırılmasında ise bağımsız örneklemler için t-testi kullanıldı.

BULGULAR

Örneklerdeki aflatoksin konsantrasyonlarına ilişkin hesaplamalar, aflatoksin kalibrasyon grafikleri kullanılarak gerçekleştirildi. AFB1 ve AFB2'nin kalibrasyon grafiklerine ait R2 değerleri her ikisi için de 0,999 olarak tespit edildi. AFB1 ve AFB2 için hesaplanan LOD değerleri her ikisi için de 0,002 olarak bulundu. Alıkonma zamanları ise AFB1 için

23,0, AFB2 için 17,5 dakika olarak belirlendi. AFB1 ve AFB2 için belirlenen geri alma oranları tüm örnekler için sırasıyla ortalama %98,0 ve %105,0 olarak hesaplandı.

Araştırmada yeşil çay, biberiye, rezene ve sinameki olmak üzere toplam 40 bitkisel çay analiz edildi. Çay örneklerinin hiçbirinde AFB2 tespit edilmedi. Bitkisel çayların %60,0'ı (n=24) ambalajsız, %40,0'ı (n=16) ambalajlı idi. Yeşil çayın %60,0'ı (n=6) ambalajsız, %40,0'ı (n=4) ambalajlı, biberiyenin %50,0'si (n=5) ambalajsız, %50,0'si (n=5) ambalajlı, rezenenin %60,0'ı (n=6) ambalajsız, %40,0'ı (n=4) ambalajlı ve sinamekinin %70,0'i (n=7) ambalajsız, %30,0'u (n=3) ambalajlı satılan çaylardan seçildi (Tablo 1).

Tablo 1. Satış şekline göre bitkisel çayların dağılımı
Table 1. Distribution of herbal teas by sales type

	Açıkta satış		**Kapalı paket**	
	n	**%**	**n**	**%**
Yeşil çay	6	60.0	4	40.0
Biberiye	5	50.0	5	50.0
Rezene	6	60.0	4	40.0
Sinameki	7	70.0	3	30.0
Toplam	24	60.0	16	40.0

Örneklerin %35,0'inde (n=14) aflatoksin tespit edilemezken, %65,0'inde (n=26) tespit edildi. Yeşil çay numunelerinin %50,0'sinde (n=5), biberiye numunelerinin %60,0'ında (n=6), rezene numunelerinin %60,0'ında (n=6), sinameki numunelerinin ise %90,0'ında (n=9) AFB1 tespit edildi. Ambalajlı satılan çaylarda daha fazla aflatoksin saptandı. Dağılım incelendiğinde ambalajsız çayların %54,2'sinde (n=13), ambalajlı çayların ise % 81,3'ünde (n=13) AFB1 bulundu (Tablo2).

Tablo 2. Satış şekline göre Aflatoksin B 1 varlığı
Table 2. Presence of Aflatoxin B 1 according to the form of sale

	Aflatoksin					
	Var		**Yok**			
	n	**%**	**n**	**%**	Ki-kare	p
Açık	13	54.2	11	45.8	3.956	0.079
Kapalı	13	81.3	3	18.8		
Toplam	26	65.0	14	35.0		

Çay türleri ve AFB1 miktarları arasında anlamlı bir fark bulunamadı (p>0,05). Ortalama AFB1 miktarları yeşil çay, biberiye, rezene ve sinameki çayları için sırasıyla; 0,0084 ng/g, 0,0235ng/g, 0,0178 ng/g ve 0,0187 ng/g idi. Ambalajsız satılan çaylarda ortalama 0,0109 ng/g, ambalajlı çaylarda ise 0,0265 ng/g AFB1 tespit edildi. Bulgulara göre satış şekline göre AFB1 varlığı bakımından anlamlı bir farklılık bulundu (p<0,05). Ambalajlı bitkisel çaylarda AFB1 miktarlarının daha yüksek olduğu belirlendi (Tablo 3).

Tablo 3. Satış şekline göre Aflatoksin B 1 miktarının karşılaştırılması
Table 3. Comparison of the amount of Aflatoxin B 1 according to the form of sale

		Aflatoksin			
Satış şekli	**n**	Ortalama (ng/g)	Standart sapma	t-testi	p
Açık	24	0.0109	0.0151	2.096	0.043*
Kapalı	16	0.0265	0.0317		

*p<0,05

TARTIŞMA

Bu çalışmada, ambalajsız satılan bitkisel çayların %54,2'sinde, ambalajlı olanların ise %81,3'ünde AFB1'e rastlandı. Ambalajsız örneklerde ortalama 0,0109 ng/g, ambalajlı satılan bitkisel çaylarda ise 0,0265 ng/g AFB1 tespit edildi. Ambalajlı satılan bitkisel çayların içerdiği aflatoksin miktarının ambalajsız satılan bitkisel çaylara göre daha yüksek olduğu belirlendi. Bitkisel çayların satış şekline göre, aflatoksin varlığı bakımından anlamlı bir farklılık olduğu bulundu ($p<0,05$). Bununla birlikte aflatoksin üreten küflerin aerobik ortamda üreyip geliştikleri bilinmektedir. Oksijen konsantrasyonunun %1'in altında düştüğü durumlarda aflatoksin üretimi büyük oranda azalmaktadır (Erzurum 2001). Bu araştırmada, ambalajlı bitkisel çayların bazılarında ambalajlamanın hava almayı önleyici şekilde yapılmadığı görüldü. Aynı zamanda, bazı örneklerin ambalajının yıpranmış olduğu ve raflarda uzun süredir beklediği anlaşıldı. Marketin ısıtma ve havalandırma koşullarına bağlı olarak, örneklerin su aktivite değerinin (aw) (optimum 0,78-0,99) yükselmiş olabileceği, özellikle bu iki koşulun aynı anda bulunmasının ambalajlı bitkisel çaylarda daha yüksek miktarda toksin oluşumuna neden olduğu sonucuna varıldı. Ambalajsız satılan bitkisel çaylar daha fazla oksijenle temas etmesine karşın, havalandırma ile birlikte azalan su oranının etkisiyle toksin oluşumunu etkilediği düşünülmektedir. Literatürde ambalajsız ve ambalajlı satılan bitkisel çayların karşılaştırıldığı başka bir çalışma örneğine rastlanılmamıştır.

Can ve Velioğlu (2018), 15 kuşburnu ve 15 ıhlamur örneğinin tamamında aflatoksinlerden en az birinin tespit edildiğini bildirmiştir. Pouretedal ve Mazaheri (2013), 40 siyah çay örneğinin 30'unun (yerli ve ithal) aflatoksinle kontamine olduğunu ve örneklerin ortalama AFB1 miktarının 10.0 ng/g olduğunu rapor etmiştir. Mannani ve ark (2020), 129 yeşil çay örneğinin %58,9'unun (n=76) aflatoksinle kontamine olduğunu ve maksimum aflatoksin miktarının (AFB1+AFB2+AFG1+AFG2) ise 116,2 ng/g düzeyinde olduğunu, eczanelerden satın alınan 60 bitkisel çayın mitotoksin analizinin yapıldığı bir başka çalışmada ise, örneklerin %20'sinde 3,40-23,7 ng/g arasında değişen miktarlarda AFB1 tespit edildiği bildirilmiştir (Reinholds ve ark. 2019). İtalya'da aromatik ve tıbbi bitkisel çay örneklerinin incelendiği bir çalışmada, örneklerin hiçbirinde aflatoksin tespit edilmediği kaydedilmiştir (Romagnoli ve ark. 2007). Hacıbekiroğlu ve Kolak (2013), İstanbul'da 62 gıda örneğinde iki bitkisel çay örneğinde (ıhlamur ve yasemin çiçeği) 1 ng/g'dan fazla AFB1 belirlemişlerdir. İspanya'da, analiz edilen 84 bitkisel çay örneğinin %96,0'sının aflatoksinle kontamine olduğu rapor edilmiştir (Santos 2009).

Bitkisel çaylara ait farklı araştırmalardan elde edilen düşük ve yüksek aflatoksin miktarlarına, örnek çeşidi, kurutma ve depolama şartları ile birlikte analiz yöntemlerinin etkili olabileceği düşünülmektedir. Bitkinin mevcut nemi, kurutma hızı, bulunduğu ortamın bağıl nemi, sıcaklığı, ortamda mevcut olan küf miktarı, küflerin toksin oluşturma yetenekleri, mikroorganizmalar arası rekabet, bitki stresi, kurutma hızı, atmosferdeki gaz bileşimi aflatoksin üretiminde etkili olan koşullardan bazılarıdır. *Aspergillus* gibi zor koşullarda dahi üreme ve gelişme yeteneğine sahip küflerin gelişimi ve toksin üretiminin engellenmesi için, hızlı ve doğru yöntemlerle yapılacak kurutma işlemi oldukça önemlidir. Ancak uzun vadede en etkili ve önemli aşama depolamadır. Depolama süresi boyunca, hava sirkülasyonu sağlanarak depo sıcaklığı ve bağıl nemi kontrol altında tutulmalıdır. Bu şekilde ürünün nem içeriğinin yükselmesi engellenebilmektedir (Stević ve ark. 2012; Pallares ve ark. 2017). Dağdelen ve ark. (2014), paketlenmiş adaçayı, ıhlamur, kuşburnu, papatya ve rezenenin aflatoksin B1, B2, G1, G2 düzeylerini HPLC yöntemi analiz etmişlerdir. Raf ömürleri boyunca periyodik olarak 1, 12, 18, 24, 28, 32 ve 36. aylarda incelenen paketlenmiş bitki çayı örneklerinde aflatoksin varlığı ve oluşumuna rastlanmadığı bulunmuştur. Hasat sonrası yapılan işlemler sayesinde ham numuneden gelen küflerin ortadan kaldırıldığı ve su aktivitesinin kritik sınır olan 0,60 aw değerinin altına indirildiği ardından yapılan bariyer özellikli paketleme sayesinde raf ömrü boyunca içeriğinin değişmediği bildirilmiştir.

Farklı süpermarketlerden alınan 12 adet siyah çay, 10 adet yeşil çay, 14 adet kırmızı çay ve sekiz adet yeşil çay-nane karışımı içeren toplam 44 adet poşet bitkisel çay örneği incelenmiş ve iki adet yeşil çay ve iki adet kırmızı çay infüzyon örneğinde AFG2 tespit edilmiştir. Yeşil çay-nane karışımında tespit edilen AFB2 değerleri 14,4-32,2 µg/L aralığında olup sınır limitlerin (10 µg/kg) üstüne olduğu belirtilmiştir (Heperkan 2006).

Ambalajlı çaylarda saklama ve depo koşullarına, açıkta satılan çaylardan daha fazla dikkat edildiği ve özellikle çeşitli kontrollerden geçtiği düşünülse de, bu çayların rafta bekleme süreleri ve paketin içerisinde oluşabilecek nem miktarına bağlı olarak aflatoksin oluşumuna elverişli bir ortam oluşabilir. Farklı çalışmalarda, ambalajlı satılan çaylarda aflatoksin içeriğinin değişkenlik gösterdiği, bazı çalışmalarda tespit edilememesine karşın, bazılarında yüksek miktarlarda bulunduğu, hatta yönetmeliklerde geçen limit değerleri aştığı görülmektedir. Kurutma ve depolamadan önce, hasat ve taşıma aşamalarında alınacak önlemler, aflatoksin oluşumu azaltmakta veya engelleyebilmektedir. Mikotoksijenik küfler toprak ve havada yaygın şekilde bulunabildiğinden, yağmur/kar öncesi ve sonrası nemli ve soğuk havalarda hasattan kaçınılmalı ve hasat işleminde bitkisel çaylar ile toprağın teması önlenmelidir. Ayrıca herhangi bir şekilde zedelenmiş olan bitkilerde aflatoksin oluşum riski sağlam bitkilere göre daha yüksek olduğundan,

böcek ve benzeri canlıların vereceği fiziksel zararlardan korunmalı, yığınlar halinde bekletmekten kaçınılmalı ve hava boşluklarının kalması için üstüne bastırılmadan muhafaza edilmelidir (Çoksöyler 1999).

Aflatoksinler, yaşamı tehdit eden toksisiteye, kanserojen özelliklere ve diğer potansiyel kronik yan etkilere sahip mantar toksinleridir. Son kanıtlar, aflatoksinin bodur çocuk büyümesinin altında yatan bir belirleyici olabileceğini ve hücre aracılı bağışıklığı azaltabileceğini ve böylece hastalığa duyarlılığı artırabileceğini düşündürmektedir. Bu nedenle, aflatoksin kontaminasyonu, maruziyeti ve düzenleme eksikliği, sağlık üzerindeki olumsuz etkilere de katkıda bulunabilir. Günümüzde bitkisel çayların, zayıflama amacıyla bilinçsiz ve yaygın bir şekilde tüketilmesi, bitkisel çaylarda güvenilirliğin önemini bir kez daha vurgulamaktadır. Bitkisel çaylar doğadan elde edilmeleri nedeniyle hasat, taşıma, kurutma, depolama işlemlerinin kontrollü koşullarda yapılamaması durumunda çeşitli kontaminantlar ile bulaşabilirler. Bununla birlikte; sıcaklık ve nem gibi uygun koşulların bulunması durumunda bitkilerde küf oluşumu hızlanmaktadır. Toksijenik küfler çeşitli besinlerde uygun koşullarda gelişip toksin üretebilirler. Küflerin metaboliti olan mikotoksinlerden, özellikle; aflatoksinler oluşturduğu akut ve kronik toksisite ve karsinojenik etkileriyle halk sağlığını tehdit etmektedir. Bitkisel çayların sıcak su ile hazırlanıyor olmaları, halk arasında, zararlı maddelerin yüksek sıcaklıkta etkisini yitirdiği düşüncesini oluşturmaktadır. Ancak; termofilik küflerin bir kısmı yüksek sıcaklıklarda da varlığını sürdürebilmekte, küflerin metabolitleri olan aflatoksinler ise ısı ile tahrip olmamaktadır. Bu şekilde, bitkisel çaylarla vücuda alımları kolayca gerçekleşebilmektedir. Türk Gıda Kodeksi Yönetmeliği'nde bazı besinlerde bulunabilecek maksimum aflatoksin miktarları belirlenmiştir. Ancak yönetmelikte çay ve bitkisel çaylara ait, aflatoksin maksimum limitlerine ilişkin bir düzenleme bulunmamaktadır. Çalışmanın sonucunda tespit edilen tüm değerler yönetmelikte belirtilen sınırın altında olmasına rağmen, ambalajlı çaylarda daha yüksek miktarda aflatoksin tespit edilmesi önemli bir bulgudur. Mevcut risklerin ve temel problemlerin belirlenebilmesi için konu ile ilgili daha fazla çalışmaya ihtiyaç vardır. Aflatoksin toksisite derecesi, tüketim miktarı ve tüketim sıklığına bağlıdır. Aflatoksinler IARC (The International Agency for Research on Cancer (Uluslarası Kanser Araştırmaları Ajansı) tarafından Grup 1 kanserojen olarak sınıflandırılmıştır ve ayrıca AFB1'in doğrudan DNA ile etkileşime giren reaktif bir bileşene sahip olması sıfırın üzerindeki bütün dozlar için güvenilirliği sorgulatmaktadır. Aflatoksinlerin bitkisel çaylarda çeşitli miktarlarda bulunduğu ve bu ürünlerde aflatoksin maksimum limitlerine ilişkin düzenlemelere ihtiyaç olduğu görülmektedir.

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RESEARCH ARTICLE

Investigation of Methicillin and Panton-Valentine Leukocidin Genes in *Staphylococcus aureus* Strains Isolated from Clotted Creams Sold in Afyonkarahisar

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ABSTRACT

This study aimed to investigate the methicillin and Panton-Valentine leukocidin genes in *Staphylococcus aureus* strains isolated from clotted cream samples produced and sold in Afyonkarahisar. A total of 110 clotted cream samples sold in public bazaars of Afyonkarahisar were collected between November 2019 and December 2020. Conventional cultural methods achieved the isolation of *S. aureus* from clotted cream samples. For the confirmation of *S. aureus* strains isolated from samples and determination of *mecA* and *pvl* genes in the strains, PCR was used. In this study, while *S. aureus* was isolated from 14 of 110 clotted cream samples by standard cultural methods, 13 (11.8%) of 110 samples were typed to be *S. aureus* by PCR. The *mecA* and *pvl* genes were found in none of the 13 *S. aureus* strains. In this study, in which the *pvl* gene was investigated for the first time in the *S. aureus* strains isolated from clotted creams in Turkey, it was thought that more research should be done to determine *mecA* and *pvl* genes in this traditional product and other dairy products.
Keywords: Clotted cream, *mecA, pvl, Staphylococcus aureus*

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Afyonkarahisar'da Satışa Sunulan Kaymaklardan İzole Edilen *Staphylococcus aureus* Suşlarında Metisilin ve Panton-Valentine Lökosidin Genlerinin Araştırılması

ÖZ

Bu çalışmada, Afyonkarahisar'da üretilen ve satılan kaymak örneklerinden izole edilen *Staphylococcus aureus* suşlarında metisilin ve Panton-Valentine lökosidin genlerinin araştırılması amaçlandı. Kasım 2019 ile Aralık 2020 tarihleri arasında Afyonkarahisar halk pazarlarında satışa sunulan toplam 110 kaymak örneği toplandı. Kaymak örneklerinden *S. aureus* izolasyonu konvansiyonel kültür yöntemleri kullanılarak gerçekleştirildi. Örneklerden izole edilen *S. aureus* suşlarının doğrulanması ve suşlarda *mecA* ve *pvl* genlerinin belirlenmesi amacıyla PZR kullanıldı. Çalışmada 110 kaymak örneğinin 14'ünden standart kültürel yöntemlerle *S. aureus* izole edilirken, 110 örneğin 13'ü (%11,8) PZR ile *S. aureus* olarak tiplendirildi. On üç *S. aureus* suşunun hiçbirinde *mecA* ve *pvl* genleri bulunmadı. Türkiye'de kaymaklardan izole edilen *S. aureus* suşlarında ilk kez *pvl* geninin araştırıldığı bu çalışmada, bu geleneksel ürün ve diğer süt ürünlerinde *mecA* ve *pvl* genlerinin belirlenmesi için daha fazla araştırma yapılması gerektiği düşünüldü.
Anahtar kelimeler: Kaymak, *mecA, pvl, Staphylococcus aureus*

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INTRODUCTION

Staphylococcus aureus is among the leading causes of foodborne infections resulting from the consumption of contaminated food (Thaker et al. 2013). The isolation of *S. aureus* has been reported from raw and pasteurized milk, cheese, ice cream, clotted cream and butter, as well as other foods of animal origin in different countries (Pamuk et al. 2012, Rahimi 2013, Basanisi et al. 2017, Dai et al. 2019, Keyvan et al. 2020). Generally, dairy animals with mastitis, mammary glands, milking equipment, animal skin, inappropriate food handling, and unhygienic environment are recognized as the contamination sources of milk and milk products (Thaker et al. 2013, Gezgen and Seker 2016, Dittmann et al. 2017).

Recently, unnecessary, prolonged, and erroneous use of antibiotics has caused *S. aureus* strains of human and animal origin to develop resistance to drugs used for therapeutic purposes. One of these resistance mechanisms is methicillin resistance encoded by the *mecA* gene (Algammal et al. 2020). The number of studies on the isolation of methicillin-resistant *S. aureus* (MRSA) from foodstuffs of animal origin has increased after these foods are identified as a potential mediator of transmission of MRSA strains to humans (Normanno et al. 2007, Basanisi et al. 2017, Saka ve Terzi Gulel 2018, Keyvan et al. 2020, Abdeen et al. 2021). Similarly, the presence of Panton-Valentine leukocidin (PVL) toxin, which is thought to be an important virulence factor in the pathogenesis of MRSA infections in humans, is being investigated, especially in the strains isolated from milk with mastitis (Zecconi et al. 2006, Türkyılmaz et al. 2010, Gezgen and Seker 2016, Şeker et al. 2019). However, the studies on the *pvl* gene prevalence in *S. aureus* or MRSA strains obtained from dairy products are limited (Papadopoulos et al. 2018).

In Turkey, Afyonkarahisar has a significant market share in the traditional dairy products production and consumption such as clotted cream. Although there are various researches on clotted cream's microbiological and chemical quality, most of these studies focused on the total bacterial load in the clotted cream. While the number of studies investigating the presence of *mecA* gene in *S. aureus* strains isolated from clotted creams is limited, there is no research on the prevalence of *pvl* gene in *S. aureus* strains obtained from these products. Therefore, we aimed to investigate the presence of *mecA* and *pvl* genes in *S. aureus* strains isolated from this traditional Turkish dairy product sold in Afyonkarahisar in the present study.

MATERIALS and METHODS

Clotted Cream Samples

In this study, a total of 110 homemade clotted cream samples (200 g each) sold in the public bazaars located in the center districts and villages of Afyonkarahisar between November 2019 and December 2020 were used. The clotted cream samples taken were immediately transported to the microbiology laboratory in aseptic conditions and in a cool box on ice on the same day. The samples were originated from cow milk (n=85), water buffalo milk (n=14) and cow and water buffalo milk (n=11).

Phenotypic Isolation and Identification of *S. aureus*

After each sample was aseptically homogenized by mixing in its container, 10 g were taken from each sample and transferred into 90 mL Brain Heart Infusion Broth (BHIB) for pre-enrichment. The broths were vortexed and then aerobically incubated at 37°C for 24-48 hours. After the incubation broths were vortexed again and 10 µL from each broth were inoculated onto Baird-Parker agar (BPA) containing egg yolk tellurite supplement added according to manufacturer's recommendation. BPA petri dishes were aerobically incubated at 37°C for 24-48 hours. Following the incubation, samples found to have grown at least five gray-black colored colonies surrounded by a dull zone on agar were considered suspicious for *S. aureus* (Singh and Prakash 2008). Gram staining, oxidase, slide and tube catalase, slide and tube coagulase, aerobic and anaerobic fermentation of glucose and mannitol tests were applied to suspicious colonies (Quinn et al. 2004, Garipcin and Seker 2015, Gezgen and Seker 2016). Strains identified to be *S. aureus* following the standard biochemical tests were stored at -20°C in BHIB containing 15% glycerol until DNA extraction.

Detection of 16S rDNA, *mecA* and *pvl* genes by PCR

DNA extraction from all strains was achieved using boiling method (Gezgen and Seker 2016). For detection of the 16S rDNA, *mecA* and *pvl* genes, the primer sets recommended by Strommenger et al. (2003), Choi et al. (2003) and Lina et al. (1999), respectively and the PCR protocols previously described by Gezgen and Seker (2016) were used in the present study. PCR amplifications were carried out in final volumes of 25 µL. All products were analyzed by 1.5% agarose gel electrophoresis and visualized using ethidium bromide under U.V. light. Molecular size markers (100-bp DNA ladder) were included in each agarose gel (Gezgen and Seker 2016).

RESULTS and DISCUSSION

In this study, after the conventional culture methods and biochemical tests were applied to the colonies for *S. aureus* identification, *S. aureus* isolation, and identification was performed from 14 (12.7%) of 110 homemade clotted cream samples. Of the 14 strains identified from clotted cream samples, seven, five, and two were obtained from clotted creams made with cow milk, water buffalo milk, and a mixture of cow and buffalo milk, respectively.

According to duplex PCR results, while 13 of 14 *S. aureus* strains were confirmed in terms of 16S rDNA gene, none of the strains harboured the *mecA* gene (Figure 1). Thus, *S. aureus* isolation rate from 110 clotted cream samples was found to be 11.8% (n=13) in this study. One of the strains isolated from cow's milk origin samples by classical methods was not confirmed as *S. aureus* by PCR. Also, *pvl* toxin gene was determined in none of 13 strains typed by PCR.

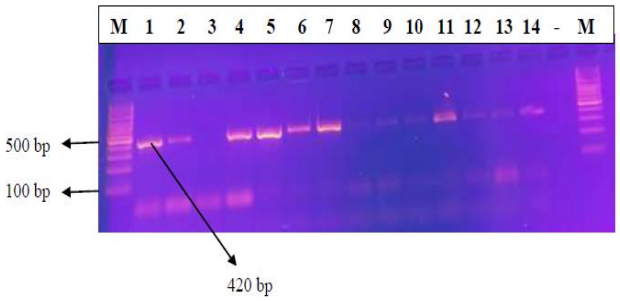

Figure 1. Duplex PCR findings for 16S rDNA ve *mecA* genes. M: DNA ladder (100 bp); lanes 1,2,4-14: 16S rDNA positive *S. aureus* test strains (420 bp); lane 3: 16S rDNA negative test strain; lanes 1-14: *mecA* negative *S. aureus* test strains; -: sterile distilled water.

Clotted cream, a traditional Turkish dairy product, is a sought-after product that benefits both the region where it is produced and the country's economy and has a high consumer share. Therefore, the various studies related to this product's microbiological and chemical quality have been reported in Turkey (Sağun et al. 2001, Pamuk et al. 2012, Sağlam and Şeker 2018, Saka ve Terzi Gulel 2018). However, most of the researches have generally focused on the total bacterial load of clotted cream. In contrast, the bacterial identification studies at the species level are limited (Sağun et al. 2001, Pamuk et al. 2012, Saka ve Terzi Gulel 2018). In a study conducted to determine the microbiological and chemical quality of dairy products offered for consumption in breakfast saloons in Van, it was reported that *S. aureus* was isolated from two (20%) of the 10 clotted cream samples (Sağun et al. 2001). Pamuk et al. (2012) from Afyonkarahisar determined the *S. aureus* was isolated from 26 (21.6%) of the 120 buffalo clotted creams samples, while in Samsun the isolation rate of *S. aureus* from 50 buffalo clotted cream samples was reported to be 18% (n=9) (Saka and Terzi Gulel 2018). In our study, a total of 110 homemade clotted cream samples sold in the public bazaars located in center districts and villages of Afyonkarahisar were examined for the presence of *S. aureus*. While *S. aureus* isolation was achieved from 14 of 110 samples by conventional culture methods, 13 of 14 isolates was confirmed to be *S. aureus* after the PCR identification. Thus, the isolation rate of *S. aureus* from 110 samples was found to be 11.8%. Of the 13 strains identified by PCR, six, five and two were obtained from clotted creams made from cow milk, water buffalo milk and the mixture of cow and buffalo milk, respectively. The isolation rate obtained in our study rate was lower than the other researcher's findings (Sağun et al. 2001, Pamuk et al. 2012, Saka and Terzi Gulel 2018). It was thought that the reason of this result may be related to the geographical region where the sampling was made, the number of samples, seasonal differences in the sampling process and the differences in the isolation and identification methods used. Although the isolation rate of 11.8% obtained in the presented study is low, the presence of this bacterium in foodstuffs or food businesses is accepted to be an indicator of inadequacy in personal hygiene practices (Tunail 2000).

The determination that foods of animal origin mediate the spread of MRSA strains has caused research to focus on this area (Wang et al. 2015, Gezgen and Seker 2016, Basanisi et al. 2017, Şeker et al. 2019). However, it seems that studies generally focus on mastitic milk samples or meat and meat products (Normanno et al. 2007, Basanisi et al. 2017, Yilmaz 2019), and there are limited studies on MRSA isolation from clotted creams (Pamuk et al. 2012, Saka and Terzi Gulel 2018). In Italy, it was emphasized that six (3.7%) of 160 *S. aureus* strains isolated from 1634 food samples containing milk and dairy products and meat and meat products carried the *mecA* gene and all MRSA strains were obtained from raw milk and cheese samples (Normanno et al. 2007). Basanisi et al. (2017) found that 40 (8.3%) of 484 *S. aureus* isolates obtained from 3760 milk and dairy product samples were genotypically MRSA. In a study carried out in Afyonkarahisar, it was reported that *mecA* positivity was found none of 23 *S. aureus* strains isolated from 602 mastitic mammary quarter milk samples (Yilmaz 2019). In one of the two studies on buffalo clotted cream samples, 9 out of 26 *S. aureus* strains isolated from 120 samples had *mecA* positivity (Pamuk et al. 2012), while this gene was found in none of 9 S. aureus strains obtained from 50 buffalo clotted creams in other one (Saka and Terzi Gulel 2018). Similar to Saka and Terzi Gulel (2018) finding, none of 13 *S. aureus* strains isolated from 110 clotted cream samples harboured the *mecA* gene in our study. Some researchers emphasized that the

prevalence of MRSA strains of animal food origin is very low compared to hospital-acquired (HA) and community-acquired (CA) MRSA strains (Normanno et al. 2007, Saka and Terzi Gulel 2018; Yilmaz 2019). In addition, it was thought that the antibiotic use histories of the animals from which the samples were obtained, the origin of the strains, the number of tested strains, and geographical differences might also be adequate on this result.

Panton-Valentine leukocidin toxin is a virulence factor thought to be important especially in the pathogenesis of MRSA infections in humans (Lina et al. 1999, Boyle-Vavra and Daum 2007). Although the role of this toxin in human infections is clear, its role in infections caused by *S. aureus* in animals has not been fully elucidated (Rainard et al. 2003, Lo and Wang 2011). Most of the studies on the presence of the *pvl* gene encoding this toxin in *S. aureus* strains in animals have focused on the strains isolated from ruminant milk with mastitis and in these researches the *pvl* gene has been reported in 0-56% of *S. aureus* strains (Zecconi et al. 2006, Türkyılmaz et al. 2010, Gezgen and Seker 2016, Şeker et al. 2019, Yilmaz 2019). However, no study investigated the presence of this gene in *S. aureus* strains obtained from clotted cream samples. In our study investigating the presence of *pvl* toxin gene in *S. aureus* strains isolated from clotted creams produced in Afyonkarahisar for the first time in Turkey the *pvl* gene was not found in any of 13 *S. aureus* strains. Several authors emphasized that the large majority of CA-MRSA more commonly harbours the *pvl* gene compared to HA-MRSA or foodborne MRSA strains and this gene has been considered the principal virulence marker (Lina et al. 1999, Boyle-Vavra and Daum 2007) or a simple determinant of CA-MRSA strains (Voyich et al. 2006). Also, the low number of strains isolated and the inability to identify MRSA strains in our study may be effective on this result.

CONCLUSION

Consequently, the isolation rate of *S. aureus* from 110 clotted cream samples was found to be 11.8% in the present study. Although this rate is not very high, it should be noted that the determination of this bacterium, especially in foods and in the food industry, may be due to inadequate personal hygiene. In addition, *S. aureus* can be found in various products made from milk animals with mastitis, which may pose a potential danger to public health. For this reason, it is important to improve the production and storage conditions of clotted creams offered for human consumption. In this study, *mecA* and *pvl* genes were not detected in any of 13 strains. However, the fact that *mecA* genes can be transferred to humans via *Staphylococcus* strains isolated from foods of animal origin is an issue that should not be ignored. Therefore, it was thought that it may be necessary to focus on studies investigating the presence of this gene in this traditional product. In our study the presence of *pvl* toxin gene was investigated in *S. aureus* strains isolated from clotted cream samples for the first time in Turkey. Although none of the strains have this toxin gene, it was thought that the acceleration of studies on the determination of this toxin gene in clotted cream and other dairy products may contribute to the detection of *pvl* gene prevalence in these products.

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

Ethical Approval: This study is not subject to the permission of HADYEK in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

Description: This study was summarized from the master thesis of first author.

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RESEARCH ARTICLE

Effects of Feeding Different Levels on Digestibility Body Weight Body Condition Score and Stool Quality in Dogs

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ABSTRACT

In this study, effects of different levels of feeding on digestibility, body weight(BW), body condition score(BCS) and some stool parameters were investigated. Fifteen adult Golden Retriever dogs were divided into 3 groups. ME content of food and ME requirements of the dogs were determined by the modified Atwater factors and the FEDIAF equation($95*BW^{0.75}$). The first group was fed 50% less than daily metabolic energy requirement(MER)(1), second group was fed 100%(2) and the third group was fed 50% more than the MER(3). Trial lasted 15 days. Stools were scored and stool samples were taken in last 4 days of the study. Dry matter(DMD) and organic matter digestibilities(OMD) were determined by acid-insoluble ash indicator method. BW and BCS values were determined on the 7th and 15th days. Changes in BW and BCS between days and groups were insignificant($P>0.05$). DMD and OMD values were the highest in groups 1 and 2($P<0.05$). Group 1 had the highest stool score($P<0.05$). There was no significant difference in stool DM levels between all groups($P>0.05$). In conclusion, modified Atwater factors and formula $95*BW^{0.75}$ were sufficient to preserve BW and BCS for this 15-day study. More studies are needed to compare the different formulas used to calculate the energy needs of dogs and the energy content in their diets. BW and BCS changes should be demonstrated with long-term trials. Effects of daily amount of food determined by the formulas on digestibility and health of dogs should also be investigated.

Keywords: Atwater factors, body condition score, digestibility, dog, metabolizable energy

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Farklı Düzeylerde Beslemenin Köpeklerde Sindirilebilirlik Canlı ağırlık Vücut Kondüsyon Skoru ve Dışkı Kalitesi Üzerine Etkileri

ÖZ

Bu çalışmada farklı düzeylerde beslemenin sindirilebilirlik, canlı ağırlık(CA), vücut kondüsyon skoru(VKS) ve bazı dışkı parametreleri üzerine etkileri araştırıldı. Onbeş yetişkin Golden Retriever köpek 3 gruba ayrıldı. Mamanın ME içeriği ve köpeklerin ME gereksinimleri, modifiye Atwater faktörleri ve FEDIAF denklemi($95*CA^{0.75}$) ile belirlendi. Birinci grup günlük metabolik enerji ihtiyacından(MEİ) %50 daha az(1), ikinci grup ihtiyacın %100'ü(2) ve üçüncü grup MEİ(3)'dan %50 daha fazla olacak düzeyde beslendi. Deneme 15 gün sürdü. Çalışmanın son 4 gününde dışkı skorlaması yapıldı ve dışkı numuneleri alındı. Kuru madde(KMS) ve organik madde sindirilebilirliği(OMS) asitte çözünmeyen kül indikatör yöntemi ile belirlendi. CA ve VKS değerlerindeki değişimler 7. ve 15. günlerde belirlendi. Günler ve gruplar arasındaki CA ve VKS değişiklikleri önemsizdi($P>0.05$). KMS ve OMS değerleri grup 1 ve 2'de en yüksekti($P<0.05$). Grup 1 en yüksek dışkı skoruna sahipti($P<0.05$). Her üç grubun dışkı KM düzeyleri arasında anlamlı bir fark yoktu($P>0.05$). Sonuç olarak, 15 gün süren bu çalışma için modifiye Atwater faktörleri ve $95*CA^{0.75}$ formülü CA ve VKS'yi korumada yeterliydi. Köpeklerin enerji ihtiyaçlarını ve diyetlerindeki enerji içeriğini hesaplamada kullanılan farklı formüllerin karşılaştırılmalı olarak değerlendirildiği daha fazla çalışmaya ihtiyaç vardır. CA ve VKS'ye etkileri uzun süren denemelerle ortaya konulmalıdır. Formüllerle belirlenen günlük mama miktarının köpeklerde sindirilebilirlik ve sağlığa etkileri de araştırılmalıdır.

Anahtar Kelimeler: Atwater faktörleri, köpek, sindirilebilirlik, metabolik enerji, vücut kondisyon skoru

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INTRODUCTION

The energy levels of the food is important as the food intake is basicly controlled by energy density of dog food or diets. Therefore, all the other nutrients in the food should be relative to the energy content (Abinaya et al. 2020). Determining metabolizable energy requirements(MER) of dogs is an important topic for pet food brands and manufacturers. Accurate data on MER of dogs allows to ensure giving accurate feeding recommendations on packaging, ensures advising customers correctly and also plays a role in animal welfare. The energy density of dog foods determines the amount of food need to be given and consequently, knowledge of food energy content is critical for the reliable usage of commercial dog foods (Castrillo et al. 2009).

It has long been established that energy requirements vary considerably from pet to pet, with factors such as activity level, breed, sex, neuter status, age, health status, temperament, body size, insulation characteristics of skin and coat, in addition to environmental factors such as housing conditions and ambient temperature all having an effect (Lund et al. 2005, German 2006, Cameron et al. 2011). Suppling energy has great importance for dogs in determining nutritive value of the food. Dogs, like other animals get their energy by partial and complete oxidation of organic molecules absorbed from the diets and tissue catabolism (Pond et al. 2005). The term of metabolizable energy (ME) is commonly used to express energy density of dog foods. The energy density of food or diet determines the amount of food need to be given daily. Therefore, food energy content is critical for the reliable usage of different kind of commercial dog foods. Energy density of food determines the concentration of other nutrients (amino acids, minerals, vitamins) must have in order to provide animals requirements (Castrillo et al. 2009). Generally ME content of commercial dry dog foods for adult dogs is varied from 3178 to 4405 kcal/kg (Hodgkinson et al. 2008).

Determining the energy requirements of pet dogs is also a particular challenge, since data from other populations, particularly those from dogs in kennelled environments, are not representative. Based upon available literature, maintenance energy requirements for adult dogs varied between 95 and 200 kcal/kg$^{0.75}$ depending on breed, level of activity or husbandry type (Bermingham et al. 2014, FEDIAF 2020).

Overfeeding is a growing underrated problem in dog nutrition all over the world. It has many connections leading not only obesity but also many metabolic disorders in dogs (Meyer et al. 1999). It is estimated that approximately 40-45% of pet dogs are overweight or obese and this rate is increasing. This is associated with medical disorders which significantly reduce life span and quality. Being overweight in dogs is generally because of too much food being offered (German 2006, Courcier et al. 2010). It is important to note that dog owners tend to underestimate the body condition of obese dogs (Colliard et al. 2006) even though dogs start to gain weight, their owners will continue to overfeed (Hodgkinson et al. 2008).

Although it is known that under and overfeeding has negative effects in dog's health, studies about their effects on digestibility and stool quality are scarce. ME measurements of dog foods are not practical or financially feasible; therefore, accepted modified Atwater equations are used to predict ME and develop feeding guidelines (Asaro et al. 2017). There are different equations for predicting ME for dogs but effects of these equations are not well determined. Atwater equations do not account for fiber or energy digestibility, so predicted ME values could differ. Therefore aim of this study was to evaluate modified Atwater equations for determination of ME density of dog food and FEDIAF equation of MER of dogs. Also, it was aimed to determine the effects of feeding at different levels on digestibility, body weight(BW), body condition score(BCS) and stool quality in dogs.

MATERIALS AND METHODS

Dogs and Trial Design

Fifteen adult (age= 5-6 years, body weight=24.5±1.5kg) Golden retriever dogs (6 male, 9 female) were used for the present study. Dogs were divided into 3 groups as having same number of female-male and similar average BW. They were housed in the individual concrete kennels with a closed (190x190 cm) and open area (510x230 cm) in XXX According to the calculated ME requirement for maintenance, daily required food was given to the 1st group at the level of 50% less than MER, 100% (full requirement) for the 2nd and 50% more than MER for the 3rd group. The trial was continued for 15 days.

Chemical Analyses and Calculation of Amount of Food

One commercial dry dog food was used and it's nutrient composition was given in Table 1. Dry matter (DM), crude ash (CA), ether extraction (EE), crude fiber (CF) and crude protein (CP) analyzes were performed according to AOAC (2003) methods. In order to determine digestibility, DM and CA analyzes were also performed on the collected faeces. The energy content of the food was determined by calculations of modified Atwater factors (FEDIAF 2020). The ME requirements of an adult dog at maintenance was calculated using the equation presented by FEDIAF (2020) as follows:

ME (kcal/kg) = 95 x $W^{0.75}$, where W represents body weight.

Energy content of the dog food used in the experiment calculated by nutrient analysis and the daily energy requirement for maintenance of the dogs. Amounts food given were determined with the following formulas:

ME in food (kcal/kg)= %CP*3.5+%EE*8.5+NFE*3.5
(Modified Atwater factors)

$$\text{Amount of food given (gr)} = \frac{\text{Dog's requirement (ME)*1000}}{\text{Food's ME density (kcal/kg)}}$$

BW=Body weight, CP=Crude protein, EE=Ether exraction, NFE,%=100-CP(%)-EE(%)-CF(%)-CA(%)-moisture(%)

Determination of Digestibility

Clean drinking water was provided ad libitum in closed part of the individual kennels. Stool samples were stored in a deep freezer (-18°C) for later analysis. Method of Alvarenga et al. (2019) was used to determine digestibility. Acid-insoluble ash(AIA) in feces and food was evaluated as an indicator. After the feces of each dog were dissolved, they were mixed and sampled for digestibility analysis. Feces were dried at 55°C for 48 hours in drying oven(VWR,Venti-line, USA) and ground in laboratory mill(Retsch SM100,Germany), after determining the stool dry matter(SDM) levels. Samples of 3g of dry feces and 10 g of food were weighed in tared porcelain crucibles and burned in a furnace (Gerhardt,Germany) at 600°C for 8 hours. They were then boiled in 2N hydrochloric acid. After this treatment, samples passed through ashless filter paper (541: Whatman, Maidstone, UK) and reburned at 600°C overnight (approximately 12 hours). The samples were weighed after the second burning to determine the percent of AIA. Digestibility was determined by AIA in 2 parallels in all 5 dogs in the three groups. The formulas given below were used to determine AIA and digestibility.

$$\text{AIA, \%} = \frac{\text{(c-a) x 100}}{\text{b}}$$

a= tare of ash pots, b= weight of stool and food, c= sample remaining after the second burning

$$\text{Digestibility, \%} = 100 - 100 \times \frac{\text{\% AIA in food}}{\text{\% AIA in feces}}$$

Determination of Body Weight and Body Condition Score

Dogs were weighed on the first, 7th and 15th days of trial (ERTE, model B1, Turkey) . The BCS evaluation was based on visual assessment and palpation according to a 9 point scale system (Laflamme, 1997). Four classes of BCS were considered in this trial as follows:

BCS 1 to 3 = lean dog; BCS 4 - 5 = ideal dog; BCS 6 - 7 = overweight dog; and BCS ≥8 = obese dog.

Determination of Stool Consistency Score

Stool consistency scoring was performed by 4 different researchers just before stool collection in the last 4 days of the trial. Stool consistencies were scored by following 1-5 system. According to this system, stools are scored by their appearance and consistency. Properly shaped and hard-consistent stools are considered high quality.

1. diarrhea-like stool, 2. soft and slightly shaped stool, 3. soft, shaped, moist and leaving marks of stool on the floor, 4. well-formed stool, non-dissolving and leaving no traces on the floor, 5. very well-formed and dry looking stools (Strickling et al. 2000).

Statistical Analysis

SPSS 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY): IBM Corp) statistics package program was used. One-way ANOVA tests were performed on BW and BCS changes, DMD, OMD, SDM and fecal consistency scores(FCS) data. Also Independent t-test (Student's t-test) test was used to compare three groups and if not met with the prerequisites, then the Bonferroni-Dunn test was performed to compare the means of the groups for all parameters examined. Values of $P<0.05$ were considered statistically significant.

RESULTS

Dry matter (DMD) and organic matter digestibilities (OMD) of tested food were the highest in group 1 (DMD:73.49%, OMD:77.8%) and 2 (DMD:72.37%, OMD:78.86%) $P<0.05$. Dog group 3 which, fed 50% more than MER ate all dog food given in fifteen minutes and no refusal was recorded. This group showed the lowest DMD (68.29%) and OMD (72.95%) (Table 2).

Dogs consumed all of their calculated amount of the food and maintained a good health throughout the study. Determined BW and BCS changes were not significant between groups and days (Table 3,4).

Results of fecal consistency score and stool dry matter were given in Table 5. There was a difference between the groups in terms of fecal scores ($P<0.05$). It was determined that the stools of the dogs in the 1st group, which ate 50% less food than they needed, had a harder consistency ($P<0.05$). There was no difference between the groups in stool dry matter levels.

Table 1. Nutrient Composition of Commercial Dry Food Fed to Dogs (%DM)

Composition	
Dry matter,%	95.95
Crude ash,%	10.64
Ether extraction,%	8.96
Crude fiber,%	9.92
Crude protein,%	20.07
ME,kcal/kg*	3142.65

*: Calculated value

Table 2. Dry matter and organic matter digestibilities of groups,%

	Groups	N	$\bar{x}$	SEM	*P*-value
DMD	1	10	73.49[a]	0.5	0.004
	2	10	72.37[a]	0.6	
	3	10	68.29[b]	1.12	
OMD	1	10	77.8[a]	0.35	0.004
	2	10	76.86[a]	0.47	
	3	10	72.95[b]	0.94	

DMD= Dry matter digestibility, OMD= Organic matter digestibility, SEM= Standart error of means
1= Group of dogs given -50% less amount of food than requirement of daily metabolizable energy of maintenance
2= Group of dogs given 100% amount of food of daily metabolizable energy of maintenance
3= Group of dogs given +50% more amount of food than requirement of daily metabolizable energy of maintenance
Means in the same column having different superscripts differ significantly (P<0.05)

Table 3. Body condition score changes of dogs given three different levels of food

		1	2	3
	N	Mean±SEM	Mean±SEM	Mean±SEM
Day 0	5	7±0.37	7.3±0.53	6.6±0.43
Day 7	5	6.5±0.34	7.6±0.55	7.2±0.33
Day 15	5	6.4±0.48	7.2±0.60	7.3±0.34
P-value		0.342	0.631	0.229

1= Group of dogs given -50% less amount of food than daily metabolizable energy of maintenance
2= Group of dogs given 100% amount of food of daily metabolizable energy of maintenance
3= Group of dogs given +50% more amount of food than daily metabolizable energy of maintenance
SEM= Standart error of means

Table 4. Body weight changes of dogs fed at three different levels

Dogs body weight parameters	N	Levels of Metabolizable energy requirements fed to dogs 50% less(1)	100%(2)	50% more(3)	*P*-value 3 vs 1	*P*-value 2 vs 1	*P*-value 2 vs 3
		Mean±SEM	Mean±SEM	Mean±SEM			
Body weight day 0, kg	5	25.82±1.02	25.54±0.49	24.6±1.19	0.635	0.615	0.645
Body weight day 7, kg	5	24.44±1.12	24.76±0.45	25.3±1.21	0.752	0.772	0.712
Body weight day 15,kg	5	23.58±1.03	24.56±0.46	25.74±1.18	0.117	0.107	0.110
Body weight change day 0 to 7, kg		-1.38	-0.78	0.7			
Body weight change day 0 to 15, kg		-2.24	-0.98	1.14			
Day 0 vs day 7	*P*-value	0.365	0.378	0.357			
Day 0 vs day 15	*P*-value	0.769	0.786	0.724			

1= Group of dogs given -50% less amount of food than daily metabolizable energy of maintenance requirement
2= Group of dogs given 100% amount of food of daily metabolizable energy of maintenance requirement
3= Group of dogs given +50% more amount of food than daily metabolizable energy of maintenance requirement
SEM= Standart error of means

Table 5. Fecal consistency scores(FCS) and stool dry matter(SDM) levels of dogs

		N	min	max	SEM	$\bar{x}$	*P*-value
FCS	1	60	3.5	5	0.06	4.21[a]	0.032
	2	60	3.5	5	0.05	4.05[b]	
	3	60	3	5	0.82	3.96[b]	
SDM	1	5			1.52	37.46	0.892
	2	5			1.46	37.82	
	3	5			1.73	39.46	

1= Group of dogs given -50% less amount of food than daily metabolizable energy of maintenance requirement
2= Group of dogs given 100% amount of food of daily metabolizable energy of maintenance requirement
3= Group of dogs given +50% more amount of food than daily metabolizable energy of maintenance requirement
SEM= Standart error of means
Means in the same column having different superscripts differ significantly (P<0.05)

DISCUSSION AND CONCLUSION

Total collection of feces and urine is the most accurate method for ME determination for dogs (Laflamme 2001, Case et al. 2011). Collection of the feces is, however complicated for dogs, and requires the dog to be maintained immobile during the process, which has animal welfare implications (Hodgkinson et al. 2008). Also, caprophagia and stepping on feces can be seen in dogs kept in kennels, which cause problems in feces collection process. Thus, AIA indicator method was used in this study for digestibility determination. Variations can be high when determining digestibility with AIA method in low ash content feedstuffs (Alvarenga et al 2019). Therefore, it is difficult to interpret the data. There is sufficient ash in the food prepared for this study (9.92%). It has been concluded in many studies that the digestibility determination in dogs with the AIA method is reliable (Zanatta et al. 2013, Alvarenga et al. 2019, Kahraman et al. 2021). Dry matter (DMD) and organic matter digestibilities (OMD) of tested food were the highest in groups given 50% less than daily MER (DMD:73.49%, OMD:77.8%) and total MER (DMD:72.37%, OMD:78.86%). Digestibility of dog foods should be more than 80%, values less than 75% are not recommended (Malca et al. 2006). Dogs fed 50% more than MER showed the lowest DMD (68.29%) and OMD (72.95%). Besides estimated amount of food given, this can be explain by increased transit time of food. In 50% less than MER given dogs showed similar DMD and OMD with 100% of MER given dogs. Fiber level in this dog food may be the reason of this result. Because satiey of dogs might increased by fiber (Pappas et al. 1989). Reduced transit time and high ileal digestibility might caused same digestibilitiy results in 50% less than MER given dogs with 100% of MER given group. There are not scientific data about effects on direct food restriction on digestibility other than BW and body composition in dogs.

Total dietary fiber could contain high levels of insoluble fiber fraction, so that increased volume and frequency of bowel movements could be reason of low

digestibility coefficients (Prola et al. 2010). DMD and OMD of dry food used were not high even given at the MER and %50 less than MER. Reason of low digestibility was probably high level of CF in used dog food (9.92%). Pet foods have fiber ranges form 0.61 to 9.40% (Hervera et al. 2007). It is the most responsible nutrient that reduces digestibility and energy content of food. High levels of fiber decreases transit time of food and digestibility in dogs (Duque-Saldarriaga et al. 2017). In a study dogs switched from a diet containing 0.6% to 14.7% of CF, DMD was reduced from 90% to 70% (Burrows et al. 1982). El-Wahab et al (2021) determined no difference of digestibility of foods contain CF levels between 1.7-2.1%. They concluded that CF was not enough to affect digestibility. Similar to this study, Brambillasca et al (2010) fed dogs with 3.33% and 9.46% CF content. They found lower DMD (71%) and OMD(75.5%) in 9.46% CF containing food.

Abinaya et al. (2020) fed dogs with three different energy levels containing foods (3021, 2697 and 2358kcal/kg food). They fed dogs *ad libitum* for 20 minutes. They reported higher DMD coefficient in high energy density food (3021 kcal/kg). Reason of this was probably lower CF and crude ash content of high energy food they used. They concluded that reduced DMD was compensated with higher DM intake of foods with lower energy density. ME of food used in this study was 3142.65 kcal/kg calculated by modified Atwater factors. 50% more MER given group's DM intake(DMI) was 20.06 gr/kg BW and 100% of MER given group's was 13.43 gr/kg BW in this study and dogs left no food in 20 minutes. Carciofi et al (2009) and Sa et al (2013) determined DMI of 16g/kg BW in adult dogs. They estimated the amount of food with the energy value of food and energy requirement of the animal, according to NRC (2006). Case et al (2011) and Laflamme (2001) also indicated that the modified Atwater calculation has shown underestimate prediction of ME of highly digestible diets and overestimate ME of those with lower digestibility. In that case, maybe daily given amount of food was not accurate for dogs used in this study for better digestibility.

Soluable and insoluable fiber fractions of dog food was not determined in this study. But it has been demonstrated that soluble fiber increases DMD (De Godoy et al. 2013). On the other hand, dog foods with CF content above 8% and a high level of fermentable non-starch polysaccharides (NSP) in the CF fraction leads underestimate prediction of energy density by modified Atwater equations (FEDIAF, 2020). NSP fraction of fiber also was not determined in this study. NRC suggests 4 step equations starts with (5.7 x % protein) + (9.4 x % fat) + [4.1 x (% NFE + % crude fibre)] calculating gross energy and there are 3 more steps to calculate ME. But modified Atwater factors suggest equation of %CP x 3.5 + % EE(fat) x 8.5 + %NFE x 3.5 (AAFCO, 2008). They are based on an average digestibility of 90% for fat, 85% for carbohydrate (NFE) and 80% for protein. Different equations did not compare with each other in this study. More studies are needed to assess equations for ME estimation of dog foods and longer feeding trials to determine their effects on BW and BSC. Soluable fiber fractions need to be determined for further studies to interpret their effects on digestibility, BW and BSC.

Dogs were kept in individial kennels which have open and closed parts and they were not allowed to run or take a walk. This was probably one of the reasons of being no digestibility coefficient difference between 1st and 2nd groups. The exact ME requirements of an individual dog will depend on its age, activity level, body condition, hair (insulating conditions), environmental temperature, acclimatization, external environmental circumstances and psychological temperament (NRC 2006). Type of husbandary has an important effect for MER estimation (Bermingham et al. 2014). Activity level was considered as"low"when calculate MER with 95 kcal*$BW^{0,75}$ in this study. The equation of 95 kcal*$BW^{0,75}$ maybe was not the best choice for the dogs kept in individual kennels. 95-130 kcal*$BW^{0,75}$ is recommended for low active dogs. But Individually housed dogs, with little opportunity to move, may have daily energy requirements as low as 70 kcal ME/$kg^{0.75}$ (FEDIAF 2020). Recommendations for MER may overestimate energy needs by 10 to 60% (NRC 2006). They often include a reasonable amount for activity.

Modified atwater factor and NRC equations have modarete accuracy for estimation of ME for wet pet foods. But NRC equations are recommened for estimation for dry dog foods (Calvez et al. 2012a). For the kibble diet, the modified Atwater calculation underestimates the ME and the NRC calculations are the most accurate predictor of ME (Tanprasertsuk et al. 2021). Furthermore, Oba et al. (2020) stated that modified Atwater factors systematically underestimate the ME content of low-fibre foods whereas they overestimate those that are high in fibre. On the other hand, Hall et al. (2013) reported that modified Atwater factors accurately predict ME concentrations in dog foods (r^2=0.97). There are conflicting results but modified Atwater factors and 95kcal*$BW^{0.75}$ equations might be the reasons of determination of low digestibility of dog food used in this study. More studies are needed for further investigations to determine MER of dog and ME of dry kibble foods.

Despite of low digestibility, equations used in this study seem effective to maintain BW and BSC. Calculated amount of food for dogs to maintain BW and BCS was acceptable for 15 days. Dogs started to lose weight but not significantly at the end of the trial. It can be interpreted that if this study has lasted for more than 1 month, BW changes would have been significant. Because dogs tend to lose %10 of their body weight in 1 month. Yamka et al. (2007) conducted a weight loss study lasted 2 months. They found significant change after 1 month of trial.

Alexander et al. (2017) offered %200 of MER to dogs and record %1.25 BW gain per week during 1 month trial. 50% more than MER given dogs gained 4.63% of initial BW end of 15 days in this study. Further studies should continue at least 1 month to determine significant BW changes.

The ideal BCS should be between 4/9 and 5/9 (FEDIAF, 2020). Initial BSC of dogs used in this study were between 6.6-7.3. These scores are considered overweight. MER for maintanence of dogs were calculated with inital body weights, ages and activity levels of dogs. After 15 days of trial, changes of BW and BSC scores of dogs were not significant. In some dogs calorie needs may further decrease as ansequence of an increase in subcutaneous fat (Meyer and Zentek 2005). According to Chandler (2011), loss of more than 2% of BW each week is undesirable because a greater proportion of lean body tissue loss often occurs. Saker and Remillard, (2005) used a weight loss program that estimates %1-4 of body weight loss in 1 week in dogs. They observed a poor accuracy of their weight loss program. Reasons were method and amount of food given. They used the equation of NRC suggests as $132*BW^{0.75}$ for MER of dogs. In this study $95*BW^{0.75}$ was used. Although it was not statistically important, 100% of MER given dogs started to lose weight after 15 days of trial. %50 more and less than daily MER given dogs were not shown any significant changes in terms of BW and BCS after 15 days of trial. These results indicates that 15 days of trial was not sufficient to determination of accuracy of modified Atwater factors. Next studies should be conducted for longer periods. Different equations are also needed to be investigated and compared to decide whether these formulas are accurate or not.

Neuter status of dogs were not considered for calculation MER. Bermingham et al (2014) reported that MER of neutered dogs lower than the requirements of intact dogs. For this study BW, age and activity levels were considered as FEDIAF (2020) suggests. As many factors affects MER, energy requirement may be below or above that suggested by NRC, AAFCO or FEDIAF and density of dog foods may need to be adjusted up or down to make sure sufficient but not excess of nutrients (Hill, 2006). In this study, ME of dog food was determined by using nutrient analysis. Quantity of the dog foods that should be fed to the animals presented on the package gives a range of quantities to take into consideration as variations between dogs. Owners often follow the recommended amounts of food labeled on the package. However, there are also obese dogs, even though they are given in the recommended amounts on the packages (Hodgkinson et al. 2008). Nuttall et al. (2017) reported that the modified Atwater factors assume constant macronutrient digestibility coefficients and have been challenged for being a source of error when calculating predicted ME. Tanprasertsuk et al. (2021) indicated that, modified Atwater calculation underestimates the ME and NRC (2006) calculation is the most accurate predictor of ME for the kibble diets of dogs. Calculated ME of dog food used in this study was 3142.65 kcal/kg. Hodgkinson et al (2008) used a commercial dog food with 2916 kcal/kg ME. They observed less BW gain. Researchers emphasized that low calorie foods should contain less than 3100 kcal/kg ME. The food used in this study was nearly a low calorie food. That could be another reason of insignificant BW gain in the group given 50% more than MER. Hodgkinson et al. (2008) used $1.2*(132*BW^{0.75})$ as NRC recommended. In this study $95*BW^{0.75}$ was used. This equation can be also considered another reason of insignificant changes in BW and BSC in all three groups. In a study, less adiposity was determined in 25% food restricted dogs (Kealy et al. 2002). Dog food used in this study had sufficient nutrient content as FEDIAF(2020) recommend. Being more than 8% fiber content may be another reason of insignificant changes for BW and BCS.

Some studies have shown that high fiber content reduces stool quality (Sunvold et al. 1995b, Wichert et al. 2002). In this study, no significant difference was found between the groups in terms of fecal score and stool dry matter parameters. Fecal scores of all groups were better than ideal range (3-4) (Inal et al. 2017). There was no difference even in dogs consumed 50% more or less of their daily MER. This might may be an indication that the formulas used are inaccurate. There are not scientific data focus on stool quality and modified Atwater factors relationships. Higher CF levels could cause wet and soft stool excreation in dogs (Brambillasca et al. 2010). Fecal consistency scores were not soft in this study even dog food had 9.92% CF. This result may be related to fiber fractions of dog food used or it may be equaiton used for determination of MER of dogs ($95*BW^{0.75}$).

In conclusion, understanding energy density of dog foods enables the development of accurate feeding programs that allow to be fed with the total recommended daily energy requirement. Accurate feeding guides help to avoid under and overfeeding. Although further studies are required, the present study indicates that the combination of modified Atwater method and $95*BW^{0.75}$ formula for estimation dog's MER, might not adequate for the food tested here. BSC and BW changes were not significant as expected at the end of 15 days trial. But relationship of low digestibility of the food with these formulas need to be investigated. Nutrient and energy contents labeled on commercial dog food packages should be evaluated in all brands. The effects of amount of food recommended by manufacturers on BW and BCS of the dogs should be determined and checked for digestibility as well. From the dog-owner and veterinary point of view, this study highlights the

importance of adjusting the quantities of dog foods that should be fed according to the body condition of the dog. Each dog's feeding program should be assessed routinely and adjustments made based on the animal's life stage and general health. This study provides scientific information to veterinarians, nutritionists, pet food manufacturers, and pet owners in understanding the importance of energy density of dog foods and energy requirements of dogs.

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RESEARCH ARTICLE

Investigation of Schmallenberg Virus Seroprevalence in Honamlı Goats from Burdur Region

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ABSTRACT

Schmallenberg virus (SBV) was first reported in cattle, in north-western Germany and east of the Netherlands in November 2011. SBV causes serious economic losses in domesticated ruminant animals. SBV is also seen in Turkey and there are few seroprevalence studies in cattle, sheep and goats. For this purpose, in this study, the prevalence of SBV was investigated in Honamlı goats in Burdur region. The study was carried out in 12 different herds that were breeding Honamlı goats in Burdur province. The animal material consisted of 186 Honamlı goats with 93 aborted and 93 normal healthy births which were aged between 2-5 years. In the findings; SBV specific antibodies were detected in only two (1.1 %) serum samples from the animals included in the study. In two goats (1.1 %), the test result was suspectable for antibody positivity. Consequently, it was determined that SBV infection has a low seroprevalence rate in Honamlı goats in Burdur province.

Keywords: ELISA, Goat, Honamlı Goat, Schmallenberg virus, Seroprevalence.

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Burdur Yöresi Honamlı Irkı Keçilerde Schmallenberg Virüs Seroprevalansının Araştırılması

ÖZ

Schmallenberg virüsü (SBV) ilk olarak Kasım 2011'de kuzeybatı Almanya'da ve Hollanda'nın doğusunda sığırlarda rapor edilmiştir. SBV evcil geviş getiren hayvanlarda ciddi ekonomik kayıplara yol açmaktadır. SBV ülkemizde de görülmektedir ve sığırlarda ve koyunlarda ve keçilerde az sayıda seroprevalans çalışmaları bulunmaktadır. Fakat Honamlı ırkı keçilerde yapılan seroprevalas çalışması yoktur. Bu amaçla bu çalışmada Burdur yöresinde Honamlı ırkı keçilerde SBV prevalansı araştırılmıştır. Bu çalışma Burdur ilinde Honamlı keçisi yetiştiren 12 farklı sürüde yürütülmüştür. Hayvan materyalini 2-5 yaşları arasında değişen 93 abortlu ve 93 normal sağlıklı doğum yapan 186 Honamlı keçi oluşturmuştur. Bulgularda; Çalışmaya dahil edilen hayvandan kan serumlarının yalnızca ikisinde (% 1.1) Schmallenberg virus spesifik antikor varlığı saptandı. iki keçide ise (% 1.1) test sonucu şüpheli antikor pozitiflik belirlenmiştir. Sonuç olarak; Burdur ilinde Honamlı ırkı keçilerde SBV enfeksiyonunun düşük seroprevalans oranına sahip olduğu belirlenmiştir.

Anahtar kelimeler: ELISA, Honamlı Keçisi, Keçi, Schmallenberg virus, Seroprevalans.

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INTRODUCTION

Schmallenberg virus (SBV) is a Shamonda /Sathuperilike RNA virus belonging to the serogroup Simbu, and located to the family Peribunyaviridae of the genus Orthobunyavirus (Elbers et al. 2012, Yilmaz et al. 2012, Wernike et al. 2018, Kauffold et al. 2021).

SBV predominantly infects domestic ruminants such as cattle, sheep, goats and, wild and exotic ruminants such as alpaca, llama, elk, water buffalo, bison, various species of deer, mouflon, chamois, wild boars and camelids. In addition, SBV antibodies have also been detected in dogs and wild boars (Brülisauer et al. 2017, Kęsik-Maliszewska et al. 2018, Kauffold et al. 2021).

SBV is mainly transmitted by biting midges of the genus Culicoides, as with other Simbu serogroup viruses. Furthermore, SBV is also transmitted by other blood-sucking vectors such as mosquitos and ticks. In addition, wind also plays a role in the spread of Culicoides midges over long distances (Kęsik-Maliszewska et al. 2018, Wernike et al. 2018, Wernike and Beer 2020).

According to the time of infection, the virus shows two different clinical courses. Especially, it produces a short-lived viremia in domestic ruminants of all age groups. Clinically, there is a history of mild transient disease with fever, diarrhea, and decreased milk yield. On the contrary, persistent infections in the fetuses of pregnant animals can cause abort, premature birth or severe congenital malformations (Endalew et al. 2019, Wernike et al. 2018).

Therefore, SBV causes serious economic losses in domesticated ruminant animals (Szeredi et al. 2020). SBV is also seen in Turkey and there are few seroprevalence studies in cattle, sheep and goats (Azkur et al. 2013, Tonbak et al. 2016, Macun et al. 2017, Elmas et al. 2018). But, there is no seroprevalence study in Honamlı goats. For this purpose, in this study, the prevalence of SBV was investigated in Honamlı goats with and without abortion in Burdur region.

MATERIALS and METHODS

This study was carried out with the permission of the Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee, dated 13.05.2021 and numbered 88/751. The study was carried out in 12 different herds that were breeding Honamlı goats in Burdur province. The animal material consisted of 186 Honamlı goats with 93 aborted and 93 normal healthy births which were aged between 2-5 years. For analysis, 8 ml of blood was taken from the vena jugularis of goats into vacuum gel tubes. The blood samples were centrifuged at 3500 RPM for 15 minutes and their serums were extracted. Extracted serums were stored in a deep freezer at -20°C until analysis.

Serological Analysis

For the determination of specific antibodies to SBV; A commercial ELISA kit (IDEXX Schmallenberg Ab Test®, IDEXX, Switzerland) was used. Control and serum samples were analyzed according to IDEXX's guidelines. In the assays, optical density (OD) was determined relative to the negative and positive controls provided by IDEXX (S/P %). According to this; S/P % = 100 x sample (OD) - negative control (OD)/Positive control (OD) - negative control (OD). S/P percentages were evaluated as follows; S/P % < 30 % negative, ≤ 30 % S/P % ≥ 40 % suspectable and ≤ 40% positive. The specificity and sensitivity of this Test Kit are 99.5% and 98.1 %, respectively (Pejaković et al. 2018).

Statistical Analysis

The findings obtained from the study were evaluated using the IBM SPSS 26.0 for Windows package program. The compositional distribution of the variables was determined using crosstabs. Spearman Correlation analysis was used to determine the relationship between variables.

RESULTS

In the findings, SBV specific antibodies were detected in only two (1.1 %) serum samples from the animals included in the study. In 2 goats (1.1 %), the test result was suspectable for antibody positivity (Table 1 and Table 2).

When the aborted goats were examined in terms of the presence of SBV specific antibodies; 1 seropositive goat was detected in Bağsaray 1 and 1 suspectable goat was detected in Ovacık 2 (Table 1 and Table 2).

When the non-aborted goats were examined in terms of the presence of SBV specific antibodies; 1 positive goat was detected in Bağsaray 1 and 1 suspectable goat was detected in Kuzköy 2 (Table 1 and Table 2).

In correlation findings, no correlation was found between abortion and SBV (r=1.00; p=1.00) (Table 3).

Table 1. Distribution of Schmallenberg Virus' in aborted goats by herds

Aborted goats		Schmallenberg Virus			
Village		Negative	Suspectable	Pozitive	Total
Kuzköy 1	Count	5	0	0	5
	%	5,4 %	0,0 %	0,0 %	5,4 %
Kuzköy 2	Count	10	0	0	10
	%	10,8 %	0,0 %	0,0 %	10,8 %
Kuzköy 3	Count	10	0	0	10
	%	10,8 %	0,0 %	0,0 %	10,8 %
Ovacık 1	Count	10	0	0	10
	%	10,8 %	0,0 %	0,0 %	10,8 %
Ovacık 2	Count	9	1	0	10
	%	9,7 %	1,1 %	0,0 %	10,8%
Çeltikçi 1	Count	5	0	0	5
	%	5,4 %	0,0 %	0,0 %	5,4 %
Çeltikçi 2	Count	9	0	0	9
	%	9,7 %	0,0 %	0,0 %	9,7 %
Bağsaray 1	Count	4	0	1	5
	%	4,3 %	0,0 %	1,1 %	5,4 %
Bağsaray 2	Count	7	0	0	7
	%	7,5 %	0,0 %	0,0 %	7,5 %
Bağsaray 3	Count	7	0	0	7
	%	7,5 %	0,0 %	0,0 %	7,5 %
Bağsaray 4	Count	7	0	0	7
	%	7,5 %	0,0 %	0,0 %	7,5 %
Bağsaray 5	Count	8	0	0	8
	%	8,6 %	0,0 %	0,0 %	8,6 %
Total	Count	91	1	1	93
	%	97,8 %	1,1 %	1,1 %	100,0 %

Table 2. Distribution of Schmallenberg Virus' in non-aborted goats by herds

Non-aborted goats		Schmallenberg Virus			
Village		Negative	Suspectable	Pozitive	Total
Kuzköy 1	Count	5	0	0	5
	%	5,4 %	0,0 %	0,0 %	5,4 %
Kuzköy 2	Count	9	1	0	10
	%	9,7 %	1,1 %	0,0 %	10,8 %
Kuzköy 3	Count	10	0	0	10
	%	10,8 %	0,0 %	0,0 %	10,8 %
Ovacık 1	Count	10	0	0	10
	%	10,8 %	0,0 %	0,0 %	10,8 %
Ovacık 2	Count	10	0	0	10
	%	10,8 %	0,0 %	0,0 %	10,8 %
Çeltikçi 1	Count	5	0	0	5
	%	5,4 %	0,0 %	0,0 %	5,4 %
Çeltikçi 2	Count	9	0	0	9
	%	9,7 %	0,0 %	0,0 %	9,7 %
Bağsaray 1	Count	4	0	1	5
	%	4,3 %	0,0 %	1,1 %	5,4 %
Bağsaray 2	Count	7	0	0	7
	%	7,5 %	0,0 %	0,0 %	7,5 %
Bağsaray 3	Count	7	0	0	7
	%	7,5 %	0,0 %	0,0 %	7,5 %
Bağsaray 4	Count	7	0	0	7
	%	7,5 %	0,0 %	0,0 %	7,5 %
Bağsaray 5	Count	8	0	0	8
	%	8,6 %	0,0 %	0,0 %	8,6 %
Total	Count	91	1	1	93
	%	97,8 %	1,1 %	1,1 %	100,0 %

Table 3. Correlation findings between Schmallenberg Virus and abortion

Spearman's rho		Schmallenberg Virus	Abortion
Schmallenberg Virus	**Correlation Coefficient**	1	,000
	Sig. (2-tailed)		1,000
	N	186	186
Abortion	**Correlation Coefficient**	,000	1
	Sig. (2-tailed)	1,000	
	N	186	186

DISCUSSION

SBV was first reported in cattle, in north-western Germany and east of the Netherlands in November 2011(Azkur et al. 2020, Szeredi et al. 2020). Within two years of being first reported, SBV spread rapidly across the European Continent and more than 8,000 outbreaks were seen in 22 European countries (Jiménez-Martín et al. 2021). Later, SBV spread to Turkey, Russia, Azerbaijan, Iran, Lebanon and China, and then to African countries including Mozambique, Namibia and Ethiopia (Kauffold et al. 2021)

A few number of seroprevalence studies have been published since the SBV infection was detected in Turkey. (Azkur et al. 2013, Yilmaz et al. 2014, Tonbak et al. 2016, Macun et al. 2017, Elmas et al. 2018, Azkur et al. 2020). The most comprehensive study in our country was carried out by Azkur et al. (2013). According to this study, they reported that the SBV seroprevalence in Turkey was 39.8 % in cattle, 1.6 % in sheep, 2.8 % in goats and 1.5 % in buffaloes. In addition, Azkur et al. (2013) also determined the prevalence of SBV in goats by province. They reported that the prevalence of SBV by provinces was 7.1 % in Sinop and 2.1 % in Samsun.. Macun et al. (2017) reported that SBV seroprevalence was 0.38 % in sheep in Kırıkkale province. Elmas et al. (2018) reported that SBV seroprevalence was 0.27 % in Akkaraman sheep in Sivas province. In parallel with this information, in our study, the prevalence of SBV in Honamlı goats in Burdur province was determined as 1.1 % (Table 1 and Table 2).

Schmallenberg virus is transmitted by blood-sucking flies (especially Culicoides spp), which is found in large numbers near rivers, waterfalls and lakes. It is also reported that the flight activities of Culicoides can be affected by many factors such as light, temperature, wind and humidity (Pawaiya and Gupta 2013, Macun et al. 2013, Lievaart-Peterson et al. 2015). Elmas et al. (2018) associated the low prevalence of SBV in Akkaraman sheep in Sivas province with the harsh continental climate of Sivas province. Because the density of blood-sucking stinging flies, Culicoides species that transmits the infection, decreases in harsh climates. Our study also strengthened the opinion that the SBV prevalence may have been low due to the grazing of goats on mountains far from the water's edge. This is supported by the high prevalence of SBV in goats in Sinop and Samsun provinces climate (Azkur et al. 2013), which are rainier and more humidity Black Sea climate.

Antibodies are formed approximately 12 to 14 days following SBV infection. The presence of the specific antibody in the serum of naturally infected adult cattle with SBV continues for at least 2 years (Conraths et al. 2013, Elbers et al. 2014, Elmas et al. 2018). However, there is any literature on the persistence of antibodies formed in natural infection with SBV in goats. In addition, Elmas et al. (2018) reported that the low SBV positive seroprevalence and the presence of three suspected seropositive samples may be due to the decrease in the titer of existing antibodies over time. In our study, the low SBV positive seroprevalence in goats and the detection of 1.1 % of goats as suspectable for SBV specific antibodies may be related to the decrease in the titer of the existing antibodies (Table 1 and Table 2).

Small ruminants show maximum sensitivity to SBV between 28-50 days of pregnancy. Abortions, stillbirths and congenital malformations are seen in infected animals during this period of pregnancy. Adult animals naturally infected with SBV have effective immunity when they subsequently recover from the infection. The level of neutralizing antibodies after immunization is effective to prevent transmission of SBV to other susceptible animals (Jiménez-Martín et al. 2021). Macun et al. (2017) reported that widespread abortion in sheep is not due to SBV. In our study, 1 goat with abortion and 1 goat without abortion were determined as SBV seropositive, and 1 goat with abortion and 1 goat without abortion were determined as suspicious in terms of SBV specific antibodies. Furtermore, statistically, there was no relationship between abortion and SBV (r=1.00; p=1.00) (Table 1, Table 2 and Table 3). These findings showed that common abortion in goats was not related to SBV.

CONCLUSIONS

Consequently, it was determined that SBV infection has a low seroprevalence rate in Honamlı goats in

Burdur province. In addition, future large-scale epidemiological studies in terms of SBV, and investigation of the presence/distribution of vectors that play a role in the transmission of infection will be beneficial for the prevention of SBV infection.

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

Ethical Approval: This study has received permission with, Mehmet Akif Ersoy University HADYEK number 88/751 and 14.04.2021 date. In addition, the authors declared that they comply with the Research and Publication Ethics.

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RESEARCH ARTICLE

Determination of Virulence Factors and Antibiotic Resistances of *Enterococcus* spp. Identified from Different Stages of Ripened (Classical) White Cheese Production

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ABSTRACT

The objective of this research was to determine the presence of virulence genes (*asa1, gelE, cylA, ace, esp, hyl and efaA*), and vancomycin resistance genes (*vanA, vanB, vanC2/C3*) and the resistance to some antibiotics of *Enterococcus* spp. isolates previously identified from different stages of ripened white cheese production. In addition, gelatinase, β-hemolytic and DNase activity, and biofilm formations were examined phenotypically. In this study, *efaA* in 95.9%, *asa1* in 89%, *ace* in 68.5%, *esp* in 52.1%, *gelE* in 78.1%, *cylA* in 16.4% and *hyl* in 23.3% of isolates were detected. Also, *vanA* in 31.5%, *vanB* in 8.2%, and in *vanC2/C3* 23.3% resistance genes were determined. β-hemolytic and DNase activity were detected in 23.2% and 16.4% of the isolates, while gelatinase activity and biofilm formation could not be detected phenotypically. Moreover, streptomycin and erythromycin resistances were found in 73.9% and in %43.8 of isolates. As a result, it was concluded that *Enterococcus* spp. may pose a risk for public health and food safety in terms of their virulence factors and antibiotic resistance. For this reason, it was suggested that the strains to be selected as starter cultures should be used after evaluating their virulence factors and resistance to antibiotics.

Keywords: Antibiotic Resistance, *Enterococcus* spp., Ripened White Cheese, Virulence Factor.

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Olgunlaştırılmış (Klasik) Beyaz Peynir Üretiminin Farklı Aşamalarından İzole Edilen *Enterococcus* spp.'nin Virülens Faktörlerinin ve Antibiyotik Dirençliliğinin Belirlenmesi

ÖZ

Bu çalışmada, daha önce klasik beyaz peynir üretiminin farklı aşamalarından identifiye edilen *Enterococcus* spp. izolatlarının virülens genleri (*asa1, gelE, cylA, ace, esp, hyl ve efaA*) ve vankomisin direnç genlerinin (*vanA, vanB, vanC2/C3*) varlığı ile bazı antibiyotiklere dirençliliklerinin belirlenmesi amaçlandı. Ayrıca jelatinaz, β-hemolitik ve DNase aktiviteleri ile biyofilm oluşumları fenotipik olarak ise incelendi. İzolatların %95,9'unda *efaA*, %89'unda *asa1*, %68,4'ünde *ace*, %52,1'inde *esp*, %78,1'inde *gelE*, %16,4'ünde *cylA* ve %23,3'ünde *hyl* virülens genleri tespit edildi. Ayrıca %31,5'inde *vanA*, %8,2'sinde *vanB* ve %23,3'ünde *vanC2/C3* direnç genleri belirlendi. Fenotipik olarak ise izolatların sırasıyla %23,2'sinde ve %16,4'ünde β-hemolitik aktivite ve DNase aktivitesi tespit edilirken, jelatinaz aktivitesi ve biyofilm oluşumu tespit edilememiştir. Bunun yanı sıra izolatların %73,9'unda streptomycin ve %43,8'inde erythromycin direnci saptandı. Sonuç olarak, *Enterococcus* spp.'nin virülans faktörleri ve antibiyotik direnci açısından halk sağlığı ve gıda güvenliği için risk oluşturabileceği kanaatine varıldı. Bu nedenle starter kültür olarak seçilecek suşların virülens faktörleri ve antibiyotiklere dirençlilik bakımından değerlendirildikten sonra kullanılmaları önerildi.

Anahtar kelimeler: Antibiyotik Direnci, *Enterococcus* spp., Klasik Beyaz Peynir, Virülens Faktör.

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INTRODUCTION

Enterococcus spp. is a group of bacteria which are Gram-positive, facultative anaerobic, catalase and oxidase negative, motile and non-spore forming. There are 58 described species in this group (García-Solache and Rice 2019) and some of these have ability to growth in environmental conditions, including wide temperature (10-45 °C), wide pH range (4.5-10) and salt concentrations up to 9.6 (Arias and Murray 2012). Natural habitat of *Enterococcus* spp. is primarily the gastrointestinal system of both humans and warm blooded animals. They are also widely found in soil, water and sewage due to their high adaptability to different environments. Therefore, they can easily contaminate foods like dairy products and milk, meat, and vegetables from these sources (Giraffa 2002, Giraffa 2003). *Enterococcus* spp., which is used as an indicator of fecal contamination, is not considered "generally recognized as safe (GRAS)" (Ogier and Serror 2008) due to biogenic amine production and its frequent association with foodborne illnesses (Riboldi et al. 2009). However, some *Enterococcus* spp. can be used as starter culture in the food industry due to their lipolytic activities, use of citrate and production of aroma compounds. On the other hand, it has been reported that *Enterococcus* spp. found in the normal microflora of various cheeses positively affect the taste, texture and general sensory profile (Giraffa 2003). In various studies it has been stated that, some species of *Enterococcus* spp. such as mainly *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus casseliflavus* and *Enterococcus durans* were detected in dairy products (Settanni et al. 2012, Gaglio et al. 2016).

The main reason of serious infections, especially nosocomial infections, caused by *Enterococcus spp*, is related to the virulence factors encoded by their virulence genes. Virulence factors are such as gelatinase (*gelE*), cytolysin (*cyl*), collagen binding protein (*ace*), aggregation factor (*asa1*), enterococcal surface protein (*esp*), hyaluranidase (*hyl*) and endocarditis antigen (*efaA*) that bacteria have (Chajęcka -Wierzchowska et al. 2017). The biofilm-forming properties of *Enterococcus* spp. both increase their resistance and facilitate contamination in the environment and food plants. At the same time, biofilms can serve as a reservoir for the antibiotic resistance genes of these species (Ch'ng et al. 2019).

Today, the development of antimicrobial resistance in bacteria causes an important problem in terms of both public health and food safety. In this context, it has been reported that genetic elements such as plasmids in their structures and chromosomal changes have a role in the development of antibiotic resistance in *Enterococcus* spp. (Hegstad et al. 2010). Because of these features, they are considered as reservoirs of antimicrobial resistance genes, as they have the potential to transmit these genes to other bacteria in food environments (Gaglio et al. 2016). The objective of this research was to determine some virulence factors and antibiotic resistance, especially vancomycin resistance of *Enterococcus* spp. isolates previously identified from different stages of ripened white cheese production.

MATERIAL AND METHOD

Enterococcus spp. Isolates

In the study, 73 *Enterococcus* spp. isolates, which were previously isolated (Pesavento et al. 2014) and identified by MALDI-TOF MS method, from different stages of ripened white cheese production between March 2020 and December 2020 were used. Fourteen of these isolates were obtained from raw milk, 11 of them from pre-ripened cheese and 48 of them from different stages (17 from 30th day of ripening, 15 from 60th day of ripening, 10 from 90th day of ripening, and 6 from 120th day of ripening) of ripening. The species of these isolates are as follows: 37 *Enterococcus faecalis*, 27 *Enterococcus faecium*, 7 Enterococcus gallinarium, 1 *Enterococcus casseliflavus* and 1 *Enterococcus durans* (Table 1). The isolates were stored in sterile glycerol (20%) at -80°C. Before use, all Enterooccus spp. isolates were activated by incubating in Tryptic Soy Broth (TSB, Merck 105459, Germany) for 24 hours at 37°C.

DNA extraction

The DNA was extracted from overnight cultures grown in TSB using the DNA extraction kit (PureLink™ Genomic DNA Mini Kit, Thermo Fisher Scientific, K1820-02, USA) following the protocol provided by the manufacturer.

Detection of Virulence Genes and Vancomycin Resistance Genes in *Enterococcus* spp. by Multiplex PCR

The presence of *asa1*, *gelE*, *cyl*A, *ace*, *esp, hyl* and *efaA* virulence genes and *vanA*, *vanB* and *vanC2/C3* vancomycin resistance genes of *Enterococcus* spp. were determined by multiplex polymerase chain reaction (multiplex PCR) according to a previously described method (Vankerckhoven et al. 2004) (Table 2). Conditions of amplification were an initial denaturing step at 94°C for 10 min, followed by 35 cycles of denaturation step at 94°C for 1 min, annealing at 55°C for 1 min, elongation at 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products were analyzed by agarose gel electrophoresis after stained with ethidium bromide (1%) and photographed under ultraviolet illumination. (Vilber Lourmat, France).

Table 1. Distribution of samples from which *Enterococcus* spp. was obtained

Sample	*Enterococcus* spp.	*E. faecalis*	*E. faecium*	*E. gallinarium*	*E. casseliflavus*	*E. durans*
Raw milk	14	13	1	-	-	-
Pre-ripened cheese	11	5	6	-	-	-
30th day of ripening	17	6	7	2	1	1
60th day of ripening	15	4	9	2	-	-
90th day of ripening	10	7	3	-	-	-
120th day of ripening	6	2	1	3	-	-
Total	73	37	27	7	1	1

Table 2. PCR primers used for the detection of virulence and vancomycin resistance genes in *Enterococcus* spp.

Gene	Targeting	Primers (5'-3')	bp	Reference
ASA1 1	*asa1*	GCACGCTATTACGAACTATGA	375	Vankerckhoven et al. (2004)
ASA1 2		TAAGAAAGAACATCACCACGA		
GEL 11	*gelE*	TATGACAATGCTTTTTGGGAT	213	Vankerckhoven et al. (2004)
GEL 12		AGATGCACCCGAAATAATATA		
CYT I	*cylA*	ACTCGGGGATTGATAGGC	688	Vankerckhoven et al. (2004)
CYT IIb		GCTGCTAAAGCTGCGCTT		
ACE-F	*ace*	GAATTGAGCAAAAGTTCAATCG	1008	Ben Omar et. al. (2004)
ACE-R		GTCTGTCTTTTCACTTGTTTC		
EFA-A-F	*efaA*	GCCAATTGGGACAGACCCTC	688	Creti et. al. (2004)
EFA-A-R		CGCCTTCTGTTCCTTCTTTGGC		
ESP 14F	*esp*	AGATTTCATCTTTGATTCTTGG	510	Vankerckhoven et al. (2004)
ESP 12R		AATTGATTCTTTAGCATCTGG		
HYL n1	*hyl*	ACAGAAGAGCTGCAGGAAATG	276	Vankerckhoven et al. (2004)
HYL n2		GACTGACGTCCAAGTTTCCAA		
Van-A-F	*vanA*	CATGAATAGAATAAAAGTTGCAATA	1030	Kariyama et al. (2000)
Van-A-R		CCCCTTTAACGCTAATACGATCAA		
Van-B-F	*vanB*	AAGCTATGCAAGAAGCCATG	536	Elsayed et al. (2001)
Van-B-R		CCGACAATCAAATCATCCTC		
Van-C2/C3-F	*vanC*	CGGGGAAGATGGCAGTAT	484	Kariyama et al. (2000)
Van-C2/C3-R		CGCAGGGACGGTGATTTT		

Reference Strains

In this study, *E. faecalis* ATCC 51299, *E. faecalis* ATCC 29212, *E. faecium* ATCC 6057 reference strains were used (Table 3).

Phenotypical Assessment of Virulence Factors Gelatinase Production, Hemolytic and DNase Activity, Biofilm Forming

Enterococcus spp. cultures were streaked on the surf*ace* of Brain Heart Infusion Agar (BHI, Merck 103870, Germany) plates containing 3% gelatin. Plates were incubated for 2-3 days at 37°C and then kept 4 hours at 4°C. Production of gelatinase was determined the appearance of a turbid halo or zone around the colonies (Perin et al. 2014).

Hemolytic activity of isolates was evaluated by culturing of fresh overnight cultures on Blood Agar (Merck 110886, Germany) containing 5% ml defibrinated sheep blood. The plates were incubated for 24-48 h at 37°C. After incubation, appearance of a clear hydrolysis zone formed around the colonies was evaluated as β-hemolysis (Gaspar et al. 2009).

Each *Enterococcus* spp. strain was spread onto the surf*ace* of DNase Agar (Merck 110449, Germany) and the plates incubated for 2 days at 37°C. After incubation 1 N HCl was flooded on the plates and colonies with clear zones were considered as DNase activity positive (Perin et al. 2014).

Table 3. PCR primers used for the detection *Enterococcus* spp. reference strains

Isolate	Primer	Sekans (5'-3')	bp	Reference
Enterococcus spp.	E1	TCAACCGGGGAGGGT	733	Deasy et al. (2000)
	E2	ATTACTAGCGATTCCGG		
E. faecalis	FL1	ACTTATGTGACTAACTTAACC	360	Jackson et al. (2004)
	FL2	TAATGGTGAATCTTGGTTTGG		
E. faecium	FM1	GAAAAAACAATAGAAGAATTAT	215	Jackson et al. (2004)
	FM2	TGCTTTTTTGAATTCTTCTTTA		
E. durans	DU1	CCTACTGATATTAAGACAGCG	295	Jackson et al. (2004)
	DU2	TAATCCTAAGATAGGTGTTTG		

The ability of the strains to form biofilms was checked using of a specially prepared medium composed of Brain Heart Infusion Broth (BHI, Merck 110493, Germany) (37 gm/l), agar number 1 (10 gm/l), Congo red dye (0.8 gm/l) and sucrose (5 gm/l). Isolates were inoculated on the medium and incubated for 24 to 48 hours at 37°C. Biofilm production was indicated by black colonies with a dry crystalline consistency (Freeman et al. 1989).

Assessment of Antibiotic Resistance

Antibiotic Resistance tests were performed by the standard disc diffusion method of Bauer et al. (1966) on Mueller-HintonAgar (Oxoid CM0337, UK). Antibiotic discs used were (all from Thermo Fisher Scientific, Oxoid, UK) ampicillin (10 mg), gentamicin (10 mg), streptomycin (10 mg), chloramphenicol (30 mg), erythromycin (5 mg), tetracycline (30 mg) and ciprofloxacin (5 mg). The diameters of the inhibition zones were measured after 18±2 h incubation at 35±1°C. The results were reported as susceptible, intermediate resistant or resistant based on the Clinical and Laboratory Standards Institute indications (CLSI, M100-ED31:2021).

RESULTS

In this study, virulence factors and antibiotic resistance of the strains of 73 *Enterococcus* spp. (37 *E. faecalis*, 27 *E. faecium*, 7 *E. gallinarium*, 1 *E. casseliflavus* and 1 *E. durans*) which are isolated from different stages of production of ripened white cheese were determined. The following virulence genes were detected from these 73 *Enterococcus* spp. isolates: 89% *asa1*, 78.1% *gelE*, 16.4% *cyl*A , 68.5% *ace* , 52.1% *esp* , 23.3% *hyl* and 95.9% *efaA*. Also from same isolates; 31.5% *vanA* , 8.2% *vanB* and 23.3% *vanC2/C3* resistance genes were determined (Table 4).

Table 4. Distribution of virulence and vancomycin resistance genes in *Enterococcus* spp. isolates

	E. faecalis **(n=37)**	***E. faecium*** **(n=27)**	***E. gallinarium*** **(n=7)**	***E. casseliflavus*** **(n=1)**	***E. durans*** **(n=1)**	***Enterococcus*** spp. **(n=73)**
Virulence genes	**%(n)**	**%(n)**	**%(n)**	**%(n)**	**%(n)**	**%(n)**
asa1	78.4(29)	100(27)	100(7)	100(1)	100(1)	89(65)
gelE	67.6(25)	85.2(23)	100(7)	100(1)	100(1)	78.1(57)
cylA	13.5(5)	22.2(6)	-	100(1)	-	16.4(12)
ace	67.6(25)	77.8(21)	28.6(2)	100(1)	100(1)	68.5(50)
esp	51.4(19)	59.3(16)	28.6(2)	-	100(1)	52.1(38)
hylA	13.5(5)	40.7(11)	-	-	100(1)	23.3(17)
efaA	94.6(35)	96.3(26)	100(7)	100(1)	100(1)	95.9(70)
Vancomycin resistance genes						
vanA	24.3(9)	40.7(11)	42.9(3)	-	-	31.5(23)
vanB	13.5(5)	3.7(1)	-	-	-	8.2(6)
vanC2/C3	8.1(3)	40.7(11)	28.6(2)	100(1)	-	23.3(17)

According to phenotypic tests, gelatinase activity could not be detected in the isolates. On the other hand, β-hemolytic activity was detected in 23.2% of the isolates. The distribution of β-hemolytic activity at the species level is; 18% of *E. faecalis*, 25% of *E. faecium*, 28% of *E. gallinarium* and 100% of *E. casseliflavus*. DNase activity was found positive in 16.4% of the isolates. The distribution of DNase activity at the species level is; 13.5% of *E. faecalis*, 22.2% of *E. faecium* and *E. durans* in 100%. However, it was determined that none of the isolates formed a biofilm.

According to the antibiotic resistance test results, it is determined that 33 of the *E. faecalis* isolates were resistant to streptomycin, 17 to ciprofloxacin and 15 to tetracycline. It is also found that 13 of the *E. faecium* isolates to streptomycin and erythromycin and 7 of the *E. gallinarium* were found to be resistant to streptomycin and erythromycin 13 of the *E. faecium* isolates had streptomycin and erythromycin; seven of the *E. gallinarium* isolates were found to be resistant to streptomycin and erythromycin. While *E. casseliflavus* isolate did not show resistance to any antibiotic, *E. durans* was found resistant to streptomycin, tetracycline and ciprofloxacin antibiotics (Table 5).

Table 5. Distribution of antibiotic resistance in *Enterococcus* spp. isolates

Antibiotic	*E. faecalis* n(%)			*E. faecium* n(%)			*E. gallinarium* n(%)			*E. casseliflavus* n			*E. durans* n		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
AMP	35(94.6)	-	2(5.4)	25(92.6)	-	2(7.4)	5(71.4)		2(28.6)	1		-	1		-
CN	27(73)	1(2.7)	9(24.3)	24(88.9)	-	3(11.1)	2(28.6)	-	5(71.4)	1	-	-	1	-	-
S	3(8.1)	1(2.7)	33(89.2)	14(51.9)	-	13(48.1)	-	-	7(100)	1	-	-	-	-	1
C	19(51.3)	17(46)	1(2.7)	24(88.9)	3(11.1)	-	4(57.1)	3(42.9)	-	1	-	-	-	1	-
E	6(16.2)	19(51.3)	12(32.4)	6(22.2)	8(29.6)	13(48.1)	-	-	7(100)	1		-	-	1	-
TE	13(35.1)	9(24.3)	15(40.5)	14(51.9)	6(22.2)	7(25.9)	-	2(28.6)	5(71.4)	-	1	-	-	-	1
CIP	7(18.9)	13(35.1)	17(45.9)	10(37)	12(44.4)	5(18.5)	2(28.6)	4(57.1)	1(14.3)	-	1	-	-	-	1

(S: Susceptible, I: Intermediate resistant, R: Resistant, AMP: ampicillin, CN: gentamicin, TE: tetracycline, E: Erythromycin, CIP: ciprofloxacin, S: streptomycin, C: chloramphenicol)

DISCUSSION

It has been reported that *Enterococcus* spp. has a role in the ripening of many cheese varieties produced by traditional methods, especially in Mediterranean countries, and contributes to the formation of typical tastes and flavors (Foulquie Moreno et al. 2006). On the other hand, the virulence genes of these bacteria and their antibiotic resistance pose a significant threat to public health (Gaglio et al. 2016). In present study, virulence factors and antibiotic resistance of the 73 *Enterococcus* spp. strains which are isolated from different stages of production of ripened white cheese were determined.

The general distribution of virulence and vancomycin resistance genes of the detected *Enterococcus* spp. isolates is presented in Table 4. In this study, *efaA*, *asa1*, *ace*, *esp* virulence genes supporting host colonization of *Enterococcus* spp. were detected in 95.9%, 89%, 68.5% and 52.1% of isolates, respectively. Domingos-Lopes et al. (2017) detected *efaA*, *ace*, *asa1*, and *esp* virulence genes of *Enterococcus* spp. isolates obtained from cheese at a rate of 33-99%. Câmara et al. (2020) reported that 100% of their *Enterococcus* spp. isolates which detected from cheeses carried *efaA*, 71% *ace*, 46% esp, and 43% *asa1* virulence genes. It is seen that our study's results are compatible with the results of these researchers. Chajęcka-Wierzchowska et al. (2017) reported the *efaA*, *asa1* and *ace* virulence genes play an important role in the attachment of *Enterococcus* spp. to host cells.

After colonization, *Enterococcus* spp. pathogen strains release toxic substances that damage host tissues. The virulence factors secreted by *Enterococcus* spp. and affecting the tissues are gelatinase (*gelE*), cytolysin (*cyl*) and hyaluronidase (*hyl*) (Chajęcka-Wierzchowska et al. 2017). By hydrolyzing gelatin and collagen, gelatinase enzyme (*gelE*) can facilitate the penetration of bacteria into the host's tissues by participating in biofilm formation (Anderson et al. 2016). Additionally, these genes cause the initiation and progression of inflammatory processes associated with *Enterococcus* spp. (Domingos-Lopes et al. 2017). In the study, *gelE* was detected in 78.1% of the isolates genotypically, although gelatinase activity was not observed phenotypically in this study. Templer and Baumgartner (2007) stated that in their *Enterococcus* spp. isolates from traditional cheese, gelatinase activity was not observed phenotypically but they detected the *gelE* gene in 76% of them genotypically. Domingos-Lopes et al. (2017) also determined 77% and İspirli et al. (2016) 75% *gelE* gene in their isolates obtained from cheeses. The results of these researchers show similarity to our study's results. Lopes et al. (2006) reported that the detection of the *gelE* gene is not sufficient for gelatinase activity in bacteria, and full expression of the fsr operon is required. However, they also reported that the fsr operon could not be detected in the laboratory as it is easily damaged during cell freezing. Contrary to the results of our study; some researchers (Fuka et al. 2017, Câmara et al. 2020, Hammad et al. 2021) stated that they detected *gelE* gene at lower rates (16.6-54%)

in their *Enterococcus* spp. isolates. Eaton and Gasson (2001) reported that genes may be lost due to the negative effects of *gelE* expression of isolates during laboratory studies and culture conditions, and in vitro tests this may cause loss of gelatinase activity.

Cytolysin (*cyl*) has a bactericidal effect against gram-negative bacteria and a toxic effect (β-hemolysis) against macrophages, erythrocytes and leukocytes (De Vuyst et al. 2003). In the present study, the *cyl*A gene was found in 16.4% of the isolates (Table 4). Fuka et al. (2017) found *cyl*A gene in 19.8% of *Enterococcus* spp. isolates and Câmara et al. (2020) in 21% of their isolates. The findings of this study are consistent with the above researchers. Domingos-Lopes et al. (2017) detected the *cyl*A gene in 32% of the isolates in their study. Avcı and Ozden Tuncer (2017) also found *cyl*A gene in 45.5% of *Enterococcus* spp. strains isolated from traditional Turkish cheeses. On the other hand, Templer and Baumgartner (2007) reported that they detected the *cyl*A gene in 6% of their *Enterococcus* spp. isolates. *cyl*A gene has been detected in *Enterococcus* spp. strains both isolated from infections and forming the commensal microbiota. It has also been reported that it is frequently detected from isolates obtained from foods (Trivedi et al. 2011).

By degrading mucopolysaccharides in connective tissue, the hyaluronidase enzyme (*hyl*) aids the transmission of bacteria and their toxins in the host (Vankerckhoven et al. 2004). In our study, hyl gene was found in 23.3% of the isolates. In species, the hyl gene was detected in *E. faecium*, *E. faecalis*, *E. casseliflavus* and *E. durans*. It has been mentioned that the *hyl* gene is commonly found in *E. faecium* and very seldom in *E. faecalis* from clinical strains (Vankerckhoven et al. 2004). Trivedi et al. (2011) determined that *E. casseliflavus* and *E. durans* species isolated from food also have the *hyl* gene. Yuksekdag et al. (2021) reported that 12% of *E. faecium* isolates obtained from white cheese samples were positive for the *hyl* gene. Vancomycin-resistant *Enterococcus* spp. (VRE) has become one of the most challenging pathogens with the rapidly spread of multidrug resistant strains with limited therapeutic options. *vanA* is the most common of them and it is dominantly found in *E. faecium* and *E. faecalis*, the species responsible for human infections (Cetinkaya et al. 2000). Also Ahmed and Baptiste (2018) reported that *vanC* genes are naturally found in *E. casseliflavus* and E. gallinarum. In this study, *vanA*, *vanB* and *vanC2/C3* genes were detected in 31.5%, 8.2% and 23.3% of *Enterococcus* spp. isolates, respectively. As well Hammad et al. (2021) stated that they have found *vanB* and *vanC* genes in 29.1% and 20.8% of milk isolates, respectively. Oruc et al. (2021) reported that while 13.63% of *Enterococcus* spp. strains isolated from traditional white cheeses harbored *vanA* gene, *vanB* gene observed only in the 31.82%. Also, Yuksekdag et al (2021) informed that 59% of *E. faecium* isolates were positive for the *vanB* gene and 6% was positive for *vanA* gene. On the contrary of our study, Domingos-Lopes et al. (2017) and Jurkovič et al. (2006) reported that a small number (<5%) of Enterococcus isolates harbored vancomycin (*vanA*, *vanB*) resistance genes.

In this study, β-hemolytic activity was detected phenotypically in 23.2% of the *Enterococcus spp* isolates. Different researchers (Domingos-Lopes et al. 2017, Adifon and Tuncer 2019, Ayhan et al. 2020, Câmara et al. 2020, Margalho et al. 2020) determined that 2.3-11.3% of the *Enterococcus* spp. isolates from cheeses showed β-hemolytic activity. β-hemolytic activity rates of these studies are lower than the rates of this study. Hemolytic activity is the least detected virulence factor in food sourced *Enterococcus* spp. (Chajęcka-Wierzchowska et al. 2017).

In present study, DNase activity was detected in 16.4% of the isolates. It has been reported that this virulence factor, which is typically researched in clinical isolates, is found at high rates in *Enterococcus* spp. and is frequently linked to the competitive characteristics of pathogenic strains (Semedo et al. 2003)

It was determined that 33 of the *E. faecalis* isolates were resistant to streptomycin, 17 to ciprofloxacin and 15 to tetracycline. Thirteen of the *E. faecium* isolates had streptomycin and erythromycin; 7 of the *E. gallinarium* isolates were found to be resistant to streptomycin and erythromycin. Also *E. durans* was found resistant to streptomycin, tetracycline, and ciprofloxacin antibiotics. De Paula et al. (2020) reported that they detected erythromycin resistance in 16.6% of *E. faecium* isolates from raw milk and traditional cheese. Templer and Baumgartner (2007) found that 30% of *Enterococcus* spp. isolates from traditional cheese made from raw milk were resistant to chloramphenicol, 50% were resistant to erythromycin, and 68% were resistant to tetracycline. According to Hammad et al. (2021); *Enterococcus* spp. identified from raw cow's milk was resistant to erythromycin (87.5%), and tetracycline (29.1%). Gaglio et al. (2016) also found that *Enterococcus* spp. isolates from artisanal cheeses were resistant to erythromycine (52.5%), ciprofloxacine (35.0%) and tetracycline (17.5%). One reason that may explain this finding is the widespread use of antibiotics in agriculture.

CONCLUSION

There is no evidence that *Enterococcus* spp. isolated from food causes infection in humans. However, there is a risk of genetically transferring virulence and antibiotic resistance genes of *Enterococcus* spp. to pathogenic bacteria. Therefore, the presence of these bacteria in foods is still debated today and is a matter

of public health concern. Especially in immunocompromised individuals, such strains are important as they pose a risk of disease. In this study, it has been revealed that *Enterococcus* spp. isolates could be a source of the transmission and spread of virulence and antimicrobial resistance genes among bacteria. This situation also reveals the necessity for a more careful valuation of strains to be used as starter cultures in the dairy industry, especially relating to their virulence characteristics and antibiotic resistance. Even if the aforementioned isolates contribute to the formation of aroma and texture in cheese production, it is considered more appropriate to use isolates that do not contain virulence genes and are sensitive to antibiotics as starter cultures.

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CASE REPORT

Aortic Valve Papillary Fibroelastoma in a Cat: Case Report

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ABSTRACT

Cardiac tumors are rare in cats. Usually the diagnosis can be made on echocardiographic examination. Papillary fibroelastoma in this case is a primary cardiac tumor and is histopathologically defined as benign. However, these types of tumors carry the risk of thromboembolism and may cause complications such as stroke or infarction. In these types of the cases the symptoms are not specific and may vary depending on the size or structure of the mass. In the report a case of papillary fibroelastoma identified echocardiographically in a male tabby cat is discussed.

Keywords: Echocardiography, Feline, Fibroelastoma, Cardiac, Tumor.

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Bir Kedide Aort Kapağı Papiller Fibroelastomu: Vaka Raporu

ÖZ

Kardiyak tümörlere kedilerde nadir rastlanır. Teşhis genellikle ekokardiyografik muayene ile yapılır. Sunulan vakadaki papillar fibroelastoma primer bir kalp tümörüdür ve histopatolojik olarak iyi huylu olarak tanımlanır. Bununla birlikte, bu tip tümörler tromboemboli riski taşır, felç ya da enfarktüs gibi komplikasyonlara sebebiyet verebilir. Olgulardaki belirtiler spesifik değildir ve kitlenin boyutu veya yapısı ile bağlantılı olarak değişkenlik gösterebilir. Sunulan raporda tekir ırkı erkek bir kedide ekokardiyografik olarak tanımlanan papiller fibroelastoma olgusu tartışıldı.

Anahtar Kelimeler: Ekokardiyografi, Kedigiller, Fibroelastoma, Kardiyak, Tümör.

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INTRODUCTION

Primary cardiac tumors are rare and can be benign or malignant (Grebenc et al. 2000). The most widest cardiac tumor is myxoma. It occurs in about half of the cases. Other benign primary neoplasms include papillary fibroelastoma which is the most common valve tumor, and also rhabdomyoma, fibroma, hemangioma, lipoma (Burke and Virmani 1996, Behairy and Gouda 2013). Papillary fibroelastomas are benign endocardial tumors that mainly affect the valves and it constitutes about three-quarters of all heart valve tumors (Edwards et al. 1991, Grebenc et al. 2000). The most well-known formation site is on the mitral and aortic valves. Papillary fibroelastomas are often small (<1cm) and appear flower-like with a narrow shaft (Aguilar and Levine 2018). Embolic clot formations may originate from the tumor or from clumps of platelets and fibrin that may form on the tumor surface (Shahian et al. 1995). The treatment of papillary fibroelastoma is not clearly defined. Surgical resection may be recommended in those with a history of embolism. In asymptomatic cases surgical removal may be considered if the mobility of the fibroelastoma is high (Aguilar and Levine 2018).

CASE HISTORY

A 2-year-old male 4.1 kg tabby cat was presented to the Pasteur Veterinary Polyclinic in Kocaeli, Turkey. The owner of the cat said that the cat is started to deep and forced breathing, stagnate and lost appetite in the last 2 days. As a result of the clinical examinations; the body temperature was 39.1 ^{o}C, heart rate was 160 beats/minute, blood pressure was 149 mm/Hg. After this examination the blood sample was taken. Also FIV and FeLV results determined were negative and has S4 degree positive reaction on FCoV. All total blood count, biochemical analyses, and T4 and NT-ProBNP results are shown in Table1 and Table2.

We have taken the X-Ray (Figure 1) and then prepared the patient for echocardiographic examination. The mass was clearly visible on echocardiography (Figure 2, 3). According to echocardiographic evaluation and M mode results also HCM was diagnosed in the cat, and turbulent flow was also observed at the level of the mitral valve. At the same time, a freely moving mass in the left ventricular outflow tract, which appeared to be originating from the aortic root, was found compatible with fibroelastoma, which was connected to the aortic root with a thin stalk, and the mass was flutters with cardiac motion almost like a yo-yo type. No thrombus was detected in the left ventricle or in the left ventricular outflow tract. The cat which was started on medication and a special cardiac diet, continues to live.

Table 1. Hemogram results of the cat

Hemogram results	
WBC	9.47
NEU	4.98
LYM	3.74
MON	0.53
EOS	0.21
BAS	0.01
NEU%	52.60
LYM%	39.50
MON%	5.6
EOS%	2.20
BAS%	0.10
RBC	9.01
HGB	11.20
HCT	30.90
MCV	34.30
MCH	12.40
MCHC	36.10
PLT	119.1
PCT	1.30

Table 2. Biochemisty results of the cat

Biochemistry results	
TP (g/dL)	8.00
ALP (U/I)	30.00
GLU (mg/dl)	115.00
ALT (U/I)	74.00
CRE (mg/dl)	0.88
BUN (mg/dl)	27.20
NT-ProBNP ng/mL	1027.4
T4 ug/dl	1.64

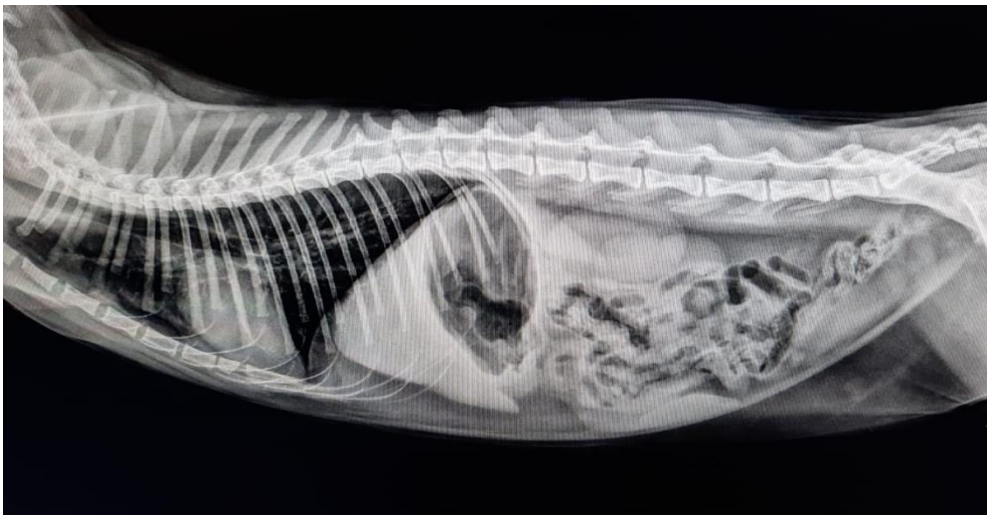

Figure 1: X-ray output of the patient

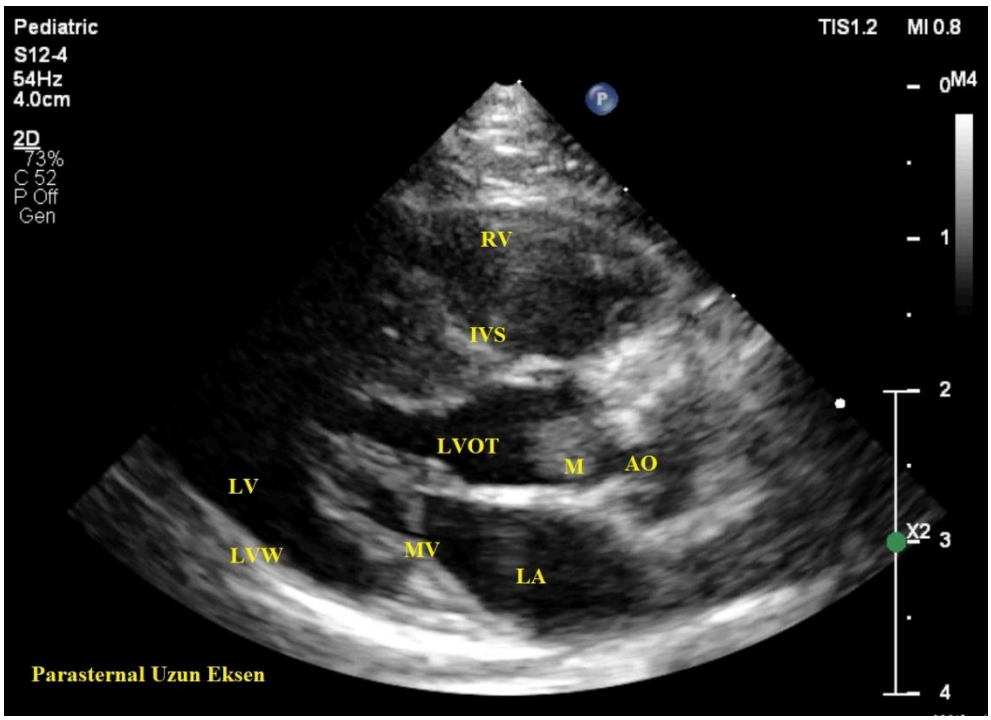

Figure 2: Echocardiographic view of the mass

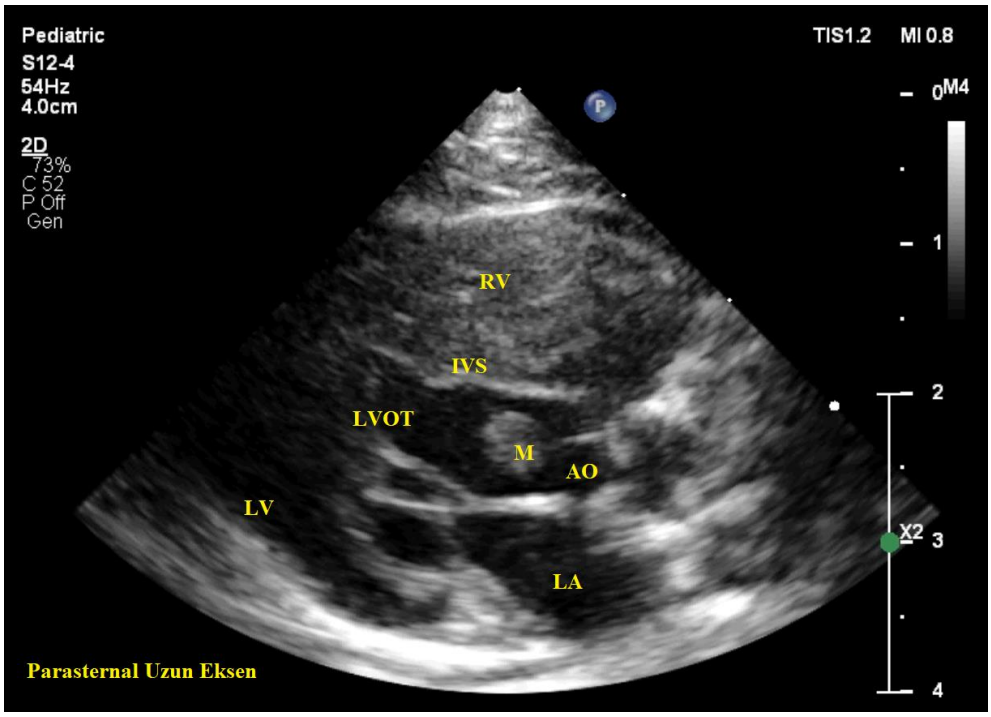

Figure 3: a. Echocardiographic view of the mass and stem

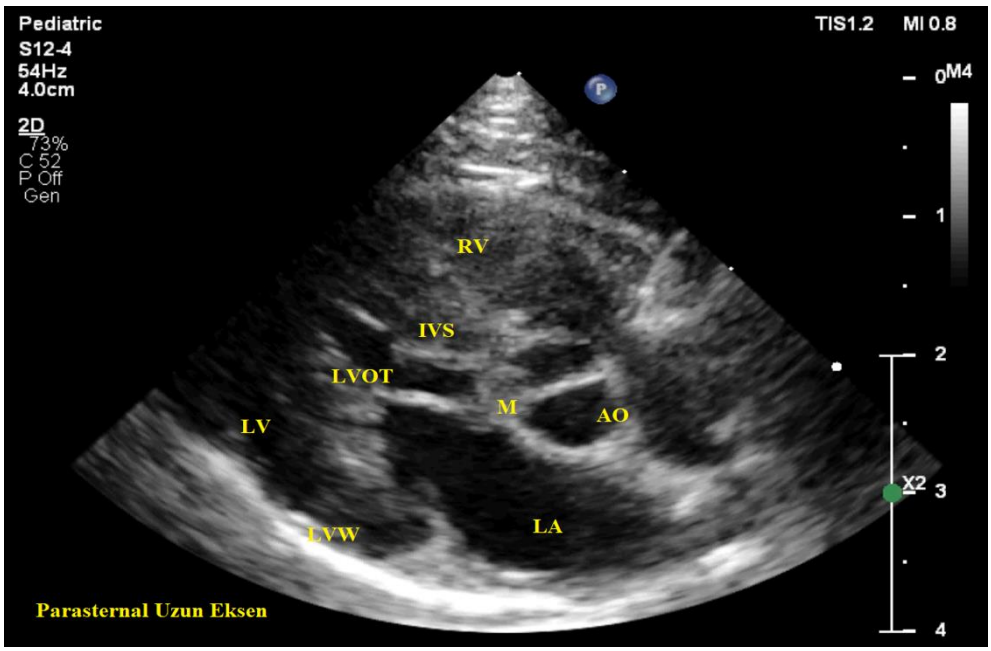

Figure 3: b. Echocardiographic view of the mass and stem

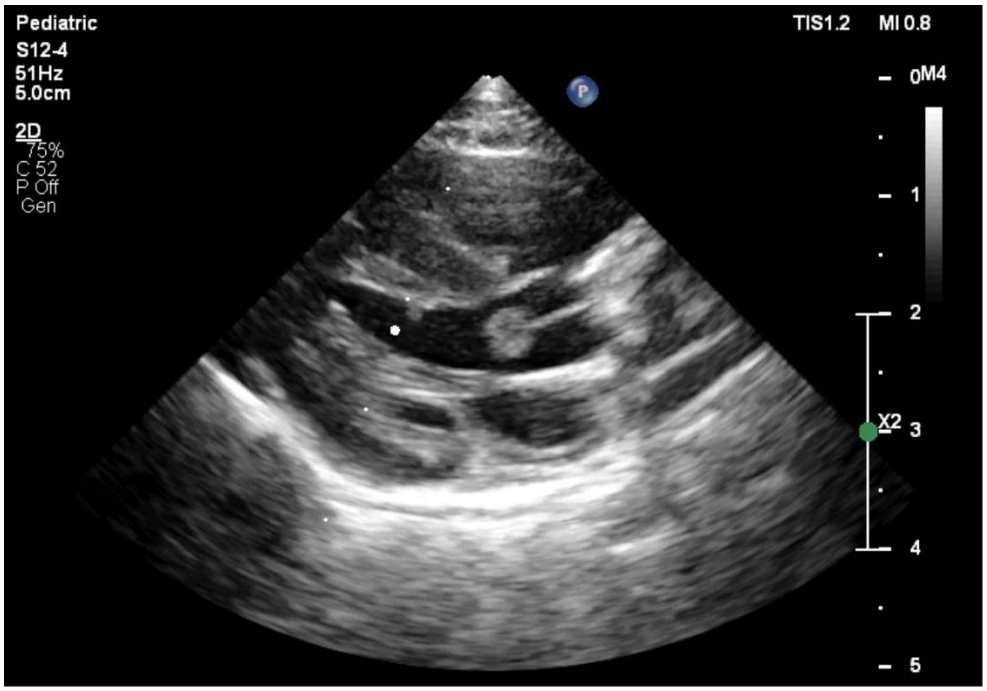

Figure 3: c. Echocardiographic view of the mass and stem

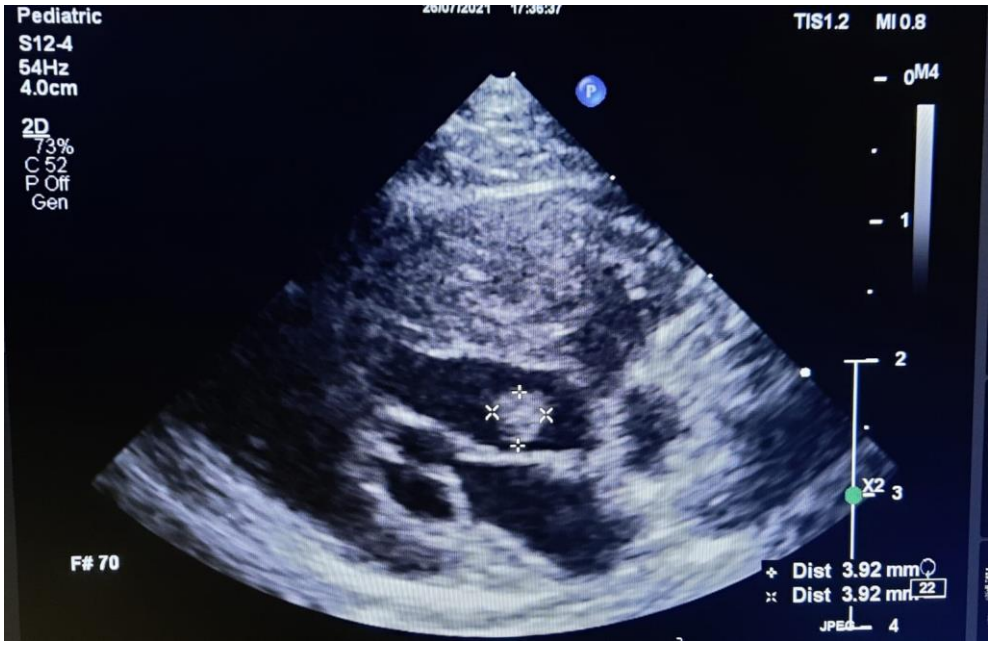

Figure 3: d. Echocardiographic measurement of the mass body

DISCUSSION and CONCLUSION

This case report was to evaluate the diagnosis and management of a cat with aortic valve fibroelastoma. Cardiac tumors have prevalence of 0.02% so they are not common (Hoffmeier et al. 2014). 75% of cardiac neoplasms are benign and %5 of these are fibroelastomas (McAllister and Fenoglio 1978). Even though fibroelastoma benign is a dangerous due to the potential for cerebral or coronary embolization (Sousa-Uva and Cardim 2018). Most papillary fibroelastoma cases are asymptomatic and the lesion is usually finds incidentally (Ikegami et al. 2015). Its etiology is unknown. They are generally with adherent thrombus thats why tends to embolize (Sousa-Uva and Cardim 2018). Papillary fibroelastomas are usually diagnosed by echocardiography. It generally exhibits a small (<1.5 cm), pedunculated, homogeneous valvular or endocardial mass, which moves with cardiac motion (Grebenc et al. 2000). In the treatment, it is recommended to treat symptomatic patients surgically. It can be said that the postoperative prognosis in these patients is very good in long-term observation. Asymptomatic patients with immobile fibroelastoma should be evaluated periodically and it can be followed closely whether the tumor has become mobile echocardiographically, surgical intervention may be considered (Gowda et al. 2003). In this case, after diagnosis mentioned above, heparin at a dose of 100 U/kg every other day, and 1 mg/kg furosemide (Diüril®, Vetaş), and 10 U interferon (Roferon®-A, Roche) for against coronavirus infection, and cardiac supplement feed additive (CardioVet®, VetExpert) for the cat, and specific cardiac diet were started. The cat continues to live asymptomatically and is routinely followed. To give more details; in the treatment, daily antibiotics (Ceftriaxone; 15mg/kg) and fluid therapy were applied and the general condition was followed. On the 3rd day, atenolol was started to be used at a dose of 2 mg/kg once a day. On the 4th day of the treatment, she started to eat dry cardiac diet. In the presence of repeated echocardiographic examinations, the treatment of the fibroelastoma, due to the fact that the mass did not become free, was continued with atenolol and antiplatelet (Plavix®, Sanofi) twice a day. In addition, heparin was used every other day for 1 more week. Because the mass did not become free and partly due to the fact that the mass is more immobile at follow-up and also general condition was much better in our patient, cardiac medications were continued and surgical intervention was not considered.

No previously reported fibroelastoma cases in cats were found in the literature review (Web of Sci.; key words: fibroelastoma, cat, cats; 29.12.2021). Therefore, this case report is important as it guides veterinarians and describes the case in detail.

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

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

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REPORT CASE

A Case of Polydactyly Encountered in A Calf and Its One-Year Results

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ABSTRACT

A 30-day-old Simental male calf brought with gait disorder and diagnosed with polydactyly as a result of clinical and radiographic examinations formed the study material. The over-formed metacarpal bone and nails were removed with operative intervention. No complications were encountered in the clinical examinations and radiographs taken on the 1st, 10th and 365th days. Thus, in such cases where the general condition of the patient is not bad, it was concluded that operative intervention is indicated to increase the quality of life of the patient and to prevent economic losses.

Keywords: Anomaly, calf, operative treatment, polydactyly, radiography

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Bir Buzağıda Karşılaşılan Polidaktili Olgusu ve Bir Yıllık Sonuçları

ÖZ

Çalışma materyalini yürüyüş bozukluğu ile getirilen, yapılan klinik ve radyografik muayeneler sonucu polidaktili tanısı konulan 30 günlük Simental ırkı erkek buzağı oluşturdu. Operatif girişim ile fazladan şekillenen metacarpal kemik ve tırnaklar uzaklaştırıldı. 1., 10. ve 365. günlerde alınan radyografiler ve yapılan klinik muayenelerde herhangi bir komplikasyon ile karşılaşılmadı. Böylece hastanın genel tablosunun kötü olmadığı bu tür olgularda, hastanın yaşam kalitesini arttırmak ve ekonomik kayıpları önlemek için operatif girişimin endike olduğu kanısına varıldı.

Anahtar sözcükler: Polidaktili, buzağı, anomali, radyografi, operatif sağaltım

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INTRODUCTION

Congenital anomalies cause anatomic distortions and functional disorders. Anomalies in animals are generally caused by genetic, environmental factors or their combination, nutritional disorders, vitamin deficiencies, stress factors, not choosing artificial insemination, mistakes in breeding selection, and teratogens (Gugjoo et al. 2013, Leipold et al. 1972, Leipold et al. 1993, Rafee and Amarpal 2016).

Polydactylism is an anomaly characterized by the presence of one or more extra digits (Belge et al. 2000, Mosbah et al. 2012, Browning et al. 2020). Polydactyly is a genetic defect that is observed in many animal species and is increasingly common in cattle (Johnson et al. 1981, Leipold and Morris 1979). In a cattle with polydactyly, lameness increases over time as the animal gets older. This situation causes a decrease in the quality of life and productivity of the animal, and results with an economic loss. Although polydactyly is a genetic disorder, its inheritance is not fully understood (Johnson et al. 1981). It is thought to be caused by the gender-linked recessive gene (Aksoy et al. 2006, Belge et al. 2000, Johnson et al. 1981, Gugjoo et al. 2013).

It has been reported that the prevalence of polydactyly in cattle is higher in the front extremity compared to the hind extremity (Belge et al. 2000, Mosbah et al. 2012, Johnson et al. 1981, Gugjoo et al. 2013, Leipold and Morris 1979). There are seven types of polydactylism seen in cattle. Unilateral polydactyly of one forelimb with additional metacarpal bones and phalanges classified as type 2 is reported in our case (Braun et al. 2019). The aim of this study is to prevent economic losses and to increase the quality of life of the patients that can undergo surgery.

MATERIAL AND METHOD

According to the anamnesis taken from the owner of the 30-day-old Simental male calf brought to our clinic; It was mentioned that there was excessive claw formation on the left forelimb, the excess claw of the animal sometimes got stuck while the animal was walking and there was lameness on the same limb.

As a result of the clinical examination, it was observed that there was a 2 cm long extra claw which was thought to be rudimentary, just above the interdigital space on the palmar side of the same limb, extending caudomedially to the distal of the metacarpal bone of the left forelimb. A bone protrusion was observed along with the metacarpal bone space in the proximal part of the claw, which was directed caudomedially, and this was thought to be an extra metacarpal bone. Radiological imaging was requested to confirm the diagnosis. Radiographs of the extremity were taken in Dorso/Palmar, Dorsomedial/Palmarolateral oblique positions (Figure 1). Radiological evaluation revealed a second metacarpal bone, one-fifth in diameter and medial to the metacarpal bone belonging to the left anterior extremity. It was found that this extra metacarpal bone was related to the medial claw. As a result of clinical and radiological examinations, Type II Polydactyly was diagnosed. Surgery was decided as these attachments were causing problems in walking.

Preoperative hemogram and biochemistry analysis results of the patient were normal and suitable for operation. 10 mg / kg intramuscular (IM) cephalexin (Cefatek® 15%, Teknovet, Turkey) for prophylaxis and 0.2 mg / kg IM morphine HCl (Morphine Hydrochloride®, Osel, Turkey) for analgesia were administered preoperatively. Diazepam (Diazem®, Deva Holding, Turkey) at a dose of 0.4 mg / kg was administered intravenously (IV) for sedation. For anesthesia induction, 2% propofol (Lipuro®, Braun, England) at a dose of 3 mg / kg was administered IV and anesthesia maintenance was provided with 2% isoflurane (Isoflurane USP®, Piramal Critical Care, USA).

A palmaromedial approach was preferred, by making a skin incision starting from the mid-diaphyseal level of the medial metacarpal bone (extra bone). Subcutaneous connective tissues were separated by blunt dissection. The surrounding tissues were completely dissected while protecting the vena digitalis palmaris communis and the Musculus extensor digitorum medialis tendon. Extra metacarpal bone was osteotomized at the mid-diaphyseal level, and the associated claw was removed completely. The subcutaneous tissue and the skin were closed in a routine manner. The rudimentary nail on the palmar surface of the foot was removed by making a circular incision on the skin and the area was closed properly. Postoperative x-rays of the foot were taken and a dry dressing was made to the operated foot (Figure 2).

Intramuscular cephalexin (Cefatek® 15%, Teknovet, Turkey) was administered at a dose of 10 mg/kg every 12 hours for the first three days and every 24 hours for the next two days. Subcutaneous meloxicam (Bavet Meloxicam, Bavet, Turkey) was administered at a dose of 0,5 mg/kg once a day for three days for pain control. Sutures were removed 10 days after the operation.

One year later, control x-rays were taken using a portable x-ray unit under field conditions in order to see what kind of changes occurred in the extremity of the animal (Figure 3). No lameness was observed in the patient, and his walk was recorded by video (Figure 4).

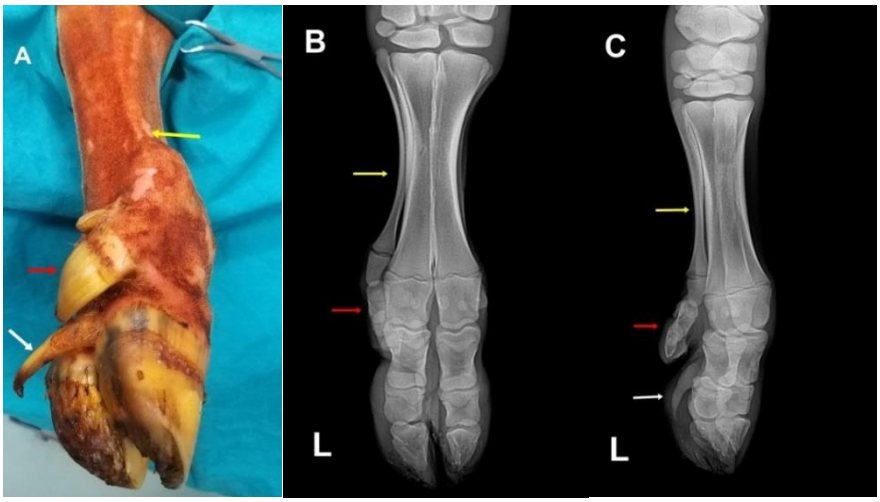

Figure 1: A. Clinical image of the claw diagnosed with polydactyly; B. D\P (Dorso/Palmar) radiography of the same claw; C. DM\PLO (Dorsomedial/Palmarolateral oblique) radiography of the same claw. Yellow arrow: Extra metacarpal bone; Red arrow: Claw connecting with metacarpal bone; White arrow: rudimentary claw.

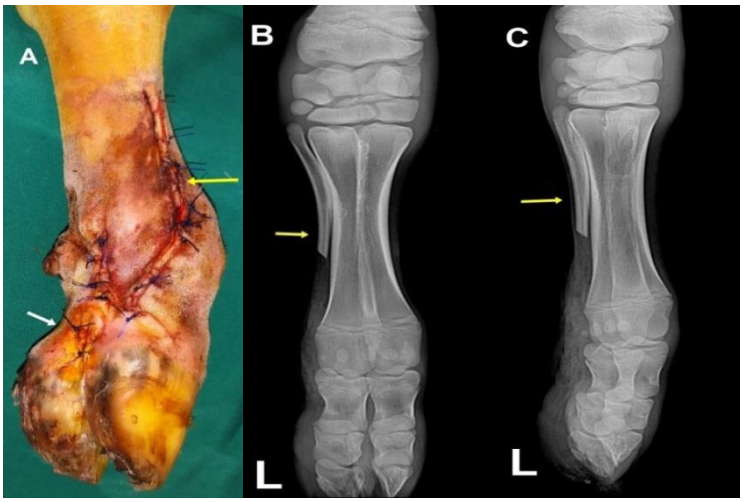

Figure 2: A. Post-operative clinical view of the claw diagnosed with polydactyly; B. Post-operative 1. day D\P radiograph of the same claw; C. Post-operative 1st day DM\PLO radiography of the same claw. Yellow arrow: Osteotomy site of the metacarpal bone; White arrow: Total extirpation of the rudimentary claw.

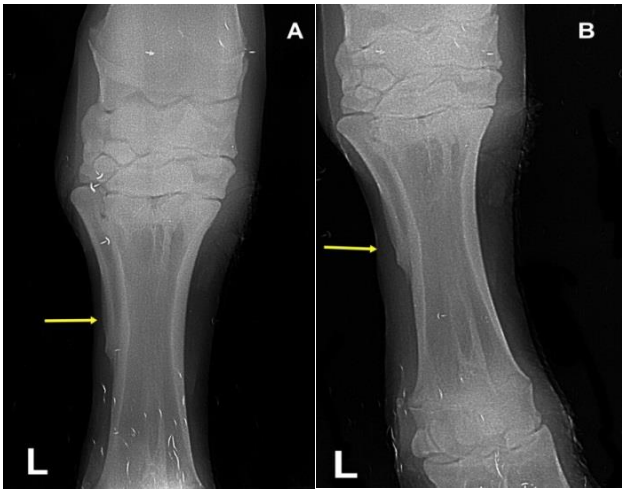

Figure3: A. Post-operative 365th day D\P radiography of the claw diagnosed with polydactyly; B. Post-operative 365 days DM\PLO radiography of the same claw. Yellow arrow: Osteotomy site of the metacarpal bone

Figure4: The patient's walking video 1 year after the operation and physical comparison of the patient with his peers.

FINDINGS AND DISCUSSION

Anomalies in animals are generally caused by genetic, environmental factors or their combination, nutritional disorders and teratogens (Gugjoo et al. 2013, Rafee and Amarpal 2016).

Polydactyly is a congenital disorder that often progresses bilaterally and rarely unilaterally (Mosbah et al. 2012). There are seven types of polydactylism seen in cattle; Type I-Bilateral polydactyly of both forelimbs with additional metacarpal bones or phalanges, Type II- Unilateral polydactyly of the forelimb or hindlimb with additional metacarpal or metatarsal bones and phalanges, Type III- Additional digits on all four limbs, Type IV- Occurs rarely, involving a bilateral duplication of digits, either of the forelimb or hindlimb, Type V- Polysyndactyly, Type VI- A bilateral incomplete formation of metacarpal II and Type VII- Polydactylism in combination with a malformation-complex. Polydactylism usually affects both forelimbs but less commonly a malformation of one or all four limbs is described (Mosbah et al. 2012).

In this study of type II polydactylism, it was observed that after surgical removal of the extra-shaped claw, the patient's quality of life was positively impacted, as mentioned in other studies (Mosbah et al. 2012, Rafee and Amarpal 2016, Özak et al. 2009). Information about the patient was obtained by phone calls at 30-day intervals for 1 year. It was reported that there was no lameness during the year.

On physical examination of the patient one year postoperatively, it was noted that cidago height and body weight of the patient was lower compared to his peers, suggesting that the patient had another anomaly other than polydactyly (Figure 4). Posture disorder in the forelimb was noted in the patient, the forelimbs were outward rotated from the distal of the carpal joint level. On radiographic evaluation performed one year postoperatively, no osteophytic growth was observed in the relevant area.

According to a study conducted in 2016 by Rafee et al., osteotomy of the over-formed metacarpal/metatarsal bone was recommended to increase the welfare of the animal and to obtain a good aesthetic appearance in these cases (Rafee and Amarpal 2016).

Results

Polydactyly is an increasingly common type of anomaly in cattle. The results are in compliance with the literature for this patient who was operated. It is recommended that the causes of this anomaly should be revealed by genetic studies, as it negatively affects animal welfare and causes economic loss.

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

Etik izin: Bu çalışma "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik" Madde 8 (k) gereği HADYEK iznine tabi değildir.

Teşekkür: Bu çalışmada hasta sahibi olan Murat CANSU'ya iş birliği için teşekkürlerimizi sunarız.

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