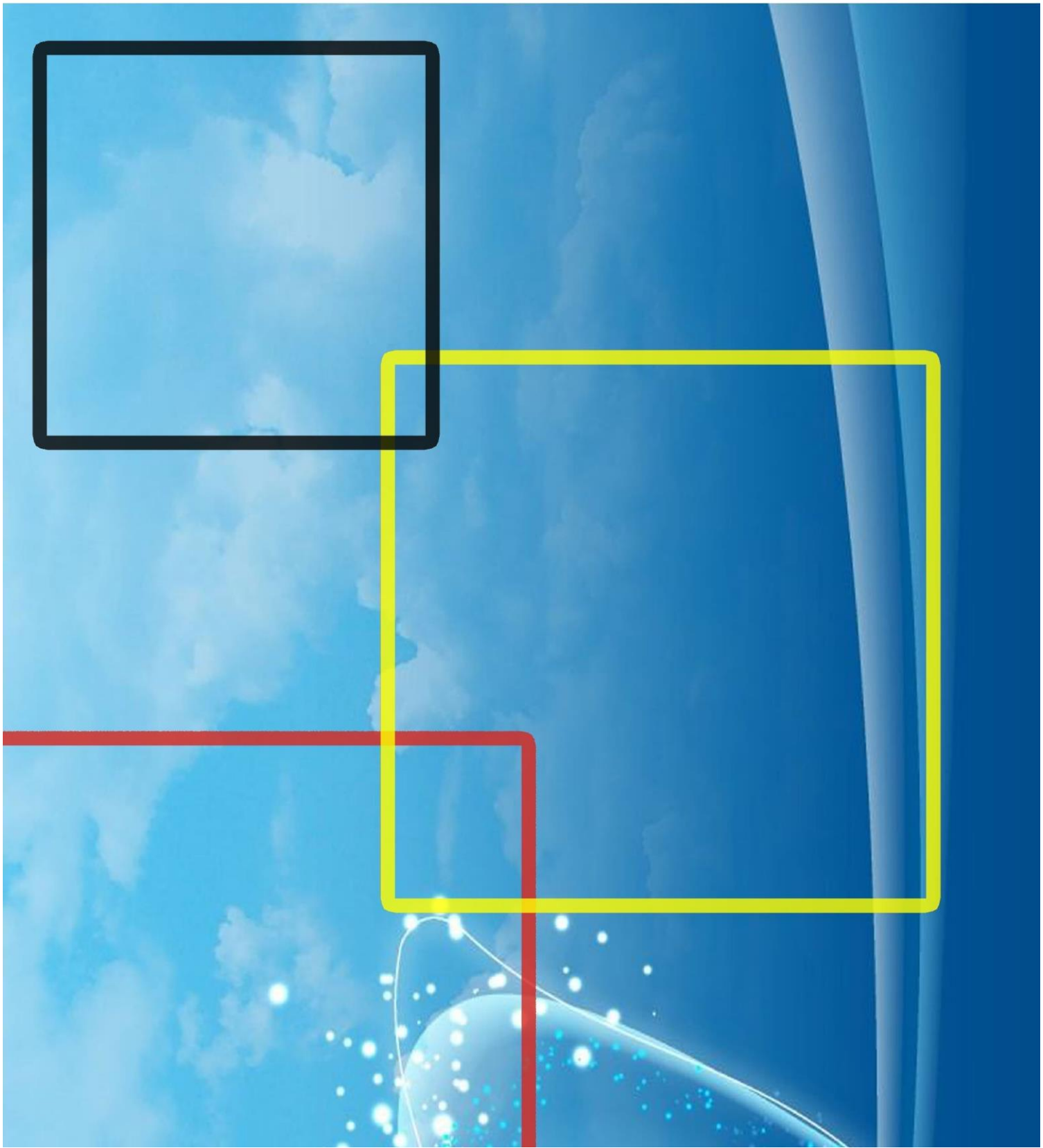
VAN VETERINARY JOURNAL

Year: 2023 / Volume: 34 / Issue: 2

ISSN : 2149-3359
e-ISSN : 2149-8644



July - 2023

VAN VETERINARY JOURNAL

This journal previously published as: **Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi**

ISSN: 2149-3359 E-ISSN: 2149-8644

This journal is published three times a year. Journal Tittle Abbreviation: Van Vet J

All articles in this journal are available free of charge from https://dergipark.org.tr/tr/pub/vanvetj

Year	Volume	Issue
2023	34	2

This journal indexed / abstracted in Asos Index, CAB Abstracts, EBSCOhost, Google Scholar, Index Copernicus, Sobiad, TR Dizin and Turkiye Atıf Dizini

July - 2023

VAN VETERINARY JOURNAL

This journal previously published as: **Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi**

This journal is published three times a year. Journal Tittle Abbreviation: Van Vet J

All articles in this journal are available free of charge from https://dergipark.org.tr/tr/pub/vanveti

Year	Volume	Issue
2023	34	2

This journal indexed / abstracted in Asos Index, CAB Abstracts, EBSCOhost, Google Scholar, Index Copernicus, Sobiad, TR Dizin and Turkiye Atıf Dizini

VAN VETERINARY JOURNAL

CONTENTS

Page

Original Articles

VAN VETERINARY JOURNAL

CONTENTS

Page

Original Articles

Review

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Taş Ş, Kozat S (2023).** The Prevalence of *Rotavirus, Coronavirus, Cryptosporidium* spp, *Escherichia coli* K 99 and *Clostridium perfringens* in Calves with Newborn Diarrhea in Muş and its Districts. *Van Vet J*, 34(2), 86-92. DOI: https://doi.org/10.36483/vanvetj.1225000

ISSN: 2149-3359 **Araştırma Makalesi** e-ISSN: 2149-8644

Muş ve İlçelerinde Yenidoğan İshalli Buzağılarda *Rotavirus*, *Coronavirus*, *Cryptosporidium* spp., *Escherichia coli* K99 ve *Clostridium perfringens* Etkenlerinin Prevalansı

Şeref TAŞ[1] Süleyman KOZAT[2,*]

[1] *Van Yüzüncü Yıl Üniversitesi, Sağlık Bilimleri Enstitüsü, İç Hastalıkları Anabilim Dalı, 65080, Van, Türkiye*
[2] *Van Yüzüncü Yıl Üniversitesi, Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, 65080, Van, Türkiye*

Gönderim Tarihi: 16.01.2023 Kabul Tarihi: 27.03.2023

ÖZ

Bu çalışma, Muş ve ilçelerinde yenidoğan ishalli buzağılarda *Rotavirus, Coronavirus, Escherichia coli K-99, Cryptosporidium* spp. ve *Clostridium perfringens* etkenlerinin prevalansını belirlemek için yapıldı. Araştırmanın hayvan materyali; sistematik bir şekilde muayeneleri yapılan Muş ili ve ilçelerinde yenidoğan farklı yaş, ırk ve cinsiyette 96 ishalli buzağıdan dışkı örnekleri alınarak yapıldı. Alınan dışkı numuneleri hızlı tanı testleriyle tekniğine uygun olarak analiz edildi. Dışkı örneklerinin hızlı diagnostik test kitleriyle incelenmesi sonucunda, buzağılardaki ishalin %10.41 *Rotavirus*, %25 *Coronavirus*, %27.08 *Rotavirus*+ *Coronavirus*, %7.29 *E. coli*, %5.37 *Cryptosporidium* spp, %12.5 *Clostridium perfringens* ve %12.5 diğer faktörlerden kaynaklandığı tespit edildi. Sonuç olarak; Muş ili ve ilçelerinde neonatal dönemdeki ishalli buzağılarda ishale neden enteropatojenlerin varlığı ve bunların dağılımı hakkında bilimsel veriler ortaya konuldu. Muş ve ilçelerinde yenidoğan ishalli buzağılar ile ilgili gelecek zamanlarda yapılacak olan bilimsel çalışmalar için yol göstereceği ve ışık tutacağı kanısına varıldı.

Anahtar Kelimeler: *İshal, Prevalans, Yenidoğan Buzağı.*

ABSTRACT

The prevalence of *Rotavirus, Coronavirus, Cryptosporidium* spp., *Escherichia coli K99* and *Clostridium perfringens* in Calves with Newborn Diarrhea in Muş and its Districts

This study was carried out to determine the prevalence of *Rotavirus*, *Coronavirus*, *Escherichia coli K-99*, *Cryptosporidium spp.* and *Clostridium perfringens* in calves with neonatal diarrhea in Muş and its districts. The material of this study was stool samples taken from 96 newborn calves of different ages, breed, and genders in Muş province and its districts. The stool samples were analyzed by rapid diagnostic tests in accordance with the technique. When stool samples were examined by rapid diagnostic test; 10.41% *Rotavirus,* 25% *Coronavirus*, 27.08% *Rotavirus+Coronavirus*, 7.29 % *E. coli*, 5.37% *Cryptosporidium spp*, 12.5% *Clostridium perfringens* and 12.5 % other factors were detected in calves with diarrhea. As a result; the presence and distribution of enteropathogens that cause diarrhea in calves with neonatal diarrhea in Muş and its districts were presented. It was concluded that this study will shed light on future scientific studies on diarrheal calves in Muş and its districts.

Keywords: *Diarrhea, Newborn calf, Prevalence.*

GİRİŞ

Dünya genelinde sığırcılık sektöründe neonatal dönem hayvan sağlığı ve verimlilik açısından önemli bir yer teşkil etmektedir. Bu dönemde görülen ekonomik kayıpların yaklaşık %75'i ishal nedeniyle oluşan kayıplar olarak bildirilmektedir. Yenidoğan buzağılarda görülen ishal en sık doğumu takiben 2-25 günlük süre içinde görülmektedir (Özkan ve Akgül 2004; Kozat ve Voyvoda 2006; Kozat ve Tuncay 2018). Buzağı ishalleri; enfeksiyöz veya non enfeksiyöz nedenlere bağlı gelişir ve dışkıdaki sıvı miktarının fazla olmasına bağlı defekasyonun normaldan çok daha sık olmasıyla karakterize bir semptomdur (Cho ve ark. 2014; Kozat ve Tuncay 2018). İshal, yenidoğan buzağılarda büyüme geriliğine neden olabileceği gibi ölümle sonuçlanarak, önemli ekonomik kayıplara da yolaçabilir. Yenidoğan buzağılarda birçok bakteri, virus ve protozoonun ishale neden olduğu, bunların yanı sıra genetik, çevresel, bakım ve beslenme gibi pek çok faktörün de hastalığa neden olabileceği bilinmektedir (Larson ve Tyler 2005). İshal; buzağıların en önemli sağlık sorunlarındandır (Radostits 2006). Özellikle immun sistemin yeterince gelişmediği hayatın ilk günlerinde ishallerin ortaya çıkması tedavide başarısızlığa neden olmaktadır. İshalin etiyolojisi birçok nedene bağlı

✉ *Corresponding author: skozat@hotmail.com

olduğundan, bu durumda hem korunma hem de tedavisi zordur (Kozat 2018; Kozat 2019). Bu araştırmada; hem seçilen alan, hemde araştırılan konu bölge hayvancılığı açısında çok önem taşımaktadır. Bu amaçla Muş ili ve ilçelerinde neonatal buzağılarda verim kaybı ve ölümlere neden olan ishal etkenlerinin tespit edilmesi hedeflendi.

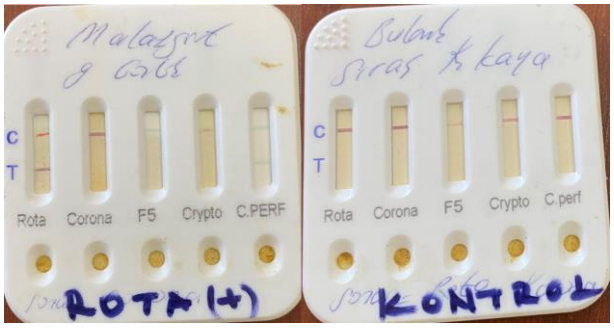
MATERYAL VE METOT

Bu araştırmanın yapılması için gerekli izinler Van Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulundan alınmıştır (Tarih: 29.04.2021 ve Karar No: 2021/04-06).
Çalışma için hayvan materyali 2021-2022 tarihleri arasında Muş ili ve ilçelerinde (Muş merkez, Bulanık, Korkut, Malazgirt, Varto ve Hasköy) bulunan 96 adet yenidoğan ishalli buzağıdan oluşturuldu. 56 baş erkek ve 40 baş dişi buzağının yaşları 2-35 günlüktür. Irk dağılımları ise 3 adet holstein, 23 montofon, 62 simental, 5 yerli kara ve 3 adet Doğu Anadolu kırmızısı buzağıdan oluşmuştu.
Hasta buzağıların klinik muayenesinde dışkının kıvamı, yoğunluğu, sıklığı, kokusu, içeriği, renginin yanı sıra buzağın yaşı, ırkı, kolostrum alıp almadığı, beden ısısı, kalp frekansı, dehidrasyon derecesi ve ishalin süresi gibi kriterler de kayıt altına alındı. Ayrıca çalışmaya alınan buzağıların ishale yakalanmadan önce herhangi bir hastalık geçirip geçirmedikleri ve ilaç tedavisi görüp görmediği ananmez biligileriyle doğrulandı. Anamnez bilgilerine göre daha önce hastalık geçirmiş veya tedavi uygulanan ishalli buzağılar araştırmaya dâhil edilmedi.

Muş ili ve ilçelerindeki işletmelerden kliniğe getirilen ishal şikâyeti olan 2-35 günlük yaştaki farklı cinsiyet ve ırka sahip 96 adet ishalli buzağının rektumundan taze dışkı numuneleri alınarak yapıldı. Araştırmada toplanan dışkı numunelerinden *Rotavirus, Coronavirus Cryptosporidium* spp. *E. coli K99* ve *Clostridium perfringens* etkenleri hızlı diagnostik ticari test kitleriyle (TMR Nutrition- Calf Test-5) belirtilen kurallara göre tespit edildi.

Dışkı örneklerinin analizi

İshalli buzağılarda dışkı numuneleri rektumdan, 20-30 gr kadar taze dışkının steril plastik kaplara toplanmasıyla gerçekleştirildi. Steril kaplara alınan dışkı numunesi test kit solüsyonuna aktarıldı. Test kit solüsyonu aktarılan dışkı solüsyonla iyice karıştırıldıktan sonra test solüsyon karışımından birkaç damla alınarak hızlı test kitinin plaklarına damlatıldı. Solüsyonun plak yüzeyine iyi temas etmesiyle 5-10 dakika bekletildikten sonra pozitif veya negatif sonuçlar değerlendirildi (Şekil 1, Şekil 2).



Şekil 1: Etiyolojik faktörlerin hızlı test kitlerindeki sonuçları.

Figure 1: Results of etiological factors in rapid test kits.

Şekil 2: Sulu sarı renkte ishal.

Figure 2: Watery yellowish diarrhea.

İstatistiksel Analiz

Elde edilen veriler tanımlayıcı istatistikler için etkenlerin oranı ve görülme sıklığı tespit edildi. Yapılan hesaplamalarda SPSS (Version-21) istatistik paket programı kullanıldı.

BULGULAR

Klinik bulgular

Kliniğe getirilen (Özel Gülçimen Veteriner Kliniği) ishalli buzağıların iştahsız, halsiz, durgun, emme refleksinin zayıf veya hiç olmadığı, ishalli buzağın ayakta duruşu, göz küresinin göz çukurluğundaki çöküş derecesi (mm) ve deri elastikiyetinin düzelme süresi (saniye) gibi klinik bulgulara göre bazı buzağıların sıvı kaybının % 7 'den fazla olduğu tespit edildi. Dışkı analiz sonuçlarına göre 7 buzağıda *E.coli K99,* 5 buzağıda *Cryptosporidium*, 10 buzağıda *Rotavirus,* 24 buzağıda *Coronavirus,* 26 buzağıda *Coronavirus+Rotavirus* ve 12 buzağıda ise *Cl. perfringens* tespit edildi. Bunlar ek olarak ishal görülen ve herhangi bir enteropatojen tespit edilemeyen 12 ishalli buzağıda diğer etkenlerden kaynaklı olabileceği düşünülerek kayıt edildi. Bu araştırmada viral etkenler ya tek başlarına ya da miks enfeksiyonlar şeklinde rastlandı ve miks enfeksiyonların olduğu vakalarda daha şiddetli klinik bulgular gözlendi. Özellikle miks enfeksiyonların *Rotavirus+Coronavirus* olarak seyrettiği tespit edildi (Tablo 1, Tablo 2).

Tablo 1: Etkenlerin birlikte seyretme durumu.

Table 1: Coexistence of etiological agents.

Etken	Sayı
Rotavirus	10
Coronavirus	24
Rotavirus+Coronavirus	26
Escherichia coli	7
Cryptosporidium spp.	5
Clostridium perfringens	12
Diğer etkenler	12

Tablo 2: Etiyolojik faktörlerin bulunma oranı.

Table 2: The presence rate of etiological factors.

Etken	Bulunma oranı (%)
Rotavirus	10/96 =%10.41
Coronavirus	24/96=%25
Rotavirus+Coronavirus	26/96=%27.08
Escherichia coli	7/96=%7.29
Cryptosporidium spp	5/96=%5.2
Clostridium perfringens	12/96=%12.5
Diğer faktörler	12/96=%12.5

İshalin karakteri ile etken arasında yapılan istatistiksel analizde sulu sarı-kanlı karakterdeki 60 ishalli vakanın 57'sinde enteropatojenler tespit edilirken, 3 ishalli vakadan ise viral veya başka etken tespit edilmedi. Sulu yeşil renkli ishalli 13 vakanın 10'unda patojenlere saptanırken, 3 ishalli vakada viral, bakteriyel ve protozoal etken tespit edilmedi. Sulu beyaz–gri karakterdeki ishalli 9 vakanın 7'sinde patojenler tespit edildi. Sulu sarı-kanlı, sulu yeşil ve sulu beyaz–gri karakterlerdeki ishallerin rengi dışında olan ve diğer karakterdeki ishaller olarak tanımladığımız ishal karakterindeki 14 vakanın 10'unda patojenlere rastlandı. Araştırma süresince elde edilen gözlem ve incelemeler doğrultusunda sulu sarı-kanlı karakterdeki ishallerde patojenler en yüksek düzeyde olduğu tespit edildi. Ayrıca ishalli buzağılarda kolostrumu yetersiz almış ya da hiç almamış buzağıların enteropatojen ajanlara bağlı ishale yakalanma oranı %90.9 iken, kolostrumu aldığı halde diğer nedenlerden dolayı ishal olan buzağı oranı %9.1 olarak saptandı (Tablo 3).

Tablo 3: Etkenlerin ishal görünümüyle ilişkisi.

Table 3: Relationship of factors with the appearance of diarrhea.

Etken	Sulu Sarı-Kanlı	Sulu Yeşil	Sulu beyaz–Gri	Diğer renk	Toplam
Rotavirus	8	0	2	0	10
Coronavirus	17	4	0	3	24
Coronavirus+ Rotavirus	17	4	3	2	26
E. coli	7	0	0	0	7
Cryptosporidium spp.	2	0	1	2	5
Clostridium perfringens	6	2	1	3	12
Diğer etkenler	3	3	2	4	12
Toplam	60	13	9	14	96

İshale neden olan etiyolojik faktörlerin yerleşim alanlarına göre dağılımları incelendiğinde, miks enfeksiyon kaynaklı ishallerin daha yaygın olduğu görüldü. Rotavirus+Coronavirus miks enfeksiyon tablosunun en fazla bulunduğu, bireysel enfeksiyonlarda ise coronavirus'ün ikinci sırada yer aldığı tespit edildi (Tablo 4).

Tablo 4: Etkenlerin bölgelere göre dağılımı.

Table 4: Distribution of factors as to residential areas.

ETKEN	Yerleşim Alanları						
	Muş	Bulanık	Varto	Malazgirt	Hasköy	Korkut	Toplam
Rotavirus	1	3	0	4	2	0	10
Coronavirus	1	9	6	3	3	2	24
Rotavirus +Coronavirus	4	13	2	1	4	2	26
E. coli	2	0	2	2	1	0	7
Cryptosporidium spp.	0	2	0	2	0	1	5
Clostridium perfringens	3	3	2	1	2	1	12
Diğer etkenler	3	4	0	1	0	4	12
TOPLAM	14	34	12	14	12	10	96

TARTIŞMA VE SONUÇ

Sığırcılık sektöründe et ve süt işletmelerinde buzağılarda ishal kaynaklı hastalıkların önemli derecede ekonomik ve verim kayıplarına yol açtığı bildirilmiştir (Kozat 2019). Dünya genelinde ve ülkemizde buzağı ishalleri ile ilgili pekçok araştırmada (Von Buenau ve ark. 2005; Al ve Balıkçı 2012; Arslan ve ark. 2015; Akyüz ve ark. 2017; Anthony ve ark. 2017) buzağılarda ishale bağlı hastalık ve ölüm oranın yüksek olduğu ve bu durumunda hayvancılık ekonomisinde önemli kayıplara neden olduğu bilinmektedir. Yenidoğan buzağılarda ishale yakalanma oranının %50'den fazla olduğu ve ishalden dolayı ölenlerin de %2-8 oranında görüldüğü rapor edilmektedir (Kozat ve Voyvoda 2006). Pekçok ülkede ve Türkiye'de buzağı ishallerinde rol oynayan etiyolojik faktörlerin identifiye

edilmesi için birçok araştırma yapılmıştır (Uzun ve ark. 2010; Kozat ve Tuncay 2018). Elde edilen sonuçların ülkemizdeki sonuçlara benzer olduğu ve neonatal dönemdeki buzağıların ishale yakalanma oranları ve buna bağlı verim kayıplarıyla ölümlerin yüksek düzeyda olduğu bildirilmektedir. Ayrıca ishale yönelik tedavi masrafları da işletmeler için önemli ekonomik kayıplara yol açtığı rapor edilmiştir (Uzun ve ark. 2010). İshalli buzağılarda verim kaybı ve ölüm oranlarını azaltmak amacıyla son yıllarda etiyolojik faktörlerin erken tespiti çok önemlidir. Bunun için klinikte pek çok laboratuvar test ve yöntemi kullanılmaktadır (Cornish ve ark. 2005; Cho ve ark. 2013; Cho ve Yoon 2014).

Neonatal dönemdeki buzağılarda ishal kaynaklı verim kaybı ve buzağı ölüm oranını azaltmak ve ishale neden olan etkenlerin kısa sürede tespit edilmesi, etkin ve hızlı tedavi için önemli bir katkı sağladığı belirtilmektedir (Murat ve Balıkçı 2012; Kozat ve Tuncay 2018).

Bu amaçla hızlı tanı test kitleri yaygın olarak kullanılmaktadır (Al ve Balıkçı 2012; Kozat ve Tuncay, 2018; Bal 2019). Yeni doğan buzağılarda ishale neden olan etiyolojik faktörlerin kısa sürede tespit edilmesi ve uygun bir tedavi sonucunda ekonomik kayıplar minimum seviyeye inebileceği belirtilmektedir (Kozat ve Voyvoda 2006). Bu çalışmada da hızlı diagnostik testlerden immunokromatografik test olan ticari in vitro Rapid Diagnostic Test (TMR Nutrition- Calf Test-5) kullanıldı. Bu test analizi için Muş ve ilçelerinde yenidoğan ishalli buzağılardaki *Rotavirus, Coronavirus, Cryptosporidium* spp., *Escherichia coli K99* ve *Clostridium perfringens* etkenleri analizi için taze dışkı örneklerinden 5-10 dakika gibi kısa bir sürede identifiye edilerek, gerekli koruyucu ve tedavi uygulamaları yapıldı.

Yenidoğan buzağılarda ishalin etiyolojik nedeni sadece enfeksiyöz ajanlar değil aynı zamanda hazırlayıcı faktörler ve bakım beslenme faktörlerinin de etkili olduğu bildirilmektedir (Cho ve Yoon 2014). Yenidoğan buzağılardaki ishalin etiyolojisi için birçok çalışma yapılmıştır. Yeni doğan buzağılarda ishale neden olan etiyolojik faktörlerle ilgili araştırmalarda en çok elde edilen ajanlar; *E.coli, Coronavirus, Rotavirus, Giardia, Toxocara, Cryptosporidium* ve *Eimeria*'ların olduğu belirlenmiştir (Khan ve Khan 1991; Langoni ve ark. 2004; Lorenz ve ark. 2011).

Rotavirus dünya çapında buzağılarda neonatal ishalin başlıca nedenlerinden biri olduğu belirtilmektedir (Karayel ve ark. 2017). Yapılan pek çok araştırmada *Rotavirus* enfeksiyonları yenidoğan buzağılarda büyümede gecikme, zayıf buzağıların doğumu ve sürülerdeki yüksek ölüm oranları nedeniyle ekonomik kayıpların temel nedenlerini oluşturmaktadır. Türkiye dâhil birçok ülkede *rotavirus* enfeksiyonunun varlığı virolojik ve serolojik yöntemlerle ortaya konmuştur (Ekik 2002). 1-4 haftalık buzağılarda ishale neden olan ajanlar çoğunlukla *Rotavirus Coronavirus ve Cryptosporidium* iken, 1-4 günlük buzağılarda daha çok *E. coli (K99/F5)* ishale neden olmaktadır. *E. coli*'nin yenidoğan buzağılardaki prevalansı %2.6-45.1 arasında, *Rotavirus* prevalansı %17.7-79.9 arasında ve *Cryptosporidium parvum* %27.8-63 arasında rapor edilmektedir (Meganck ve ark. 2014). Çabalar ve ark. (2007) Van'da ishalli buzağılarla ilgili yapmış oldukları araştırmasında *Rotavirus* %17.97, *Coronavirus* %1.12 düzeyde tespit ettiklerini rapor etmişlerdir.

Bu araştırmada ishalli buzağılarda *rotavirus* enfeksiyonu %10.41 oranında tespit edildi. *Rotavirus* enfeksiyonun 10-15 günlük buzağılarda yoğun olarak saptandı. Bu çalışmanın *Rotavirus* ile ilgili verileri araştırıcıların (Ekik 2002; Çabalar ve ark. 2007; Meganck ve ark. 2014; Kozat ve Tuncay 2018) verileriyle benzerlik arz etmektedir.

Coronavirus tipik olarak buzağıları doğumdan sonraki ilk 3 haftada etkiler ve en yüksek insidans 7. ile 10. günler arasında ortaya çıkar (Kozat ve Tuncay 2018). Neonatal dönemdeki ishalli buzağılarda *coronavirus* enfeksiyonun prevalansı ilgili pek çok araştırmalar yapılmıştır. Bu araştırmalarda; Reynolds ve arkadaşları (1984), 74 ishalli buzağı gaitasından *Coronavirus* %21.3 oranında, İskoçya'da ishalli buzağıların %3.6'ında *Coronavirus* tespit ettiklerini bildirmişlerdir (Snodgrass ve ark., 1986). Abraham ve arkadaşları (1992), 108 adet ishalli buzağı için 5 farklı ishal ajanı taspit ettikleri araştırmalarında *Coronavirus* %38.9 ve Fransa'nın güney batı bölgesinde yapılan bir araştırmada ise ishalli buzağılarda Coronavirus %16.5 düzeyinde tespit etmişlerdir (Bendali ve ark.1999). İspanya'da ishalli buzağılarda Coronavirus etkenini %10.7 oranında rapor etmişlerdir (Garcia ve ark. 2000). Lanz Uhde ve ark. (2008) ise İsviçre'de 1-21 günlük ishalli buzağılarda Coronavirus etkenini %7.8'inde tespit ettiklerini bildirmişlerdir. İran'da 126 ishalli buzağıda *coronavirus* %3.17 (Mayameei ve ark. 2010), Norveç'te (Gulliksen ve ark. 2009), 68 ishalli buzağının gaitasında *Coronavirus* %4.2 oranın tespit ettiklerini bildirmişlerdir. Eskiizmirliler ve arkadaşları (2001), ishalli buzağılardan alınan 185 dışkı örneğinin %13'ünde *coronavirus* tespit ettiklerini belirtmektedirler. Bu çalışmada 96 adet ishalli buzağıda *Coronavirus* oranı %25 (24/96) düzeyinde bulundu. İshalli buzağılarda *coronvirus* enfeksiyonun prevalansıyla ilgili bazı araştırmacıların (Reynolds ve ark.1984; Abraham ve ark. 1992) verileriyle paralel arz ederken, bazı araştırmacıların (Bendali ve ark. 1999; Garcia ve ark. 2000; Lanz Uhde ve ark. 2008) oranlarından yüksek bulundu.

En yaygın hastalıklardan biri olan buzağı ishali, etkilenen buzağılarda morbidite ve mortalite, tedavi maliyetleri ve düşük büyüme oranları nedeniyle önemli maddi kayıplar yapan kompleks bir sendromdur (Garaicoechea ve ark. 2006; Mayameei ve ark. 2010). *Rotavirus* ve *Coronavirus*, dünya çapında çocuk ve birçok hayvan türünde şiddetli ishalin iki ana nedenidir (Mayameei ve ark. 2010). Yenidoğan bazı buzağılarda ishale neden olan etkenler birden fazla etiyolojik faktör şeklinde görülmektedir. Miks enfeksiyon olarak tanımlanan bu kompleks ajanlar farklı etkenlerlerin bir arada görülmesidir. İshalin gelişiminde virus, bakteri ve protozoonlar gibi enterik patojenler en önemli ajanlardır. Bazı ishal vakalarında tek bir etken bile ishale sebep olabilirken, bazılarında ise birden fazla etkenin ishale neden olduğu bilinmektedir (Cho ve Yoon 2014). İspanya'da ishalli buzağılardan alınan dışkı örneklerinin %42.7'sinde *Rotavirus*, %7.3'ünde *Coronavirus* ve *Rotavirus+Coronavirus* miks enfeksiyonu toplam dışkı örneklerinin %5.1'inde tespit ettiklerini rapor etmişlerdir (Fuente ve ark. 1998). Yeni doğan buzağılarda neonatal dönemde (0-4 haftalık) özellikle 0-2 haftalık süreçte ishal vakaların %80'inin enfeksiyöz kaynaklı olduğu ve %50'sinden fazlasında birden çok etkene bağlı, %31'i ise iki etkenden kaynaklı olduğu tespit edilmiştir (Cho ve ark. 2010; Al ve Balıkçı 2012).

Elazığ'da yapılan bir çalışmada enfekte buzağıların %30'u (n=9) *Rotavirus*, %13'ü (n=4) *Coronavirus*, %17'si (n=5) *E. coli*, %6'sı (n=2) miks enfeksiyon, %33 (n=10) diğer etkenlerden dolayı ishal oldukları tespit ettiklerini rapor etmişlerdir. Siirt ilinde yapılan çalışmada *Rotavirus+Cryptosporidium* %10 oranında tespit ettiklerini belirtmişlerdir (Kozat ve Tuncay, 2018). Bu araştırmada ise *Rotavirusu ile Coronavirus* miks olarak saptandı. *Rota+Coronavirus* miks enfeksiyon oranı ise %27.08

(26/96) olarak tespit edilmiştir. Araştırmada miks enfeksiyonlarla ilgili elde edilen veriler değerlendirildiğinde miks enfeksiyonlarda viral kökenli ajanların yaygın olduğu ve en yüksek oranda miks enfeksiyonların ishalin oluşumunda rol oynadığı ortaya konuldu. Araştırmada miks enfeksiyonlarla ilgili elde edilen veriler araştırıcıların verilerini desteklenmektedir (Cho ve ark. 2010)

Kriptosporidiozis buzağıların önemli ishal etkenlerinden biridir. Protozoonların neden olduğu genellikle ruminantlarda neonatal dönemde görülen zoonotik bir enfeksiyondur (Şimşek ve ark. 2012). Kriptosporidium etkenleri hem yenidoğan hem de genç buzağıların intestinal sistemine yerleşip ishal yapan parazitlerdir (Özkan ve ark. 2001). Kriptosporidiozisli buzağılarla ilgili bir araştırmada 1-2 aylık buzağılarda en yüksek ve %23.2 düzeyinde tespit ettiklerini belirtmişlerdir. Aynı araştırmada cinsiyetlere göre dağılımında ise enfeksiyonların dişi buzağılardaki oranı %19,7 iken erkek buzağılarda ise bu oranı %21.5 olarak rapor edilmişlerdir (Şimşek ve ark. 2012). Meganck ve arkadaşları (2014) ise ishalli buzağılarda *Cryptosporidium* etkenlerini %27.63 oranında tespit ettiklerini bildirmişlerdir. Türkiye'de çeşitli araştırmalar sonucunda; Ankara'da %35.8, Elazığ'da %7.2, Karacabey'de ise %26.7 oranında (Al ve Balıkçı 2012), Kars ilindeki çalışmada %5.9 oranında tespit ettiklerini rapor etmişlerdir (Aydın ve ark. 2001). Bu çalışmada Muş ve ilçelerinde *Cryptosporidium* etkenleri %5.2 düzeyinde tespit edildi. Elde edilen bulgular araştırıcıların (Aydın ve ark. 2001) verileriyle paralellik arzederken, bazı araştırıcıların (Al ve Balıkçı 2012; Şimşek ve ark. 2012) verilerine göre ise düşük olduğu tespit edildi.

Günümüzde buzağı ishallerin etiyolojileri ilgili yapılan araştırmalarda paraziter etkenlerden *Cryptosporidium, Eimeria* ve *Toxocara*'ların, viral etkenlerden *Rotavirus* ile *Coronavirus* ve bakteriyel etkenlerden ise *Escherichia coli* en önemli patojenler olduğu rapor etmişlerdir (Lorenz ve ark. 2011; Akyüz ve ark. 2017). *E. coli* doğumdan sonraki ilk günlerinde (1-4. gün) ishale neden olup dünya genelinde ishalin en önemli nedeni olarak tespit edilmiştir (Guliksan ve ark. 2009). Türkiye'de neonatal dönemdeki ishalli buzağılarda *E. coli* varlığını araştırmak için pekçok araştırma yapılmıştır. Bu araştırmalarda Tokat bölgesinde *E. Coli'ye bağlı enfeksiyon* oranını %7.48 (Kaya, 2017) ve Aydın ve arkadaşları (2001) ise Kars bölgesinde %69.3 olarak tespit ettiklerini rapor etmişlerdir. Diğer bir araştırmada ise ishalli buzağılarda *E. coli* için prevalansın %2.6-45.1 oranında olduğunu tespit ettiklerini belirtmektedir (Meganck ve ark.,2014). Siirt yöresinde yapılan çalışmada *E. coli* %6 oranında, miks seyreden *E. coli*+*Rotavirus*%5 oranında, *E. coli*+*Coronavirus* %7 olarak tespit ettiklerini bildirmektedir (Kozat ve Tuncay 2018). Bu araştırmada ise tek etken olarak görülen *E. coli* %7.29 oranında tespit edildi. Bu da neonatal dönemde ishale neden olan *E. coli* etkenin yenidoğan buzağılarda yaygın bir dağılıma sahip olduğu ve hem tek başına hem de miks enfeksiyonlar şeklinde görülebileceği kanısına varıldı. Bu araştırmada *E. coli* enfeksiyonunun oranının bazı araştırıcıların (Kozat ve Tuncay 2018; Kaya 2017) verilerin benzerlik arzederken, bazı araştırıcıların (Aydın ve ark. 2001) bulgularından daha düşük tespit edildi. Araştırmamızda *E. coli* etkenin düşük çıkmasının nedeni ise sadece *K99* suşuna bakıldığından kaynaklanabileceği düşünülmektedir.

Clostridium perfringens, evcil hayvanlarda enteritisin önemli bir nedeni olup, etkenin virülansı büyük ölçüde toksikasyona dayanmaktadır (Ferrarezi ve ark. 2008). Sığırlarda *Clostridium perfringens A, B, C, D* ve *E* tipleri olmak üzere beş farklı şekilde enterotoksemiye neden olmaktadır (Uzal ve ark. 2010). *Clostridium perfringens* tip A'nın α ve β2 toksinleri ile birlikte yaptıkları etki sonucu intestinal sistemde doku hasarına bağlı lezyonlar ve ince bağırsaklarda kanlı ishale neden olmaktadır (Songer 2010; Uzal ve ark. 2010). Sivas ilinde neonatal ishalli buzağılarla ilgili yapılan bir araştırmada *Clostridium perfringens*'e neonatal dönemin tamamında rastlanıldığı ve prevalansın %38 (53/138) düzeyinde olduğu bildirilmektedir (Kuliğ ve Coşkun, 2019). Bu araştırmada ise *Clostridium perfringens* %12.5 olarak tespit edildi.

Yenidoğan buzağılar annelerinin plasenta yapısı özelliğinden dolayı (epitelyokordial) hipo veya agammaglobulinemik olarak doğarlar. Ancak bu dönemde annelerinden yeterli düzey kolostrum aldıklarında immuniteleri gelişir ve enfeksiyonlara karşı direnç kazanırlar (Foster ve Smith, 2009). Kolostrum, yenidoğan buzağıların neonatal dönemdeki enfeksiyonlara karşı immun destek görevi sağlayan birçok önemli bileşiği içermektedir. Doğum yapmış ineklerdeki IgG oranı, kolostral immunoglobulinin G (IgG) %90'ından daha fazladır. Doğumdan hemen sonraki ilk sağımla beraber ortalama IgG yoğunluğu yaklaşık 60 gr/L iken, doğumdan sonraki 12. saatte yapılan sağımda bu oran belirgin bir düşüşle yaklaşık 1 gr/L'ye düşer. İlerleyen sağımlarda normal yoğunluğu 0.5 gr/L'ye kadar düşmektedir. Yeni doğmuş bir buzağıda pasif immun yetmezlik gelişmemesi için normal serum immunoglobulin G yoğunluğu 10 g/L'den yüksek olmalıdır. Bunun için en az 2 litre kolostrum alması gerektiği belirtilmektedir (Kozat 2019). İshal, neonatal dönemde özellikle 6 haftalıktan küçük süt buzağılarının en önemli morbidite ve mortalite nedenidir. Buzağının bağışıklığını; patojen maruziyetini ve ardından hastalık riskini etkileyen temel değişkenler arasında çevresel koşullar, sürü yönetimi ve beslenme de yer alır. Hastalık, konakçı-patojen etkileşimlerinin doruk noktasını yansıtmaktadır (Izzo ve ark. 201). Yenidoğanlarda ve genç buzağılarda ishal etkenleri olarak; bakteriler, virusler ve paraziter ajanlar ile çevresel faktörler, bakım ve beslenme bozuklukları gibi nedenler etkili olmaktadır (McGuirk 2008; Kozat ve Tuncay 2018). Yapılan bu araştırmada ise Muş yöresindeki ishalli buzağılarda ishalin etiyolojisini belirlemek amacıyla hızlı diagnostik test (TMR Nutrition-Calf Test-5) kitleri kullanılarak, etiyolojik farktörler belirlendi. Elde edilen etkenlerin yüzdelik dağılımları belirlenerek enteropatojen ajanların sayıları kayıt altına alındı. Bu araştırmada ishalli buzağılarda kolostrumu yetersiz almış ya da hiç almamış buzağıların enteropatojen ajanlara bağlı ishale yakalanma oranı %90.9 iken, kolostrumu aldığı halde diğer nedenlerden dolayı ishal olan buzağı sayısı ise %9.1 oranında saptanmıştır. Özellikle ishal vakalarının yoğun olduğu hanelerde bakım ve barınma koşullarının kötü olması ve kolostrum sütünün uygun miktarlarda verilmesiyle ilgili yeterli bir bilgiye sahip olmadıkları araştırma sürecince tespit edildi. Bu da araştırıcıların (Izzo ve ark. 2011; Kozat ve Tuncay 2018; Kozat 2019) kolostrum yönetimi ve bakım, beslenme ve barınma koşullarıyla ilgili ortaya koymuş oldukları düşünceleri destekler nitelik olduğu sonucuna varılmıştır.

Sonuç olarak Muş ili ve ilçelerinde neonatal ishalli buzağılarda ishalin etiyolojik ajanları ve bu ajanların oransal dağılımı hakkında veriler tespit edilmiştir. Elde edilen bu verilerle şunlar amaçlanmıştır:

a) Muş ili ve ilçelerinde buzağı ishalinde rol oynayan etkenlerin varlığı göz önünde bulundurularak gerekli bilgilendirme ve kontrol programlarının yapılmasına öncülük etmek ve ishal kaynaklı ekonomik kayıpları en aza indirilmesine yönelik çalışmalara destek olmak,

b) Bu verilerle gerek veteriner hekim ve gerekse sığırcılık işletme sahiplerinin bilinçlendirilerek, neonatal dönemdeki buzağıların hastalıktan korunması ve sağlıklı buzağıların yetiştirilmesine katkı sağlanması,

c) Bu veriler yörede yapılacak benzer araştırmalara kaynak sağlanmasının yanı sıra, ülkemizde neonatal dönemdeki ishal vakalarıyla ilgili verilerin yaygınlaşması ve çiftçilerin daha fazla bilgilendirilmesi amacına yöneliktir.

d) Ayrıca bu tür vakalarda akılcı yaklaşımların çoğaltılması ve etkin koruyucu tedbirlerin alınmasına katkı sağlamak amaçlanmıştır.

ÇIKAR ÇATIŞMASI

Yazarlar bu çalışma için herhangi bir çıkar çatışması olmadığını beyan eder.

TEŞEKKÜR VE BİLGİLENDİRME

Bu çalışma Şeref TAŞ isimli yazarın yüksek lisans tezinden özetlenmiştir.

YAZAR KATKILARI

Fikir/Kavram: ŞT, SK

Denetleme/Danışmanlık: SK

Veri Toplama ve/veya İşleme: ŞT

Analiz ve/veya Yorum: SK, ŞT

Makalenin Yazımı: ŞT

Eleştirel İnceleme: SK, ŞT

KAYNAKLAR

Abraham G, Roeder PL, Zewdu R (1992). Agents associated with neonatal diarrhoea in Ethiopian dairy calves. *Trop Anim Health Prod,* 24 (2), 74-80.

Akyüz E, Naseri A, Erkılıç EE ve ark. (2017). Neonatal buzağı ishalleri ve sepsis. *Kafkas Üniv Fen Bilimleri Enstitüsü Derg,* 10 (2), 181-191.

Al M, Balıkçı E (2012). Neonatal ishalli buzağılarda rotavirus, coronavirus, *E. coli K99 ve Cryptosporidium parvum*'un hızlı test kitleri ile teşhisi ve enteropatojen ile maternal immünite ilişkisi. *F Ü Sağ Bil Vet Derg,* 26 (2), 73-78.

Anthony SJ, Johnson CK, Greig DJ, et al. (2017). Global patterns in coronavirus diversity. *Virus Evol,* 3 (1), 1–15.

Arslan MÖ, Kırmızıgül AH, Parmaksızoğlu N, Erkılıç EE (2015). Eimeria zuernii ile doğal enfekte buzağılarda kış coccidiosisi olgusu. *Atatürk Üniv Vet Bil Derg,* 10 (3), 193-197.

Aydın F, Umur Ş, Gökçe G, Genç O, Güler MA (2001). Kars yöresindeki ishalli buzağılardan bakteriyel ve paraziter etkenlerin izolasyonu ve identifikasyonu. *Kafkas Üniv Vet Fak Derg,* 7 (1), 7-14.

Bal D (2019). Manisa yöresinde neonatal buzağı ishalleri üzerine etiyolojik araştırmalar. Yüksek lisans tezi. Kocatepe Üniversitesi Sağlık Bilimleri Enstitüsü, Afyon, Türkiye.

Bendali F, Bichet H, Schelcher F, Sanaa M (1999). Pattern of diarrhoea in newborn beef calves in sauth-west France. *Vet Res,* 30, 61-74.

Cho YI, Han JI, Wang C, et al. (2013). Case–control study of microbiological etiology associated with calf diarrhea. *Veterinary Microbiolog* 166 (3-4), 375-385.

Cho YI, Kim WI, Liu S, Kinyon JM, Yoon KJ (2010). Development of a panel of multiplex real-time polymerase chain reaction assays for simultaneous detection of major agents causing calf diarrhea in feces. *J Vet Diagn Investig,* 22 (4), 509-517.

Cho YI, Yoon KJ (2014). An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. *J Vet Sci,* 15 (1), 1-17.

Cornish TE, Van Olphen AL, Cavender JL, et al. (2005). Comparison of ear notch immunohistochemistry, ear notch antigen-capture ELISA, and buffy coat virus isolation for detection of calves persistently infected with bovine viral diarrhea virus. *J Vet Diagn Investig*, 17 (2), 110-117.

Çabalar M, Kaya A, Arslan S (2007). Yeni doğan buzağıların ishal olgularında *rotavirus* ve *coronavirus* araştırılması. *Vet Bil Derg,* 23 (3-4), 103-106.

Ekik M (2002). Konya bölgesinde yenidoğan ishalli buzağılardan rotavirus antijenlerinin ELISA ile Belirlenmesi ve Annelerinden Rotavirus 93 antikorlarının tespiti. S.Ü. Sağlık Bilimleri Enstitüsü Viroloji Anabilim Dalı. Doktora Tezi, Konya.

Eskiizmirliler SN, Öncel T, Beyazıt A, Mısırlıoğlu OZ (2001). Türkiye'nin değişik illerindeki ishalli buzağılarda rotavirus, coronavirus ve crytosporidiosisin yayılışı. *Vet Hek Mikrobiy Derg,* 2, 35-42.

Ferrarezi MC, Cardoso TC, Dutra IS (2008). Genotyping of Clostridium perfringens isolated from calves with neonatal diarrhea. *Anaerobe,* 14 (6), 328-331.

Foster DM, Smith GW (2009). Pathophysiology of diarrhea in calves. *Vet Clin North Am Small Anim Pract,* 25 (1), 13-36.

Fuente R, Garcia A, Ruiz-Santa-Quiteria JA ve ark. (1998). Proportional morbidity rates of enteropathogens among diarrheic dairy calves in Central Spain. *Prev Vet Med,* 36 (2), 145–152.

Garaicoechea L, Bok K, Jones LR ve ark. (2006). Molecular characterization of bovine rotavirus circulating in beef and dairy herds in 556 Argentina during a 10-year period (1994–2003). *Vet Microbiol,* 118, 1–11.

Garcia A, Ruiz-Santa-Quiteria JA, Orden JA (2000). Rotavirus and concurrent infections with other enterapathogens in noenatal diarrheic dairy calves in Spain. *Comp Immun Microbiol Infect Dis,* 23, 175 – 183.

Gulliksen SM, Jor E, Lie KI ve ark. (2009). Enteropathogens and risk factors for diarrhea in Norwegian dairy calves. *J Dairy Sci*, 92 (10), 5057-5066.

Izzo MM, Kirkland PD, Mohler VL, et al. (2011). Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. *Aust Vet J,* 89 (5), 167-173.

Karayel I, Fehér E, Marton S, et al. (2017). Putative vaccine breakthrough event associated with heterotypic rotavirus infection in newborn calves. *Vet Microbiol,* 201, 7-13.

Kaya U (2017). Tokat Bölgesindeki Neonatal Buzağı İshallerinin Etiyolojisinin Belirlenmesi Yüksek lisans Tezi, Cumhuriyet Üniversitesi Sağlık Bilimleri Enstitüsü, Sivas.

Khan A, Khan MZ (1991). Aetiopathology of neonatal calf mortality. *J Isl Acad Sci,* 4 (2), 159- 165.

Kozat S, Tuncay İ (2018). Siirt yöresindeki yenidoğan ishalli buzağılarda *Rotavirus, Coronavirus, Cryptosporidium* spp, *Escherichia coli K 99* ve *Giardia lamblia* etkenlerinin prevalansı. *Van Vet J,* 29 (1), 17-22.

Kozat S, Voyvoda H (2006). İshalli buzağılarda kristalloid (laktatli ringer) ve kolloidal + kristalloid (% 6 dekstran - 70 + laktatlı ringer) infüzyon solüsyonlarinin rehidratasyon etkinliği. *Van Sağ Bil Derg,* 9 (1), 139-151.

Kozat S (2018). Hypothermia in newborn calves. *J İstanbul Vet Sci*, 2 (1), 30-37.

Kozat S (2019). Yenidoğan buzağılarda kolostrum yönetiminin önemi. *Ataturk Univ Vet Bilim Derg,* 14 (3), 343-353.

Kuliğ CC, Coşkun A (2019). Sivas ve ilçelerindeki neonatal ishalli buzağılarda E. coli, *Cryptosporidium, Clostridium perfringens, Rotavirus* ve *Coronavirus* Prevalansı. *Turk Vet J,* 1 (2), 69-73.

Langoni H, Linhares AC, De Avila FA, Da Silva AV, Elias AO (2004). Contribution to the study of diarrhea etiology in neonate dairy calves in São Paulo state, Brazil. *Braz J Vet Res Anim Sci,* 41, 313-319.

Lanz Uhde F, Kaufmann T, Sager H, et al. (2008). Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. *Vet Rec,* 163 (12), 362-366.

Larson RL, Tyler JW (2005). Reducing calf losses in beef herds. *Vet Clin North Am Food Anim Pract*, 21 (2): 569-584.

Lorenz I, Fagan J, More SJ (2011). Calf health from birth to weaning. II. Management of diarrhoea in pre-weaned calves. *Irish Vet J*, 64 (9), 1-6.

Mayameei A, Mohammadi G, Yavari S, Afshari E, Omidi A (2010). Evaluation of relationship between *Rotavirus* and *Coronavirus* infections with calf diarrhea by capture ELISA. *Comp Clin Path,* 19 (6), 553-557.

McGuirk SM (2008). Disease management of dairy calves and heifers. *Vet Clin North Am Food Anim Pract*, 24 (1), 139-153.

Meganck V, Hoflack G, Opsomer G (2014). Advances in prevention and therapy of neonatal dairy of diarrhoea: a systematic review with emphasis on colostrum management and fluid therapy. *Acta Vet Scand,* 56 (1), 75.

Murat A, Balıkçı E (2012). Neonatal ishalli buzağılarda rotavirus, coronavirus, E. coli K99 ve Cryptosporidium parvum'un hızlı test kitleri ile teşhisi ve enteropatojen ile maternal immünite ilişkisi. *F Ü Sağ Bil Vet Derg*, 26 (2), 73–78.

Özkan C, Akgül Y (2004). Neonatal ishalli buzağılarda hematolojik, biyokimyasal ve elektrokardiyografik bulgular. *YYÜ Vet Fak Derg,* 15 (1-2), 123-129.

Özkan M, Gıcık Y, Metin H, Sarı B (2001). Prevence of cryptosporidium spp oocysts in diarrhoeic calves in Kars Province, Turkey. *Turk J Vet Anim Sci,* 25, 161-164.

Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2006). Veterinary Medicine: A Textbook of the diseases of catle, sheep, goats, pigs and horses. 10th ed. Saunders Co. London.

Reynold SM, Chasey D, Scott AC, Bridger JC (1984). Evaluation of ELISA and EM for detection of coronavirus and rotavirus in bovine faeces. *Vet Rec,* 114, 397-401.

Snodgrass DR, Terzolo HR, Sherwood D, Campell I, Menzies JD. (1986). Aetiology of diarrhoea in young calves. *Vet Rec,* 119, 31-34.

Songer JG. Enteric Clostridia In: Gyles CL, Prescott JF, Songer JG., Thoen CO (2010). Pathogenesis of bacterial infections in animals. 4th edition, Wiley-Balckwell, USA. Pp. 211-229.

Şimşek AT, İnci A, Yıldırım A, Çiloğlu A, Bişkin Z, Düzlü Ö (2012). Nevşehir yöresindeki yeni doğan ishalli buzağılarda cryptosporidiosis' in real time PCR ve Nested PCR yöntemleri ile saptanması. *Erciyes Üniv Vet Fak Derg*, 9 (2), 79-87.

Uzal FA, Vidal JE, McClane BA, Gurjar AA (2010). Clostridium perfringens Toxins Involved in mammalian veterinary diseases. *The Open Toxinology Journal*; 3, 24-42.

Von Buenau R, Jaekel L, Schubotz E, Schwarz S, Stroff T, Krueger M (2005). *Escherichia coli* strain nissle 1917: significant reduction of neonatal calf diarrhea. *J Dairy Sci,* 88 (1), 317-323.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Daldaban F, Arslan K, Akyüz B (2023).** The Relationship Between *CACNA2D1* Gene and Subclinic Mastitis in Holstein Breed Cattle. *Van Vet J*, 34(2), 93-97. DOI: https://doi.org/10.36483/vanvetj.1242270

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

The Relationship Between *CACNA2D1* Gene and Subclinic Mastitis in Holstein Breed Cattle

Fadime DALDABAN[1,*] Korhan ARSLAN[1] Bilal AKYÜZ[1]

[1]*Erciyes University, Faculty of Veterinary Medicine, Department of Genetics, 38280, Kayseri, Türkiye*

Received: 25.01.2023 Accepted: 17.05.2023

ABSTRACT The *CACNA2D1* gene encodes the *CACNA2D1* protein and, this protein is involved in the excitation-contraction mechanism of the muscle cells during milk withdrawal, helps the nipples to open and close. Because of this role in physiological mechanism and its relationship with quantitative trait locus (QTL) regions, *CACNA2D1* gene is known to be associated with mastitis resistance. In this study, it was aimed to investigate the relationship between different three SNP (C367400T, A496561G and G519663A) on the *CACNA2D1* gene, and subclinical mastitis in Holstein breed cattle reared in Develi district of Kayseri province. SNPs were genotyped from DNA samples by PCR-RFLP method. In the study, California mastitis test (CMT) data, and distributions of genotypes of the three SNPs on the *CACNA2D1* gene were calculated. In the study, genotype distributions were determined in terms of C367400T, A496561G and G519663A SNPs found on the *CACNA2D1* gene according to CMT status. The difference between the C367400T, A496561G and G519663A SNPs was not significant ($p>0.05$). In the study group examined the Chi-square ($\chi 2$) analysis conducted, it was observed that the Holstein cattle were in the Hardy-Weinberg equilibrium (HWE) in terms of C367400T and A496561G SNPs, deviation from HWE for the G519663A SNP ($p<0.05$). As a result, it was thought that the *CACNA2D1* gene and these SNPs should be evaluated with more samples and different mastitis indicator data in studies on mastitis resistance.

Keywords: *Bovine mastitis, CACNA2D1, Cattle, Genetic marker.*

ÖZ

Holştayn Irkı Sığırlarda *CACNA2D1* Geni ve Subklinik Mastitis Arasındaki İlişki

CACNA2D1 geni, *CACNA2D1* proteinini kodlar ve sütün indirilmesi sırasında kas hücrelerinin uyarılma-kasılma mekanizmasında görev alarak meme uçlarının açılıp kapanmasına yardımcı olur. Fizyolojik olarak üstelendiği bu görev ve kantitatif özellik lokusu (QTL) bölgelerine yakınlığı nedeni ile *CACNA2D1* geninin mastitis direnci ile ilişkili olduğu bilinmektedir. Bu çalışmada Kayseri ili Develi ilçesinde yetiştirilen sağmal Holştayn sığırlarda *CACNA2D1* geninde bulunan üç farklı SNP bölgesi ile subklinik mastitis durumları arasındaki ilişkinin araştırılması amaçlandı. Çalışma grubunu hepsi üçüncü laktasyonda 151 baş sağmal Holştayn sığır ırkı oluşturdu. Çalışmada California mastitis test (CMT) durumuna göre *CACNA2D1* geni üzerinde bulanan üç SNP (C367400T, A496561G ve G519663A) yönünden genotip dağılımları belirlendi. Yapılan istatistiki analizler sonucunda C367400T, A496561G ve G519663A SNP genotipleri arasında fark bulunmadı ($p>0.05$). Yapılan Chi-Kare analizinde incelenen çalışma grubunda C367400T ve A496561G kodlu SNP'ler yönünden Hardy-Weinberg dengesinde (HWE) oldukları, G519663A kodlu SNP yönünden ise HWE'den saptıkları ($P<0.05$) gözlemlendi. Sonuç olarak mastitis direnci ile ilgili çalışmalarda *CACNA2D1* geni ve bu SNP'lerin daha fazla örneklemle ve farklı mastitis göstergesi verilerle değerlendirilmesi gerektiği düşünüldü.

Anahtar Kelimeler: *CACNA2D1, Genetik belirteç, Sığır, Sığır mastitisi.*

INTRODUCTION

The survival of livestock enterprises depends on the continuity of production and profitability. Production and profitability in a dairy farm are ensured by having animals with high milk and reproductive efficiency. In dairy cattle breeding, the longer the productive period of the existing animal stock, the higher the enterprises will have a chance to benefit from dairy cattle (Mundan and Karabulut 2008). In recent years, innovations in the field of molecular genetics have been used in the improvement of yield characteristics such as milk and fertility, disease resistance, etc. and the data obtained from these areas are reflected in the cattle breeding. These methods have contributed to both the identification of the genetic

✉ *Corresponding author: fadimeozdemir@erciyes.edu.tr

structure of the animal existence and the selection of high-yielding animals in modern breeding practices (Narayana et al. 2022).

Mastitis is a disease characterized by inflammation in the mammary tissue, causing major economic losses in dairy cattle enterprises and affecting all farm animals raised for milk yield worldwide (Bronzo et al. 2020). The financial loss in severe mastitis cases in dairy cattle farms in Türkiye is equivalent to 710 L of milk per infected animal, and this rate corresponds to 22.6% of lactation milk yield (Sarıözkan 2019). The financial loss due to mild/moderate mastitis in dairy cattle farms is reported to be equivalent to 310 L of milk per infected animal and this rate corresponds to 9.9% of lactation milk yield (Sarıözkan 2019). Subclinical mastitis is a form of mastitis in which there is no visible change in the breast tissue and milk (Alaçam 1997; Krishnamoorthy et al. 2021). For this reason, subclinical mastitis, which is difficult to diagnose, persists in the infected animal for a long time and causes the spread of the disease in the herd (Baştan 2019). On the other hand, the cost of treatment of sick animals, the decrease in milk yield of sick animals, the destruction of milk obtained from treated animals and the removal of high-yielding animals from the herd cause significant economic losses in dairy cattle enterprises (Sabuncuoğlu and Çoban 2006; Sarıözkan 2019).

The researchers on the genetic basis of mastitis resistance in dairy cattle reported an association between some genes and polymorphisms with mastitis resistance which can use in breeding studies on mastitis resistance (Youngerman et al. 2004; Asaf et al. 2014a; Asaf et al. 2014b; Tolone et al. 2016; Jacob et al. 2020; Kirsanova et al. 2020). However, these genes and single nucleotide polymorphisms (SNPs) need to be validated in different breeding herds. One of these genes is the *CACNA2D1* gene, which encodes the calcium channel voltage-dependent alpha-2/delta subunit 1 protein, which is involved in the excitation-contraction mechanism in muscle and glial cells and neurons (Yuan et al. 2011a; Yuan et al. 2011b; Magotra et al. 2017; Magotra et al. 2019). *CACNA2D1* protein, which is involved in the excitation-contraction mechanism of the muscle cells during milk withdrawal, helps the nipples to open and close (Gabashvili et al. 2007). The *CACNA2D1* gene mapped in cattle was reported to be associated with 7 different quantitative trait locus (QTL) regions, including the somatic cell score (SCS), an indicator of mastitis (Buitkamp et al. 2003; Rupp and Boichard 2003; Zhang et al. 1998). Moreover, a QTL region was also identified near this gene, associated with somatic cell number (SCC) (Longeri et al. 2006). Also, Zhang et al. (2021) suggested that the *CACNA2D1* gene may be a candidate gene for mastitis in cattle.

This study aimed to investigate the relationship between subclinical mastitis and three SNP (C367400T, A496561G and G519663A) found on the *CACNA2D1* gene, which is reported as a candidate gene for mastitis in Holstein cattle.

MATERIAL AND METHODS

Animal Material

Animal materials used in the study were approved by the Animal Experiments Local Ethics Committee (11.12.2013 date and 13/157 number). The animal material consisted of 151 Holstein cattle breed with an average age of 5.9 years which were raised in a dairy cattle farm in Develi district of Kayseri province. Blood and milk samples were collected from animals, all in their third lactation. Subclinical mastitis status of animals was determined by from milk samples and total DNA was isolated from blood samples by phenol: chloroform: isoamyl extraction method.

Subclinical Mastitis Test

Subclinical mastitis status of the cattle was determined by the California Mastitis Test (CMT) by the farm's responsible veterinarian from the milk samples. Mastitis status of animals was noted as suggested by Daldaban et al. (2021) and Fthenakis (1995).

DNA Isolation and PCR-RFLP

Genomic DNA was extracted from whole blood using the phenol: chloroform: isoamyl extraction method (Puttaraju et al. 2020). DNA concentration was quantified by using a Nano Drop (Synergy H1Hybrid MultiMode Microplate Reader, BioTek, USA) and stored at −20 °C until use. Primer pairs and restriction enzymes were obtained from Yuan et al. (2011b) (Table 1). Polymerase chain reaction (PCR) analysis was performed for C367400T, A496561G and G519663A SNPs with DNA samples.

PCR was performed to determine the genotypes of cattle in C367400T, A496561G and G519663A SNPs. PCR was carried out using 60 ng of genomic DNA in a total reaction volume of 20 µL containing 10× PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 10 pM of forward and reverse primers (Yuan et al. 2011b), and 0.5 U of Taq DNA polymerase (Thermo Fisher Scientific, WA, USA). Amplification reactions were performed 5 min at 94^0C, followed by 35 cycles of 30 s at 94^0C, 30 s of Table 1 annealing temperature and 30 s at 72^0C with an extension step of 8 min at 72^0C. The amplicons obtained at the end of PCR were screened by a 1.5% agarose gel (Prona, Biomax, Ankara, Türkiye) and visualized (Kodak Gel Logic Imaging System, New York, ABD, USA).

These amplicons obtained with PCR were analysed by Restriction Fragment Lenght Polymorphism (RFLP). Restriction endonuclease enzymes (Thermo Fisher Scientific, WA, USA) were applied to identify genotype. All enzymes were incubation PCR device at a temperature of suitable temperature for 10 h. Enzymes and incubation times used for the PCR-RFLP are indicated in Table 1. The RFLP digestion products were checked with 2% agarose gel electrophoresis (120 V for 40 min).

Table1: Primer and amplicon sizes of *CACNA2D1* gene SNP regions.

SNP	Primer Sequence	AT	C	A	RE	RET
C367400T	F TGAAAGGGTTGTCCTGCCATC R GTGCTTGTTGTTCCCATTGCC	61	35	322	*Rsa*I	37
A496561G	F CCATATCTGTGTCCTGTGTCT R GGTAAAGTAAAGTGGAAGTCG	59	35	386	*Taq*I	65
G519663A	F TCTAACGCCTTATTGACATC R CTTACTGTTTCCTTTGGTTC	54.6	35	269	*Hpa*II	37

F: Forward, R: Reverse, AT: Anneling temperature, C: Cycle, A: Amplicon RE: Restriction endonulease enzyme, RET: Restriction endonulease enzyme digestion temperature.

Statistical Analysis

Proportional distributions of *CACNA2D1* gene C367400T, A496561G and G519663A coded SNP loci were calculated according to the subclinical mastitis status of the cows used in the study [no mastitis, M (-); and existence of mastitis, M (+)]. Statistical significance-control was

performed with Pearson Chi-square test. IBM SPSS program was used for statistical analysis.

RESULT

The PCR-RFLP analysis performed for the *CACNA2D1* gene, PCR bands of 322 bp, 386 bp, and 269 bp were observed for C367400T, A496561G and G519663A respectively (Figure 1).

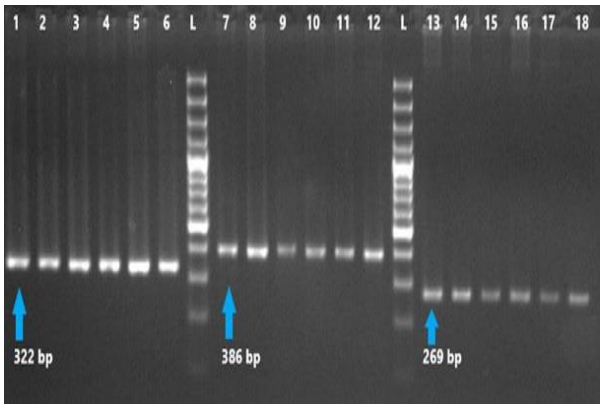


Figure 1: PCR amplicon sizes of C367400T, A496561G and G519663A SNPs. (1-6: 322 bp (C367400T SNP), 7-12: 386 bp (A496561G SNP) and 13-18: 269 bp (G519663A SNP); L: 100 bp ladder; bp: base pair).

Three genotypes for the C367400T SNP with *Rsa*I restriction enzyme digestion were detected: 322 bp for the TT genotype; 322, 236 and 86 bp for the CT genotype; 236 and 86 bp for the CC genotype (Figure 2a). After digestion with the *TaqI* restriction enzyme digestion, the AA (386 bp), AG (386, 229 and 157 bp) and GG (229 and 157 bp) genotypes were observed for the A496561G SNP (Figure 2-b). The polymorphism in the G519663A SNP was identified by digestion of the PCR product with *HpaII* restriction enzyme. After digestion with restriction enzyme digestion, the AA (269 bp), AG (269, 175 and 64 bp) and GG (175 and 64 bp) genotypes were observed for the G519663A SNP (Figure 2-c).

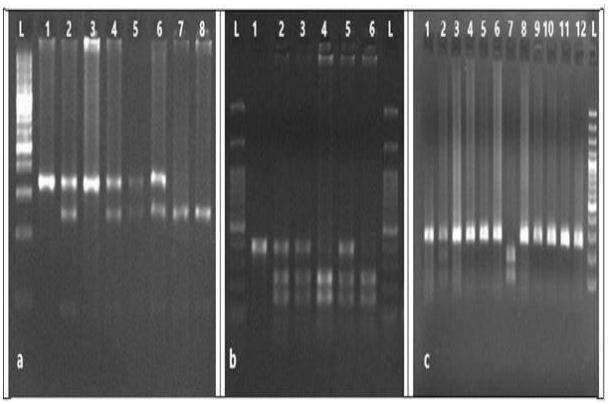


Figure 2: PCR-RFLP results of C367400T, A496561G and G519663A SNPs. a: PCR-RFLP results of C367400T (lanes 1,3: genotype TT; lanes 2, 4, 5 and 6: genotype CT; lanes 7 and 8: genotype CC); b: PCR-RFLP results of A496561G *(*lanes 1: genotype AA; lanes 2, 3, 5: genotype AG; lanes 4 and 6: genotype GG*)*; c: PCR-RFLP results of G519663A (lanes 1,3-6 and 8-12: genotype AA; lanes 2: genotype AG; lanes 7: genotype GG)*;* Lanes L: 100 bp ladder.

The difference between the subclinical mastitis status of the examined Holstein breed milk cows and the proportional distributions of the genotypes for C367400T, A496561G and G519663A coded SNPs in the *CACNA2D1* gene was not found to be significant (p>0.05). However, 49.4% (37/75) of subclinical mastitis positive (M+) cattle were found in CT genotype in terms of C367400T polymorphism; 54.6% (71/130) were in GG genotype in terms of G519663A polymorphism, and 53.4% (71/133) were in the GG genotype in terms of A496561G polymorphism (Table 2).

The most common genotypes were CT (0.497) for the C367400T SNP region, GG (0.861) for the A496561G SNP region, and AA (0.881) for the G519663A SNP region in the examined Holstein dairy cows. The examined Holstein cattle were in HWE in terms of C367400T and A496561G coded SNP regions, and they deviated from HWE (p<0.05) in terms of G519663A coded SNP region (Table 3).

Tablo 2: Proportional distribution of C367400T, A496561G and G519663A SNPs.

SNP	Genotype	M (-)	M (+)	n	χ2	p value
C367400T	CC	10 (45.4%)	12 (54.6 %)	22	1.756 *NS*	0.416
	CT	38 (50.6%)	37 (49.4%)	75		
	TT	21 (38.9%)	33 (61.1%)	54		
G519663A	AA	0 (0 %)	1 (100.0 %)	1	0.996 *NS*	0.608
	AG	10 (50.0 %)	10 (50.0%)	20		
	GG	59 (45.4%)	71 (54.6)	130		
A496561G	AA	62 (46.6 %)	71 (53.4%)	133	0.426 *NS*	0.808
	AG	6 (40.0 %)	9 (60.0%)	15		
	GG	1 (30.0 %)	2 (60.0%)	3		

M (-): Mastitis positive, M (+): Mastitis negative, NS: Not Not significant, χ2: Chi-square.

Table 3: Allele and genotype frequency of C367400T, A496561G and G519663A SNPs.

SNP	Genotype	n	Genotype Frequency	Allele	Allele Frequency	X^2
C367400T	CC	22	0.146	C	0.39	0.243^{NS}
	CT	75	0.497	T	0.61	
	TT	54	0.357			
G519663A	AA	133	0.881	A	0.93	**8.151***
	AG	15	0.099	G	0.07	
	GG	3	0.02			
A496561G	AA	1	0.007	A	0.07	0.057^{NS}
	AG	20	0.132	G	0.93	
	GG	130	0.861			

X^2: Chi-Square value, *NS*: Not significant, *:p<0.05.

DISCUSSION AND CONCLUSION

Mastitis is one of the most common health problems in dairy farms. Delay in diagnosing the disease along with the difficulties in diagnosing the animals with subclinical mastitis, place mastitis in the disease group with high treatment costs for dairy cattle farms. Therefore, it is important for dairy cattle enterprises to prevent economic losses due to cause by mastitis. Identifying and keeping mastitis-resistant animals in the herd has important advantages in preventing losses caused by subclinical mastitis in farms. Thanks to the development of molecular genetic methods, it has become easier to search for genes or SNPs associated with mastitis resistance. A number of genes, including the *CACNA2D1* gene, was reported in studies conducted for this purpose (Yuan et al. 2011a; Yuan et al. 2011b).

This study investigated the relationship between C367400T, A496561G and G519663A SNPs in the *CACNA2D1* gene and subclinical mastitis in Holstein cattle. The Chi-square (χ2) analysis performed at the end of the study showed that Holstein cattle were HWE regarding the SNPs coded C367400T and A496561G but deviated from HWE regarding the SNP coded G519663A. The results obtained in the study showed that genetic variation continued regarding the studied SNPs. The genotypes with high rates in terms of mastitis resistance of the studied SNPs were TT genotype in C367400T, AA genotype in A496561G and GG genotypes in G519663A, respectively.

Yuan et al. (2011b) found that the frequency of T allele frequence (0.55) was high in cattle in which *CACNA2D1* gene C367400T SNP was analyze. Bagheri et al. (2013) reported that T allele frequency (0.94) was high in C367400T SNP in Holstein breed in Iran. Similarly in this study, T allele frequency (0.61) was found to be high in Holstein cattle examined. The study findings are consistent with Yuan et al. (2011b) and Bagheri et al. (2013).

Yuan et al. (2011b) in A496561G coded SNP in the *CACNA2D1* was reported to be the AG least common genotype (0.16 in Holstein breed; 0.22 in Sanhe breed; 0.22 in Simmental breed). Bagheri et al. (2013) reported that AA was the least common genotype (0.31) in the A496561G SNP in their study on Holstein breed in Iran. This study found the AA genotype (0.007) as the least common genotype. The study findings are consistent with Bagheri et al. (2013). However, the literature review revealed that the number of studies was quite limited that examined the A496561G coded SNP in the *CACNA2D1* gene for Holstein and other cattle breeds. Therefore, more studies are needed to reveal the general status of A496561G SNP in the Holstein cattle breed and to obtain more reliable results.

Yuan et al. (2011b) investigated the genotype and allele frequencies of G519663A coded SNP and they reported that AA was the most common genotype. Magotra et al. (2018) was reported by that the GG genotype for G519663A coded SNP was the most common genotype in Karan Fries cross-breed cattle, a native Sahiwal (*Bos taurus indicus*) and a Holstein×Tharparkar (*Bos taurus indicus*) cross-breed in India. In this study, the most observed genotype in Holstein (*Bos taurus typicus*) cattle breed was AA (0.881). The study findings are consistent with Yuan et al. (2011b). On the other hand, the reason the results of this study differ from Magotra et al. (2018) is thought to be due to the origin of the cattle breeds.

Various studies were conducted to investigate the relationship between SNPs on the *CACNA2D1* gene and mastitis in different cattle breeds. In one of these studies, Yuan et al. (2011a) investigated the relationship between *A526745G* coded SNP in the *CACNA2D1* gene and mastitis and reported a significant relationship between this SNP and SCS. Another study reported a statistically significant correlation between another SNP coded *C367284A* in the *CACNA2D1* gene and SCS (Deng et al. 2011). A study by Magotra et al. (2019) reported a relationship between mastitis and another SNP coded *G38819398A* in the *CACNA2D1* gene and argued that cattle with GG genotype were more resistant to mastitis.

As a results, there are various studies investigating the relationships between mastitis and different SNPs on the *CACNA2D1* gene in different cattle breeds (Deng et al. 2011; Yuan et al. 2011a; Yuan et al. 2011b; Magotra et al. 2019). In this study, the relationship between subclinical mastitis and C367400T, A496561G and G519663A SNPs in the *CACNA2D1* gene were investigated for Holstein cattle raised in Türkiye. However, no relationship was found between the mentioned SNPs and subclinical mastitis in the examined samples. The results obtained from this study may have resulted from the number of samples examined. Considering the QTL region where the *CACNA2D1* gene is located and the physiological processes in which this gene is involved, it is suggested to conduct comprehensive studies on mastitis resistance in which this gene and the SNPs in this gene are evaluated with larger samples.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENT

We would like to thank Specialist Veterinarian Murat AKKAYA for taking blood samples of the animals used in the study and applying the California Mastitis Test (CMT) test.

In this study, blood samples taken in the TYL-2017-7703 coded project conducted by Bilal AKYÜZ were used.

AUTHOR CONTRIBUTIONS

Idea / Concept: FD
Supervision / Consultancy: KA, BA
Data Collection and / or Processing: FD
Analysis and / or Interpretation: FD, KA, BA
Writing the Article: FD
Critical Review: BA

REFERENCES

Alaçam E (1997). Meme Hastalıkları. Alaçam E ve Şahal M (Ed). Sığır Hastalıkları (s. 389- 425). Medisan Yayınevi, Ankara.

Asaf VNM, Bhushan B, Panigrahi M et al. (2014a). Association study of genetic variants at single nucleotide polymorphism rs109231409 of mannose-binding lectins 1 gene with mastitis susceptibility in Vrindavani crossbred cattle. *Vet World*, 7 (10), 807-810.

Asaf VNM, Kumar A, Rahim A et al. (2014b). An overview on single nucleotide polymorphism studies in mastitis research. *Vet World,* 7 (10), 416–421.

Bagheri M, Miraie-Ashtiani R, Moradi-Shahrbabak M et al. (2013). Selective genotyping and logistic regression analyses to identify favorable SNP-genotypes for clinical mastitis and production traits in Holstein dairy cattle. *Livest Sci*, 151 (2-3), 140-151.

Baştan A (2019). İneklerde Meme Sağlığı ve Sorunları: Baştan A (Ed). Sütün Önemli Fiziksel ve Kimyasal Özellikleri (s. 34). Üçüncü Baskı. Hacattepe TAŞ, Ankara.

Bronzo V, Lopreiato V, Riva F et al. (2020). The role of innate immune response and microbiome in resilience of dairy cattle to disease: the mastitis model. *Animals,* 10 (8), 1397.

Buitkamp J, Ewald D, Masabanda J, Bishop MD, Fries R (2003). FISH and RH mapping of the bovine alpha (2)/delta calcium channel subunit gene (*CACNA2D1*). *Anim Genet*, 34 (4), 309–310.

Daldaban F, Arslan K, Akçay A, Sohel MMH, Akyüz B (2021). Association of BRCA1 (G22231T, T25025A, C28300A) polymorphisms with subclinical mastitis and milk yields in Holstein Cattle. *Harran Üniv Vet Fak Derg*, 10 (1), 12–19.

Deng G, Yuan Z, Gao X et al. (2011). Identification mutation of the *CACNA2D1* gene and its effect on somatic cell score in cattle. *J Appl Anim Res*, 39 (1), 15–18.

Fthenakis GC (1995). California Mastitis Test and Whiteside Test in diagnosis of subclinical mastitis of dairy ewes. *Small Rumin Res*, 16 (3), 271–276.

Gabashvili IS, Sokolowski BHA, Morton CC, Giersch A (2007). Ion channel gene expression in the inner ear. *J Assoc Res Otolaryngol*, 8 (3), 305-328.

Jacob KK, Radhika G, Aravindakshan TV (2020). An in-silico evaluation of non-synonymous single nucleotide polymorphisms of mastitis resistance genes in cattle. *Anim Biotechnol,* 31 (1), 25-31.

Kirsanova E, Boysen P, Johansen GM et al., (2020). Expression analysis of candidate genes for chronic subclinical mastitis in Norwegian Red cattle. *J Dairy Sci*, 103 (10), 9142-9149.

Krishnamoorthy P, Goudar AL, Suresh KP, Roy P (2021). Global and countrywide prevalence of subclinical and clinical mastitis in dairy cattle and buffaloes by systematic review and meta-analysis. *Res Vet Sci*, 136, 561-586.

Longeri M, Polli M, Strillacci MG, Samore AB, Zanotti M (2006). Quantitative trait loci affecting the somatic cell score on chromosomes 4 and 26 in Italian Holstein cattle. *J Dairy Sci*, 89 (8), 3175–3177.

Magotra A, Gupta ID, Verma A et al. (2017). Characterization and validation of point mutation in exon 19 of *CACNA2D1* gene in Karan Fries (Bos taurus x Bos indicus) cattle. *Indian J Anim Res*, 51 (2), 227–230.

Magotra A, Gupta ID, Verma A et al. (2018). Characterization and validation of point mutation in Exon 19 of Calcium channel, voltage-dependent, Alpha-2/Delta subunit 1 (*CACNA2D1*) gene and its relationship with mastitis traits in Sahiwal. *Indian J Anim Res*, 52 (1), 61-64.

Magotra A, Gupta ID, Verma A et al. (2019). Candidate SNP of *CACNA2D1* gene associated with clinical mastitis and production traits in Sahiwal (*Bos taurus indicus*) and Karan Fries (*Bos taurustaurus × Bos taurus indicus*). *Anim Biotechnol,* 30 (1), 75–81.

Mundan D, Karabulut O (2008). Sütçü sığırlarda damızlıkta kullanma süresi ve uzun ömürlülüğün ekonomik açıdan önemi. *YYÜ Vet Fak Derg*, 19 (1), 65–68.

Narayana SG, de Jong E, Schenkel FS et al. (2022). Underlying genetic architecture of resistance to mastitis in dairy cattle: A systematic review and gene prioritization analysis of genome-wide association studies. *J Dairy Sci,* 100 (1), 323-351.

Puttaraju HP, Prakash BM, Keshava Murthy BC (2020). Molecular Biology and Biochemistry: A Lab Manual. Puttaraju HP, Prakash BM, Keshava Murthy BC (Ed). Nucleic Acid Extraction (pp. 51-52). New India Pblishing Agency, New Delhi.

Rupp R, Boichard D (2003). Genetics of resistance to mastitis in dairy cattle. *Vet Res*, 34, 671–688.

Sabuncuoğlu N, Çoban Ö (2006). Mastitis ekonomisi. *Atatürk Üniversitesi Vet Bil Derg,* 1 (1-2), 1-5.

Sarıözkan S (2019). Türkiye'de süt sığırcılığı işletmelerinde mastitis nedeniyle oluşan finansal kayıpların tahmin edilmesi. *Harran Üniv Vet Fak Derg*, 8 (2),147–151.

Tolone M, Mastrangelo S, di Gerlando R et al. (2016). Association study between β-defensin gene polymorphisms and mastitis resistance in Valle del Belice dairy sheep breed. *Small Rumin Res*, 136, 18-21.

Youngerman SM, Saxton AM, Oliver SP, Pighetti GM (2004). Association of CXCR2 polymorphisms with subclinical and clinical mastitis in dairy cattle. *J Dairy Sci*, 87 (8), 2442-2448.

Yuan ZR, Li J, Liu L et al. (2011a). Single nucleotide polymorphism of *CACNA2D1* gene and its association with milk somatic cell score in cattle. *Mol Biol Rep*, 38 (8), 5179–5183.

Yuan ZR, Li J, Zhang LP et al. (2011b). Novel SNPs polymorphism of bovine *CACNA2D1* gene and their association with somatic cell score. *Afr J Biotechnol*, 10 (10), 1789-1793.

Zhang Q, Boichard D, Hoeschele I et al. (1998). Mapping quantitative trait loci for milk production and health of dairy cattle in a large, outbred pedigree. *Genetics,* 149 (4), 1959-1973.

Zhang H, Liu A, Wang Y et al. (2021). Genetic parameters, and genome-wide association studies of eight longevity traits representing either full or partial lifespan in Chinese Holsteins. *Front Genet,* 12, 634986.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Tavalı ZE, Çak B (2023).** The Current Situation of Goat Breeding in Manavgat District and the Effects of the Pandemic Process (COVID-19 Epidemic). *Van Vet J*, 34 (2), 98-108. DOI: https://doi.org/10.36483/vanvetj.1242817

ISSN: 2149-3359 **Araştırma Makalesi** e-ISSN: 2149-8644

Manavgat İlçesinde Keçi Yetiştiriciliğinin Mevcut Durumu ve Pandemi Sürecinin (COVID-19 Salgını) Etkileri

Zeki Emre TAVALI[1,*] Bahattin ÇAK[2]

[1] *Tarım ve Orman Bakanlığı, Manavgat İlçe Tarım ve Orman Müdürlüğü, 07600, Antalya, Türkiye*
[2] *Van Yüzüncü Yıl Üniversitesi, Veteriner Fakültesi, Zootekni Anabilim Dalı, 65080, Van, Türkiye*

Geliş Tarihi: 26.01.2023 Kabul Tarihi: 09.06.2023

ÖZ

Bu çalışmada, Manavgat ilçesindeki keçi yetiştiricilerinin mevcut durumları ve sorunlarının tespiti ile COVID-19 salgınının sektöre olan etkileri incelenmiştir. Manavgat ilçesindeki 50 mahallede bulunan küçükbaş hayvancılık işletmeleri bu araştırmanın materyalini oluşturmuştur. Çalışma, Tarım Orman Bakanlığı Manavgat ilçesi TÜRKVET sistemine kayıtlı işletmelerde yürütülmüştür. Araştırmanın yapıldığı işletmeler için anket formu düzenlenmiş ve işletme sahipleri ile bireysel olarak görüşmeler yapılarak veriler toplanmıştır. Çalışmadaki anket uygulaması, Haziran/2021 ve Mart/2022 tarihleri arasında yapılmıştır. Araştırma bulgularına göre; Yetiştiricilerin %91.4'ü erkek, %8.6'sı ise kadın oldukları; yetiştirici yaşının, %48.6 ile 36-50 yaş aralığında oldukları; yetiştiricilerin %74.3'ü ilkokul mezunu; %91.4'ün evli olduğu saptanmıştır. Pandemi (COVID-19 salgını) hakkında yetiştiricilerin (%68.6) iyi düzeyde bilgi sahibi olduğu tespit edilmiştir. COVID-19 salgınının hayvancılığı olumsuz etkilediği fikrine yetiştiricilerin %44.3 oranında katıldığı; COVID-19 salgını öncesi ve sonrası arasında gelirlerinin olumsuz etkilendiği sorusuna %57.1 oranında kesinlikle etkilendiği dedikleri; Salgının uzun yılların sürmesi durumunda gıda talebinin karşılanmasında hayvancılığa olan talebin artacağı görüşüne yetiştiricilerin %47.1'inin katılıyorum dedikleri ayrıca pandeminin uzun yıllar devam etmesi durumunda keçi yetiştiriciliğinin sona ereceği fikrine ise %34.3'ünün katılmıyorum yanıtını verdikleri tespit edilmiştir.

Anahtar Kelimeler: *Anket, Keçi, Pandemi.*

ABSTRACT

The Current Situation of Goat Breeding in Manavgat District and the Effects of the Pandemic Process (COVID-19 Epidemic)

In this study, the current situation and problems of goat breeders in Manavgat district and the effects of the COVID-19 outbreak on the sector were examined. Small ruminant farms in 50 neighborhoods in Manavgat district formed the material of this research. The study was carried out in the enterprises registered in the TÜRKVET system in the Manavgat district of the Ministry of Agriculture and Forestry. A questionnaire was prepared for the businesses where the research was conducted, and data were collected by making individual interviews with the business owners. The survey application in the study was carried out between June, 2021 and March, 2022. According to the research findings; 91.4% of the breeders were male and 8.6% were female; the age of the breeder was between 36-50 years with 48.6%; 74.3% of the breeders are primary school graduates; It was also determined that 91.4% were married. It has been determined that the breeders (68.6%) have a good level of knowledge about the pandemic (COVID-19 outbreak). 44.3% of the breeders agreed with the idea that the COVID-19 epidemic negatively affected livestock; To the question that their income was negatively affected between before and after the COVID-19 outbreak, they said that it was definitely affected by 57.1%; 47.1% of the breeders agree with the view that the demand for animal husbandry will increase in order to meet the food demand if the epidemic lasts for many years. In addition, it was determined that 34.3% of them gave the answer that they do not agree with the idea that the goat manager will end if the pandemic continues for many years.

Keywords: *Goat, Pandemic, Survey.*

✉ *Corresponding author: emretavali@gmail.com

GİRİŞ

Keçi; diğer canlıların yeterince yararlanamadığı niteliği daha düşük olan mera bölgeleri, çalılık ile fundalık alanlardan mümkün olduğunca en iyi şekilde faydalanarak et süt ve diğer hayvansal ürünlere çevrilmesini sağlayan bakım ve besleme maliyeti oldukça düşük bir hayvandır (Kaymakçı ve Aşkın 1997). Keçi sütünün bileşiminin sahip olduğu kendine özgü özelliğinden dolayı, keçi sütü dünyanın birçok ülkesinde diğer sütünden faydalanılan hayvanlara nazaran ciddi öneme sahiptir (Şentürklü ve Arslanbaş 2010).

Keçi yetiştiriciliğinin ülkemizde yeterli seviyeye gelerek ilerisi için sürdürülebilir hale getirilmesi ve ticari açıdan katkı sağlanabilir duruma ulaştırılması amacıyla yetiştirici birliklerinin daha işlevsel çalışmaları gerekmektedir. Ülkemizde süt keçilerinde uygulanan entansif yetiştirme yönteminin kıl keçilerinde de uygulanması gerekmektedir. Bu konu ile ilgili ıslah çalışmaları yanı sıra üretim teknikleri getirilmeli ilgili kurum ve kuruluşlar ile teşvik ve destekleri sağlanmalıdır (Günlü ve Alaşahan 2010).

Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO) istatistiklerine göre Dünya 2011 yılı keçi sayısı 932 166 441 baş iken, 2020 yılı keçi sayısı 1 128 106 236 baş olmuştur. FAO (2022), istatistiklerine göre Türkiye'nin 2011 yılı keçi sayısı 6 293 233 baş iken, 2020 yılı keçi sayısı 11 985 845 baş olmuştur. Bu verilere göre keçi sayısında artış olduğu görülmektedir. Dünya Sağlık Örgütünün 12 Mart 2020 tarihinde pandemi diye ilan ettiği COVID-19 salgını; tüm insanların fiziksel durumu, ruhi ve sosyal hayatlarını olumsuz olarak tehdit etmeye devam etmektedir (Budak ve Korkmaz 2020). COVID-19 salgınının, insan ve hayvan refahı ile sağlığı üzerine hâlâ büyük ölçüde bilinmeyen muhtemel etkilerinin yanında tarımsal ve hayvansal üretim üzerindeki etkileri de araştırılmalıdır. Genel olarak bu sektöre ait ürünlerin arzında ve talebinde bir daralma, olabileceği ve tüketiciye ulaştırılmasında ise bazı sorunlarla karşılaşılması olasıdır.

Bu çalışmada, Antalya İli Damızlık Koyun ve Keçi Yetiştiricileri Birliği'ne kayıtlı Manavgat ilçesindeki keçi yetiştiriciliği yapan işletmelerin mevcut durumları ile sorunlarının belirlenmesi ve pandemi sürecinin (COVID-19 salgını) keçi yetiştiriciliği yapılan hayvancılık işletmelerindeki yansımaları ele alınmıştır.

MATERYAL VE METOT

Antalya ili Manavgat ilçesine bağlı 50 mahallede bulunan 70 küçükbaş (koyun-keçi) hayvancılık işletmeleri bu araştırmanın materyalini oluşturmuştur. Çalışma, Tarım Orman Bakanlığı Manavgat ilçesi TÜRKVET sistemine kayıtlı olan işletmelerde, Haziran/2021 – Mart/2022 tarihleri arasında yürütülmüştür.

Bu çalışma, Van Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulunun, 29.04.2021 tarih ve 2021/04-32 sayılı kararı ile araştırma onayı gerektirmeyen belgesi almıştır.

Manavgat ilçesi keçi yetiştiriciliğinin genel özellikleri ve pandeminin (COVID-19) keçi yetiştiriciliği üzerine olan etkilerini belirlemek için işletmelere anket uygulanmıştır. İşletme sahipleri ile bireysel olarak (yüz yüze) görüşmeler yapılarak çalışma verileri toplanmıştır. Yetiştiricilere anket uygulanmadan önce deneme anketi yapılmıştır.

Araştırma anketi: Yetiştirici bilgisi; İşletme/hayvan materyali bilgisi; Keçi işletmelerinde sağım, Hayvan besleme, Kasaplık satışlar; Mera-Yayla dönemleri; İşletmelerde uygulanan sağlık yöntemi, Keçi yetiştiriciliğinde yaşanan sorunlar ve Pandeminin (COVID-19) keçi yetiştiriciliği üzerindeki etkilerini içeren 7 bölüm ve toplam 67 sorudan oluşmuştur.

İstatistiksel Analiz

Araştırmada ele alınan özellikler için tanımlayıcı istatistikler, sayı ve yüzde olarak verilmiştir. Tüm analizler SPSS (ver: 21) istatistik paket programı ile yapılmıştır.

BULGULAR

Yetiştiricilik Bilgileri

Araştırma neticesinde yetiştiricilerin %91.4'nün erkek olduğu; %48.6'nın, 36-50 yaşlar arasında olduğu; %74.3'nün ilkokul mezunu oldukları; %91.4'nün evli bireylerden oluştuğu; %58.6'nin 4-6 kişilik aile birey sayısına sahip oldukları; %78.6'nın geçimlerini hayvancılık ile sağladığı; %55.7'nın ailenin geçimi için hayvancılığın tek başına yetersiz olduğunu; %54.3'nin bulundukları çevre ve iklim koşullarının elverişli olmasının tercih sebebi olduğu tespit edilmiştir.

İşletme/Hayvan Materyali Bilgileri

İncelenen işletmelerde yetiştiricilerin; %74.3'nün barınak tipi olarak yazın açık kışın kapalı ağılı tercih ettikleri; %100'nün aileden çoban temini yaptıkları; %57.1'nin işletmelerinde 50-200 baş keçi olduğu; %70.0'nın damızlıkta kullanma yaşı olarak 18 aylık yaşta olduğu; %44.3'nün oğlaklara su vermeye 5. Haftada başladıkları; %100.0'nün serbest aşım yöntemini tercih ettikleri; %61.4'nün teke katım süresi olarak 45.günü seçtikleri; %60.0'nın doğum zamanının nisan-mayıs aylarında olduğu; %72.9'nun sütten kesim zamanı 3. Ay olduğu; %100.0'nün emiştirme süresi 60 gün süre olduğu; %70.0'nın emiştirme şekli olarak sabah-akşam olduğu; %57.1'nin kaba ve kesif yeme başlama zamanının 5. Hafta olduğu; işletmelerdeki mevcut keçi ırkının %100.0'nün kıl keçisi olduğu; yetiştiricilerin %71.4'nün en çok kıl keçisine sahip olmak istediği ve yetiştiricilerin %34.3'nün "kayıt tutmak olmazsa olmaz" sorusuna katılıyorum cevabını verdikleri tespit edilmiştir.

Keçi İşletmelerinde Sağım, Hayvan Besleme, Kasaplık Satışlar

Yetiştiricilerin; %74.3'nün mayıs ayında sağıma başladığı; %58.6'nın sağım süresinin 4 ay ve üzeri olduğu; %98.6'nın sağımı kadınların yaptığı; %98.6'sının keçileri beslemenin mera ve elden besleme şeklinde yapıldığı; yetiştiricilerin %100.0'nün keçilere kesif yem verdikleri; %70.0'nin kesif yemi dışardan aldıklarını; yetiştiricilerin %100.0'nün kaba yem olarak samanı tercih ettikleri; %50.0'nin teke katım döneminde ek yemleme yapmak verimi artırmaktadır sorusuna katılıyorum cevabını verdikleri; %78.6'ı damızlık seçimini oldukça önemli buldukları; %57.1'i gebelik döneminde ekstra yemleme yapılmalıdır sorusuna kararsızım cevabı verdikleri; %52.9'nın ağız sütü alımının sağlanmasını önemli buldukları; %51.4'ü yeni doğanlarda göbek bakımının yapılmasını buldukları; %100.0 kasaplık satış dönemi olarak 1 yaşında cevabını verdikleri; %91.4'ü kırkım yaptıklarını; %88.6'ı keçilerde kırkım zamanı olarak temmuz ayı olduğu; yetiştiricilerin %100.0'ü en önemli gelir kaynağı olarak keçi sütü + et cevabını verdikleri belirlenmiştir.

Keçi sütünü nasıl değerlendiriyorsunuz? sorusuna katılımcıların birden fazla şıkları tercih ederek cevap verebilecekleri ifade edilmiş ve alınan sonuçlar değerlendirildiğinde; %100.0 oranında peynir yaptıkları, %92.9 oranında yavru beslemesinde kullandıkları ve %81.4'nün ise aile ihtiyacında değerlendirildiği

saptanmıştır. Hangi yem hammaddelerini kullanıyorsunuz? sorusuna yine birden fazla şıkları tercih ederek cevap verebilecekleri ifade edilmiş ve alınan sonuçlar değerlendirildiğinde; %100.0'nın arpa cevabını verdikleri, %65.7'nin kepek cevabını verdiği ve %54.3'nün ise küspe cevabını tercih ettikleri tespit edilmiştir.

Mera-Yayla Dönemleri

Yetiştiricilerin %72.9'nun mera imkânlarının olduğu; %61.4'ü mera mülkiyeti kime ait sorusuna orman ve tarla kenarlarında cevabını verdikleri; %48.6'nın 5. Ayda mera-yaylaya çıktıkları; %67.1'i 10. Ayda mera-yayla döndükleri; %68.6'nın yaylaya ulaşım şekli olarak araç + yaya cevabını verdikleri; %70.6'nin keçileri kendilerinin otlattığı tespit edilmiştir.

İşletmelerde Uygulanan Sağlık Yöntemi

Yetiştiricilerin %95.7'i Sürü sağlığının korunması açısından belirli periyotlarda Veteriner Hekimlerden bilgi aldıkları; %95.7'i koruyucu hekimlik yönünden aşılamayı oldukça önemli buldukları; %54.3'ü Yetiştiriciliğini yaptığınız hayvanların refah durumunu nasıl değerlendiriyorsunuz? Sorusuna iyi cevabını verdikleri; Yetiştiricilerin %100.0'nün koyun-keçi vebası, %87.1'nin Brusella aşısı ve %70.0'nin ise koyun-keçi çiçek aşısı yaptırdıkları tespit edilmiştir.

Keçi Yetiştiriciliğinde Yaşanan Sorunlar

Yetiştiricilerin %51.4'ü Damızlık hayvan teminini önemli buldukları; %58.6'ı Ağıl ve barınak sorununu önemli buldukları; %82.9'u Kesif yem sorununu kesinlikle önemli buldukları; %97.1'i İşçi/çoban ve işçilik sorununu önemli buldukları; %72.9'u Doğum sorunlarını önemli buldukları; %97.1'nin Oğlak bakımı ve büyütme sorusuna önemli cevabı verdiği; %84.3'nün Örgütlenme sorusuna, kesinlikle katılıyorum cevabını verdiği; %71.4'nün Pazarlama sorununa kesinlikle katılıyorum cevabını verdiği ve yetiştiricilerin %68.6'nın Sağlık-İlaç ve Veteriner hekim giderlerinin pahalı oluşu sorusuna ise kesinlikle katılıyorum cevabını verdikleri saptanmıştır.

Tablo 1: Bazı demografik özellikler için tanımlayıcı istatistikler (sayı ve yüzde).

Table 1: Descriptive statistics for some demographics traits (number and percentage).

Özellikler		N	%
Cinsiyet	Kadın	6	8.6
	Erkek	64	91.4
Yetiştiricinin yaşı	20-35	8	11.4
	36-50	34	48.6
	51 ve üzeri	28	40
Yetiştiricinin eğitim düzeyi	Yok	7	10
	İlkokul	52	74.3
	Ortaokul	9	12.9
	Lise	-	-
	Üniversite	2	2.9
Yetiştiricinin medeni durumu	Bekar	6	8.6
	Evli	64	91.4
	Boşanmış/Dul	-	-
Yetiştiricinin aile kişi sayısı (Ebeveyn + Çocuk)	1-3	25	35.7
	4-6	41	58.6
	7 ve üzeri	4	5.7
Geçim kaynağınız nedir?	Tarım	-	-
	Hayvancılık	55	78.6
	Tarım ve Hayvancılık	15	21.4
	Diğer	-	-
Sizin ve ailenizin geçimi açısından hayvancılık tek başına yeterli mi?	Kesinlikle yeterli	-	-
	Yeterli	9	12.9
	Fikrim yok	16	22.9
	Yetersiz	39	55.7
	Kesinlikle yetersiz	6	8.6
Keçi yetiştiriciliğini tercih etmenizin sebebi nedir?	Ekonomik olması	2	2.9
	Bulunduğum çevre ve iklim koşullarının elverişli olması	38	54.3
	Kültürel Sebepler	27	38.6
	Pazar payının yüksek olması	-	-
	Hepsi	3	4.3

Tablo 2: İşletme/Hayvan materyali için tanımlayıcı istatistikler (sayı ve yüzde).
Table 2: Descriptive statistics for Business/Animal material (number and percentage).

Özellikler		N	%
Kullandığınız barınak tipi nasıldır?	Kapalı ağıl	2	2.9
	Yarı açık ağıl	16	22.9
	Yazın açık kışın kapalı ağıl	52	74.3
Çoban temin durumu	Aileden	70	100
	Yurt dışından	-	-
	Köy içinden	-	-
	Başka İlden	-	-
İşletmelerdeki keçi sayısı	1-50 baş	9	12.9
	50-200 baş	40	57.1
	201-499 baş	21	30
	500 baş	-	-
	501- 1000 baş	-	-
Damızlıkta kullanma yaşı	15 ay	21	30
	18 ay	49	70
	24 ay	-	-
	Diğer	-	-
Oğlaklara su verme haftası	2. Hafta		
	3. Hafta	1	1.4
	4. Hafta	25	35.7
	5. Hafta	31	44.3
	5. Haftadan sonra	13	18.6
Teke katım yöntemi	Elde aşım	-	-
	Serbest aşım	70	100
	Sınıf usulü aşım	-	-
Teke katım süresi	30 Gün	-	-
	45 Gün	43	61.4
	60 Gün	27	38.6
	Yıl boyu	-	-
Doğum zamanı	Aralık-Ocak	-	-
	Şubat-Mart	28	40
	Nisan Mayıs	42	60
Sütten kesim zamanı	2 ay	2	2.9
	3 ay	51	72.9
	4 ay	17	24.3
	5 ay	-	-
Emiştirme süresi	30 gün	-	-
	60 gün	70	100
	120 gün	-	-
Emiştirme şekli	Akşam	-	-
	Sabah	21	30
	Sabah-Akşam	49	70
	Sabah- Öğle- Akşam	-	-
Kaba ve kesif yeme başlama zamanı	4. Hafta	-	-
	5. Hafta	40	57.1
	5. Haftadan sonra	30	42.9
İşletmenizdeki mevcut keçi ırkı?	Kıl keçisi	70	100
	Saanen keçisi	-	-
	Honamlı keçisi	-	-
	Şam keçisi	-	-
	Malta keçisi	-	-
	Kilis keçisi	-	-
Hangi keçi ırkını sahip olmayı tercih ederdiniz?	Kıl keçisi	50	71.4
	Saanen keçisi	4	5.7
	Honamlı keçisi	16	22.9
	Şam keçisi	-	-
	Malta keçisi	-	-
	Kilis keçisi	-	-
	Diğer	-	-
Hayvan yetiştiriciliğinde kayıt tutmak olmazsa olmazdır" fikrine katılıyor musunuz?	Kesinlikle katılıyorum	15	21.4
	Katılıyorum	24	34.3
	Kararsızım	18	25.7
	Katılmıyorum	13	18.6
	Kesinlikle katılmıyorum	-	-

Tablo 3: Keçi işletmelerinde sağım, hayvan besleme, kasaplık satışları için tanımlayıcı istatistikler (sayı ve yüzde).
Table 3: Descriptive statistics for milking, animal feeding, butchery sales in goat farms (number and percentage).

Özellikler		N	%
Sağıma başlama dönemi	Mart	-	-
	Nisan	10	14.3
	Mayıs	52	74.3
	Haziran	8	11.4
Sağım yapma Süresi	2 ay	5	7.1
	3 ay	24	34.3
	4 ay ve üzeri	41	58.6
Sağımı yapan kişi	Kadın	69	98.6
	Erkek	1	1.4
	Kadın-Erkek	-	-
Keçi sütünü nasıl değerlendiriyorsunuz?	Peynir	70	100
	Çiğ süt	0	0
	Aile ihtiyacı	57	81.4
	Yavru beslemesinde	65	92.9
	Diğer	0	0
Hayvanlarda beslemeyi nasıl yapıyorsunuz?	Meraya dayalı olarak	-	-
	Mera ve elden besleme	69	98.6
	Sürekli ağılda (Elden besleme)	1	1.4
Hayvanlara kesif yem veriyor musunuz?	Evet	70	100
	Hayır	-	-
Kesif yemi nerden alıyorsunuz?	Kendim hazırlıyorum	21	30
	Yemciden alıyorum	49	70
Hangi yem hammaddelerini kullanıyorsunuz? (Birden fazla seçenek işaretlenebilir).	Arpa	70	100
	Buğday	0	0
	Küspe	38	54.3
	Kepek	46	65.7
	Diğer	0	0
Kaba yem(ot) veriyor musunuz? (Birden fazla seçenek işaretlenebilir).	Fiğ	-	-
	Yonca	-	-
	Saman	70	100
	Mısır silajı	-	-
	Diğer	-	-
Teke katımı döneminde ek yemleme yapmak verimi arttırmaktadır	Kesinlikle katılıyorum	12	17.1
	Katılıyorum	35	50
	Kararsızım	23	32.9
	Katılmıyorum	-	-
	Kesinlikle katılmıyorum	-	-
Sürünün veriminin arttırılmasında damızlık tekelerin seçimi oldukça önemlidir.	Kesinlikle önemli	12	17.1
	Önemli	55	78.6
	Kararsızım	3	4.3
	Önemsiz	-	-
	Kesinlikle önemsiz	-	-
Gebelik döneminde keçilere ekstradan yemleme yapılmalıdır.	Kesinlikle katılıyorum	12	17.1
	Katılıyorum	18	25.7
	Kararsızım	40	57.1
	Katılmıyorum	-	-
	Kesinlikle katılmıyorum	-	-
Yeni doğanlarda ağız sütünün alınımının sağlanması önemli mi?	Kesinlikle önemli	10	14.3
	Önemli	37	52.9
	Kararsızım	23	32.9
	Önemsiz	-	-
	Kesinlikle önemsiz	-	-
Yeni doğanlarda göbek bakımının yapılması önemli mi?	Kesinlikle önemli	3	4.3
	Önemli	36	51.4
	Kararsızım	24	34.3
	Önemsiz	7	10
	Kesinlikle önemsiz	-	-
Kasaplık Satışların Dönemi	Oğlak	-	-
	1 Yaşında	70	100
	2 Yaşında	-	-
	Diğer	-	-
Kırkım yapıyor musunuz?	Evet	64	91.4
	Hayır	6	8.6
Keçilerde kırkım zamanı	Haziran	8	11.4
	Temmuz	62	88.6
	Ağustos	-	-
	Bilmiyorum	-	-
En önemli gelir kaynakları	Keçi Sütü	-	-
	Keçi Sütü + Et	70	100
	Damızlık satışı	-	-
	Diğer	-	-

Tablo 4: Mera-Yayla dönemleri için tanımlayıcı istatistikler (sayı ve yüzde).
Table 4: Descriptive statistics for the Pasture-Yayla periods (number and percentage).

Özellikler		N	%
Mera imkânınız bulunmakta mıdır?	Evet	51	72.9
	Hayır	19	27.1
Meranın mülkiyeti kime aittir?	Kendi malı	-	-
	Köy ortak malı	3	4.3
	Orman ve tarla kenarlarında	43	61.4
	Hazine arazisinde	5	7.1
Yetiştiricilerin mera-yayla çıkış zamanı	2. Ay	-	-
	3. Ay	-	-
	4. Ay	16	22.9
	5. Ay	34	48.6
	Yok	1	1.4
Yetiştiricilerin mera-yayla dönüş zamanı	8. Ay	-	-
	9. Ay	-	-
	10. Ay	47	67.1
	11. Ay	4	5.7
	Yok	-	-
Yaylaya ulaşım şekli	Araç	-	-
	Yaya	3	4.3
	Araç + Yaya	48	68.6
Keçileri otlatan kişi	Kendim	36	70.6
	Çoban	0	0
	Ben + Çoban	16	29.4

Tablo 5: İşletmelerde uygulanan sağlık yöntemi için tanımlayıcı istatistikler (sayı ve yüzde).
Table 5: Descriptive statistics for the health method applied in businesses (number and percentage).

Özellikler		N	%
Hayvanlarda sağlık korumayı nasıl yapıyorsunuz?	Yapmıyorum	0	0
	Yılda bir kez iç-dış parazit mücadelesi yapıyorum	46	65.7
	İlkbahar ve sonbaharda iç-dış parazit mücadelesi yapıyorum	38	54.3
	Vitamin takviyesi yapıyorum	69	98.6
	İhtiyaç durumunda veteriner hekime başvuruyorum	39	55.7
Sürü sağlığının korunması açısından belirli periyotlar ile veteriner hekimlerden bilgi alınmalı.	Kesinlikle Katılıyorum	3	4.3
	Katılıyorum	67	95.7
	Kararsızım	-	-
	Katılmıyorum	-	-
	Kesinlikle Katılmıyorum	-	-
Koruyucu hekimlik yönünden Aşılama oldukça önemlidir.	Kesinlikle önemli	3	4.3
	Önemli	67	95.7
	Kararsızım	-	-
	Önemsiz	-	-
	Kesinlikle önemsiz	-	-
Hangi tür hastalıklara karşı aşılama yaptırıyorsunuz?	Koyun-keçi vebası	70	100
	Mavi Dil	0	0
	Koyun-keçi çiçek	49	70
	Brusella	61	87.1
	Hepsi	0	0
Yetiştiriciliğini yaptığınız hayvanların refah durumunu nasıl değerlendiriyorsunuz?	Çok iyi	27	38.6
	İyi	38	54.3
	Orta	5	7.1
	Kötü	-	-
	Çok kötü	-	-

Tablo 6: Keçi yetiştiriciliğinde yaşanan sorunlar için tanımlayıcı istatistikler (sayı ve yüzde).
Table 6: Descriptive statistics for problems in goat farming (number and percentage).

Özellikler		N	%
Damızlık hayvan temini	Kesinlikle Önemli	12	17.1
	Önemli	36	51.4
	Kararsızım	22	31.4
	Önemsiz	-	-
	Kesinlikle Önemsiz	-	-
Ağıl ve barınak sorunu	Kesinlikle Önemli	29	41.4
	Önemli	41	58.6
	Kararsızım	-	-
	Önemsiz	-	-
	Kesinlikle Önemsiz	-	-
Kesif yem sorunu	Kesinlikle Önemli	58	82.9
	Önemli	12	17.1
	Kararsızım	-	-
	Önemsiz	-	-
	Kesinlikle Önemsiz	-	-
Mera-Yayla sorunu	Kesinlikle Katılıyorum	2	2.9
	Katılıyorum	60	85.7
	Kararsızım	8	11.4
	Katılmıyorum	-	-
	Kesinlikle Katılmıyorum	-	-
İşçi/çoban ve işçilik sorunu	Kesinlikle Önemli	2	2.9
	Önemli	68	97.1
	Kararsızım	-	-
	Önemsiz	-	-
	Kesinlikle Önemsiz	-	-
Doğum sorunları	Kesinlikle Önemli	3	4.3
	Önemli	51	72.9
	Kararsızım	16	22.9
	Önemsiz	-	-
	Kesinlikle Önemsiz	-	-
Oğlak bakımı ve büyütme	Kesinlikle Önemli	2	2.9
	Önemli	68	97.1
	Kararsızım	-	-
	Önemsiz	-	-
	Kesinlikle Önemsiz	-	-
Örgütlenme	Kesinlikle Katılıyorum	59	84.3
	Katılıyorum	7	10
	Kararsızım	4	5.7
	Katılmıyorum	-	-
	Kesinlikle Katılmıyorum	-	-
Pazarlama sorunu	Kesinlikle Katılıyorum	50	71.4
	Katılıyorum	20	28.6
	Kararsızım	-	-
	Katılmıyorum	-	-
	Kesinlikle Katılmıyorum	-	-
Sağlık-İlaç ve Veteriner hekim giderlerinin pahalı oluşu	Kesinlikle Katılıyorum	48	68.6
	Katılıyorum	22	31.4
	Kararsızım	-	-
	Katılmıyorum	-	-
	Kesinlikle Katılmıyorum	-	-

Pandeminin (COVID-19) Keçi Yetiştiriciliği Üzerindeki Etkileri

Yetiştiricilerin %68.6 'ı tüm dünyada etkisini sürdüren pandemi (COVID-19 salgın) hakkında bilgi düzeyiniz? sorusuna iyi cevabını verdikleri; Tüm dünyayı etkileyen COVID-19 salgınının hayvancılığı olumsuz yönde etkilediği fikrine katılıyor musunuz? sorusuna %44.3 oranında katılıyorum, %42.9 oranında ise kesinlikle katılıyorum cevabını verdikleri; yetiştiricilerin %57.1'i COVID-19 salgını öncesi ve sonrası arasında kazancınız olumsuz etkilendi mi? sorusuna kesinlikle katılıyorum cevabı verdikleri; Salgının uzun yıllar sürmesi halinde oluşacak gıda talebinin karşılanmasında hayvan yetiştiriciliğine talep artar fikrine katılıyor musunuz? sorusuna yetiştiricilerin %47.1'i katılıyorum,%27.1'i ise kararsızım cevabını verdikleri ve Pandeminin (COVID-19 Salgını) daha uzun yıllar sürmesi halinde keçi yetiştiriciliğinin biteceği fikrine katılıyor musunuz? sorusuna ise yetiştiricilerin %34.3'ü katılmıyorum,%31.4'ü kararsızım ve %24.3'ü ise katılıyorum cevabını verdikleri tespit edilmiştir.

Tablo 7: Pandeminin (COVID-19) keçi yetiştiriciliği üzerindeki etkileri için tanımlayıcı istatistikler (sayı ve yüzde).

Table 7: Descriptive statistics for the effects of the pandemic (COVID-19) on goat farming (number and percentage).

Özellikler		N	%
Tüm dünyada etkisini sürdüren Pandemi (COVID-19 salgın) hakkında bilgi düzeyiniz?	Çok iyi	1	1.4
	İyi	48	68.6
	Orta	19	27.1
	Kötü	2	2.9
	Çok kötü	-	-
Tüm dünyayı etkileyen COVID-19 salgınının hayvancılığı olumsuz yönde etkilediği fikrine katılıyor musunuz?	Kesinlikle Katılıyorum	30	42.9
	Katılıyorum	31	44.3
	Kararsızım	9	12.9
	Katılmıyorum	-	-
	Kesinlikle Katılmıyorum	-	-
COVID -19 salgını öncesi ve sonrası arasında kazancınız olumsuz etkilendi mi?	Kesinlikle Katılıyorum	40	57.1
	Katılıyorum	24	34.3
	Kararsızım	6	8.6
	Katılmıyorum	-	-
	Kesinlikle Katılmıyorum	-	-
Salgının uzun yıllar sürmesi halinde oluşacak gıda talebinin karşılanmasında hayvan yetiştiriciliğine talep artar fikrine katılıyor musunuz?	Kesinlikle Katılıyorum	4	5.7
	Katılıyorum	33	47.1
	Kararsızım	19	27.1
	Katılmıyorum	13	18.6
	Kesinlikle Katılmıyorum	1	1.4
Pandeminin (COVID-19 Salgını) daha uzun yıllar sürmesi halinde keçi yetiştiriciliğinin biteceği fikrine katılıyor musunuz?	Kesinlikle Katılıyorum	6	8.6
	Katılıyorum	17	24.3
	Kararsızım	22	31.4
	Katılmıyorum	24	34.3
	Kesinlikle Katılmıyorum	1	1.4

TARTIŞMA VE SONUÇ

Bu çalışmada, işletmecilerin %74.3'nün ilkokul mezunu, %12.9'nun ortaokul mezunu, %10.0'nın eğitiminin olmadığı ve çok düşük düzeyde de (%2.9) üniversite mezunu olduğu saptanmıştır.

Yapılan bazı çalışmalarda; Bilginturan (2008), yetiştiricilerden ilkokul mezunu olanların oranı %97.5 iken lise mezunlarının oranının ise %2.5 olduğunu, Kandemir ve ark. (2015), dağdaki işletmecilerin eğitimlerinin ilköğretim (%81.8) düzeyinde olduğunu, Erdem (2019), Uşak ilinde ise ilkokul mezunlarının oranı %86.4 iken ortaokul ve lise mezunlarının oranları sırasıyla; %9.8 ve %3.8 olduğunu, Ceyhan ve ark. (2015) göre Niğde ilinde %89.5'i ilkokul, %5.3'ü ortaokul ve %5.3'nün de lise mezunu olduğunu, bildirmişlerdir. Bu literatürlerdeki sonuçların çalışmamız bulgularından daha yüksek olduğu gözlenmiştir. Karakuş ve Akyol (2013), Van ilinde %46.10'nun ilkokul mezunu, %17.88'nin ise ortaokul mezunu olduklarını, Koyuncu ve ark. (2006), Çanakkale'de yaptıkları çalışmada ilkokul, ortaokul ve lise mezunlarının oranları sırasıyla; %10, %10, %25 belirlenmiştir. Şimşek (2019), işletmecilerin %61'nin ilkokul, %35'nin lise, %1'nin ön lisans ve %1'nin ise lisans mezunu olduğunu, hiç eğitim almayanların ise %1'lik bir oranda olduğunu bildirmişlerdir. Bu literatürlerde bildirilen araştırma bulguları çalışmamız sonuçlarından daha düşük olduğu belirlenmiştir.

Çalışmada, yetiştirici yaşları; %48.6 ile 36-50 yaş aralığında; %40.0 ile 51 ve üzeri yaş aralığında ve %11.4 ile 20-35 yaş aralığında oldukları tespit edilmiştir.

Şimşek (2019), %58 oranında 38-58 yaşta olduğu, %21'nin 18-38 yaş yaşta, 58-78 yaş ve üzerinde olanları ise %17 olarak tespit etmiştir. Bu literatürdeki %58'lik değere sahip olan yaş grubunun çalışmamız bulgusuyla yaklaşık olarak benzer olduğu. Acar (2010), yetiştiricilerin %57.58'inin 19 ile 45 yaş arası, %42.42'sinin de 46 yaş ve üzeri olduğunu bildirmiştir. Bu kaynakta bildirilen sonuçlar çalışmamız bulguları ile yaklaşık olarak benzer olduğu söylenebilir. Kandemir ve ark. (2015) göre yaptıkları çalışmada, altmış yaşının altında olanların oranının %90, kırk yaşının altında olanların oranının %21.8 olduğunu belirlemişlerdir. Çalışmamız sonuçları ile karşılaştırıldığında, 50 yaşın altındaki oranın bu literatürdeki sonuçlardan düşük, 40 yaş altı için bildirilen değerden yüksektir. Karakuş ve Akyol (2013)'a göre, Van ilinde ortalama yaşı 45 olarak tespit

etmişlerdir. Bu sonuç çalışmamız ile yaklaşık benzer olduğu söylenebilir.

Bu çalışmada yetiştiricilerin %91.4'nün erkek, %8.6'nın ise kadın oldukları tespit edilmiştir.

Şimşek (2019) göre, Kırşehir'de %93'nün erkeklerin kadınların ise %7 olduğunu saptamışlardır. Çalışmamız bulguları ile değerlendirildiğinde her iki cinsiyet için bildirilen değerlerin yaklaşık benzer olduğu söylenebilir. Kandemir ve ark. (2015) göre, %97.4'nün erkek olduğunu bildirmişlerdir. Bu literatüre ait %97.4'lük değer çalışmamız bulgusundan daha yüksektir.

Bu çalışmada, aile kişi sayısı (Ebeveyn + Çocuk) bulguları; %58.6'sı 4-6 kişilik aile; %35.7'si 1-3 kişilik aileye ve %5.7'si ise 7 ve üzeri aile sayısına sahip oldukları belirlenmiştir.

Bilginturan (2008), Burdur İlinde, üç kişiden (%45) oluştuğunu tespit etmiştir. Çalışmamız bulgusu ile değerlendirildiğinde literatürde bildirilen üç kişiden oluşan %45'lik değerin daha yüksek olduğu gözlenmiştir. Şimşek (2019), Kırşehir'de, evde çalışan kişi sayısının %85'lik oranıyla 0-2 kişi olduğu, bunu %12 sinin üç veya dört kişi olduğu, ailede %1'lik oran ile de yediden fazla çalışan kişi olduğunu tespit ettiklerini bildirmiştir. Bu literatürde bildirilen 1-3 kişilik aile fertleri ile 7 ve üzeri fert sayısı için bildirilen literatür değerleri çalışmamız değerinden düşüktür.

Çalışmamızda, koruyucu hekimlik yönünden aşılamaya önemli diyenlerin oranı %95.7 olduğu; Yetiştiricilerin %100.0'nın koyun-keçi vebası, %87.1'nin Brusella aşısı ve %70.0'nin ise koyun-keçi çiçek aşısı yaptırdıkları tespit edilmiştir.

Yetiştiricilerin işletmelerde, hayvan sağlığı ve refahı ile ilişkili olarak aşı uygulaması, iç ve dış parazit mücadelesi, vb. uygulamalar konusunda bilinçli olması önemlidir.

Şimşek (2019) göre, işletmelerin %98'nin aşı yaptırdıklarını belirtmişlerdir. Literatürde bildirilen bulguların çalışmamız sonuçları ile yaklaşık benzer olduğu söylenebilir. Acar (2010), çalışmalarında, %98.18'inin düzenli aşı yaptırdığı, aşı uygulamasını ve ilaç danışmasının %36.79'unun kendilerinin yaptığı, %63.03'ününde veteriner hekimin yaptığını tespit etmişlerdir. Literatürde bildirilen %98.18'lik değer, çalışmamızda ki %100.0'nın koyun-keçi vebası bulgusundan düşük, %87.1'lik Brusella aşısı ve %70.0'lik koyun-keçi çiçek aşısı yaptıranların değerinden yüksektir. Bu literatür bulguları ile çalışmamız bulguları benzer olduğu söylenebilir.

Karakuş ve Akyol (2013) göre, Van ilinde, aşı yaptıranların %68.52'sinin rastgele bir aşılama planına sahip olduğunu, tüm işletmelerin yalnızca %46.19'u hayvanlarına enterotoksemi, çiçek, Brusella ve şap aşılarının tamamını yaptırdıklarını bildirmişlerdir. Bu literatür sonuçları çalışmamız değerlerinden düşüktür.

Bu çalışmada işletmelerdeki mevcut keçi ırkının %100.0 ile kıl keçisi olduğu saptanmıştır.

Ceyhan ve ark. (2015) göre, çalışmalarında, işletmelerin tamamının kıl keçisi yetiştirdiğini bildirmişlerdir. Bu sonuç çalışmamız bulgusuyla benzerdir. Erdem (2019), Uşak'ta yaptığı bir çalışmada keçi ırklarının sırasıyla Kilis, Saanen, Kıl Keçisi oranları; %3.8, %3.8 ve %89.4 olduğunu, Kandemir ve ark. (2015) göre keçi türünde "Kıl ve melezleri" (%20.8) ile "Saanen ve melezleri" (%9.8) öncelikli yetiştirildiğini, Karakuş ve Akyol (2013), ise Van ilinde yaygın ırkın %79.68'nin Kıl keçisi ve %20.32'nin Norduz keçisi olduğunu bildirmişlerdir. Bu literatürlerde tespit edilen bulgu sonuçları çalışmamız bulgusundan daha düşük olduğu gözlenmiştir.

Bu çalışmada yetiştiricilere yöneltilen "Mera-Yayla sorunu" sorusuna %85.7'nin katılıyorum cevabını verdikleri saptanmıştır.

Ceyhan ve ark. (2015) göre, Niğde ili keçi işletmelerinin %97.4'ünün kendi merası bulunduğunu, sürülerin yaylalara genellikle Nisan ve Mayıs aylarında çıkmakta ve Ağustos ayı sonu, Kasım ayı başında geri dönmekte olduklarını, yayla dönüşünden sonra işletmeler hayvanlara (%86.7) az miktarda ek yemleme yaptıklarını bildirmişlerdir. Erdem (2019), Uşak'ta yaptığı bir çalışmada yaylacılığın yetiştiriciler arasında çok tercih edilmediğini (%87.1) bildirmiştir.

Bu çalışmada yetiştiricilere yöneltilen "İşçi/çoban ve işçilik sorunu" sorusuna %97.1'nin önemli cevabını verdikleri, yetiştiricilerin %100'nün ise çobanı kendi ailesinden temin ettikleri tespit edilmiştir.

Ceyhan ve ark. (2015) göre, Niğde ilinde %73.7'si çobanı kendi ailesinden temin ettiklerini, Acar (2010), Isparta ilinde, çobanların %93.94'nün aile fertlerinden olduklarını, Erdem (2019), Uşak ilinde ise çoban büyük oranda (%93.2) aileden temin edildiğini tespit etmişlerdir. Kandemir ve ark. (2015) göre çalışmalarında işletmelerin genelinde (%88.6), işletme sahibinin (%62.7) ya da aile bireylerinden birisi tarafından (%25.9) çobanlık yapıldığını, Karakuş ve Akyol (2013), Van ilinde, mera döneminde çoban tutma eğiliminde olan işletmelerin %70.12'sinde yalnızca bir çoban tutulmakta olduğunu bildirmişlerdir. Çalışmamızda elde edilen, çobanı kendi ailesinden temin ettikleri değeri bu literatür bildirişlerinden daha yüksek olduğu belirlenmiştir.

Bu çalışmada yetiştiricilere yöneltilen "Kullandığınız barınak tipi nasıldır?" sorusuna %74.3'nün yazın açık kışın kapalı ağıl; %22.9'nın yarı açık ağıl ve %2.9'nın ise kapalı ağıl cevabını verdikleri saptanmıştır.

Ceyhan ve ark. (2015) göre, Niğde ilindeki keçi ağıllarının %60.5'inin yarı açık, %34.2'sinin ise kapalı olduğunu bildirmiştir. Acar (2010), Isparta ilindeki keçi yetiştiriciliği yapan işletmelerin %76.97'sinde yarı açık ağıl kıl çadırı önünde tel ile çevrilmiş alanları olduğu, %20'sinin kapalı ağıl ve kıl çadırlardan olduğu, %3.03'ünün ise sadece tel ve taş ile çevrili açık ağıllardan oluştuğunu bildirmiştir. Erdem (2019), Yetiştiricilerin %60.6'sı kapalı barınak, %39.4'ü yarı açık ağıl tipinde barınak kullandığını saptamıştır. Karakuş ve Akyol (2013). Van ilinde yaptıkları bir çalışmada işletmelerin tamamının bir barınağa sahip olduğu bunun %98.84'nun kapalı tipte olduğunu bildirmişlerdir.

Bu çalışmamda yetiştiricilere yöneltilen "Damızlık hayvan temini" sorusuna %51.4'nün önemli cevabını verdikleri saptanmıştır. Ayrıca, Ağıl ve barınak sorunu sorusuna %58.6'nın önemli; Kesif yem sorunu sorusuna %82.9'nın kesinlikle önemli; İşçi/çoban ve işçilik sorunu sorusuna %97.1'nin önemli; Doğum sorunları sorusuna %72.9'nun önemli; Oğlak bakımı ve büyütme sorusuna %97.1'nin önemli; Örgütlenme sorusuna %84.3'nün kesinlikle katılıyorum; Pazarlama sorunu sorusuna %71.4'nün kesinlikle katılıyorum; Sağlık-İlaç ve Veteriner hekim giderlerinin pahalı oluşu sorusuna %68.6'nın kesinlikle katılıyorum cevabını verdikleri saptanmıştır.

Ceyhan ve ark. (2015) göre, Niğde ilindeki damızlık hayvanları işletmelerin %84.2'sinin kendi sürüsünden temin ettiğini bildirmişlerdir. Acar (2010), Isparta'daki keçi yetiştiricilerinin %100'nün damızlık hayvan ihtiyaçlarını başka işletmelerden karşıladığını belirlemiştir. Erdem (2019), Uşak'ta işletmelerin damızlık hayvan temin şekli olarak %72.8'inin kendi sürüsünden, Karakuş ve Akyol (2013) ise Van ilinde yetiştiricilerin %62.30'u damızlık

ihtiyacını kendi işletmelerinden temin ettiklerini bildirmişlerdir.

Bu çalışmada yetiştiricilere yöneltilen "Hayvan yetiştiriciliğinde kayıt tutmak olmazsa olmazdır" fikrine katılıyor musunuz? sorusuna yetiştiricilerin %34.3 ile en yüksek oranda katılıyorum cevabını verdikleri tespit edilmiştir.

Ceyhan ve ark. (2015) göre, Niğde'de %86.8'inin kayıt tuttuklarını, Erdem (2019), Uşak ilinde %81.9'nun kayıt tutmadığını belirlemiştir. Karakuş ve Akyol (2013) Van ilinde hayvanları ile ilgili herhangi bir kayıt tutan işletmelerin payının yalnızca %38.05 olduğunu bildirmişlerdir. Bu literatür sonuçları çalışmamız bulgusundan yüksektir.

Bu çalışmada, yetiştiricilerin tamamının %100.0 teke katım yöntemi olarak serbest aşım yöntemini tercih ettikleri saptanmıştır.

Kandemir ve ark. (2015) göre, İzmir'de yaptıkları bir çalışmada, işletmelerin tamamında (%100) koç/teke katım yöntemi serbest aşım olduğunu bildirmiştir. Literatüre ait bu değer ile çalışmamız sonuçları benzerdir. Acar (2010), Niğde ili keçi yetiştiriciliği yapan işletmelerde teke katımında %76.97'sinin serbest aşım yöntemin, tercih ettiklerini tespit etmiştir. Ceyhan ve ark. (2015) göre, Niğde'de %76.3'ü serbest teke katımı yaptıklarını tespit etmişlerdir. Karakuş ve Akyol (2013). Van'da işletmelerin %95.08'inde koç/teke katımı serbest aşım şeklinde yapıldığını bildirmişlerdir. Bu literatürlerde belirtilen değerler çalışmamız sonucundan daha düşük olduğu gözlenmiştir.

Bu çalışmada damızlıkta kullanma yaşı en yüksek %70.0 oran ile 18 aylık yaş olarak tercih edildiği tespit edilmiştir.

Ceyhan ve ark. (2015) göre, Niğde ilindeki işletmelerde erken yaşta damızlıkta kullanım oranı %5.3 iken, 12 aylıktan yukarı damızlıkta kullananların oranı %94.7'dir. İlk defa damızlıkta kullanım yaşı 12 ay (%2.6), 15 ay (%64.2), 18 ay (%50) ve 24 aylık yaş (%13.2) olarak belirlemişlerdir. Çalışmamız sonuç değeri (%70) bu literatürde belirtilen 18 ay (%50) oranından oldukça yüksektir. Acar (2010), Niğde ili keçi yetiştiriciliği işletmelerindeki dişi çepiçlerin %38.18'i 8 ile 12 aylık dönemde iken, %11.52'si ise 24 aylık yaşa ulaştıklarında damızlıkta kullanıldığını bildirmiştir. Çalışmamız bulgusu bu literatürdeki değerden farklı ve yüksektir. Erdem (2019), Uşak ilinde dişilerin ilk damızlıkta kullanılma yaşı %44.7 oranında 15 aylık, %32.6 oranında 18 aylık, %18.9 oranında da 12 aylık olarak belirlemiştir. Bu literatürdeki sonuç, çalışmamız bulgu değerinden oldukça düşüktür.

Kandemir ve ark. (2015) göre, İzmir ilinde (%97.1) koç/tekelerin sürüde bulunma süresi 6 ay ve üzeridir. İşletmelerin genelinde yaşı 12 ay ve üzerinde erkek (%85.9) ve dişi hayvanlar (%60.7) damızlıkta kullanılmakta olup, dağdaki işletmelerde bu oranlar (%100 ve %91.4) ovadakilere (%70.6 ve %29.4) göre daha yüksek olarak bildirmişlerdir. Bu literatür değeri ile çalışmamız değeri oldukça farklıdır.

Bu araştırmada doğum zamanının %60.0 oranı ile nisan-mayıs aylarında olduğu saptanmıştır.

Ceyhan ve ark. (2015) göre, Niğde ilinde doğumların daha çok (%68.4) Şubat-Mart aylarında olmaktadır. Bunu %23.7'lik oran ile Nisan-Mayıs ayları izlediğini bildirmişlerdir. Bu araştırmaya ait bulgu ile çalışmamız sonucu benzerdir.

Bu çalışmada, emiştirme süresi %100.0 ile 60 gün sürdüğü; emiştirme şeklinin ise %70.0 ile sabah-akşam olduğu tespit edilmiştir.

Acar (2010), Niğde ilinde oğlakların %93.33'ünün emiştirme yaptıkları, %95.15'inin emiştirmeyi sabah-akşam yaptıkları belirlenmiştir. Bu literatürdeki emiştirme şekli bulgu değeri çalışmamız değerinden oldukça yüksektir. Ceyhan ve ark. (2015) göre, işletmedeki oğlakları (%52.6) sabah ve akşam emiştirirken, günde 3 defa emzirenlerin oranı %34.2, sadece sabah emiştirenlerin oranı %5.3 ve sadece akşam emiştirenlerin oranı da %7.9 olarak tespit etmişlerdir. Bu literatürde saptanan emiştirme şekli bulgu değeri çalışmamız değerinden daha düşüktür.

İşletme gelirleri; bu çalışmada, yetiştiricilerin tamamının %100.0 işlemedeki en önemli gelir kaynağı olarak keçi sütü + et cevabını verdikleri belirlenmiştir.

Ceyhan ve ark. (2015) göre, işletme gelirlerinin %50'si keçi sütünden sağlandığını belirlemişlerdir. Literatüre ait sonuçlardan çalışmamız bulgusu farklı ve yüksektir.

Bu çalışmada, beslemenin %98.6 ile mera ve elden besleme şeklinde yapıldığı; %100.0 ile keçilere kesif yem verdikleri; %70.0'nin kesif yemi yemciden aldıklarını; kaba yem olarak samanı tercih edenlerin %100.0 olduğu; teke katım döneminde ek yemleme yapmak verimi artırmaktadır sorusuna katılımcıların %50.0 ile katılıyorum cevabını verdikleri tespit edilmiştir.

Kandemir ve ark. (2015) göre, işletmelerin yarısına yakınında (%45.2) kullanılan kaba yemi saman oluştururken, kalan kısmında kuru ot ve mısır silajı gibi diğer yem kaynakları oluşturmaktadır. Araştırma alanı genelinde işletmelerin küçük bir bölümünde (%14.9) hiç karma yem kullanılmaz iken, büyük bir bölümünde (%70) ise besi yemi kullanıldığını bildirmişlerdir. Çalışmamıza ait bul değerleri bu literatür bildirişinden farklıdır.

Bu çalışmada, katılımcıların %91.4'ü kırkım yapıyor musunuz? sorusuna evet dedikleri; keçilerde kırkım zamanı olarak ise %88.6 ile temmuz ayı olduğu tespit edilmiştir.

Erdem (2019), Uşak ilinde %87.1 oranı ile kırkım yaptıklarını ve bunu %52.3 oranı ile Haziran ayında yaparlarken %33.3 oranı ile Temmuz ayında yaptıkları saptanmıştır. Bu literatür sonucuna göre, çalışmamız sonuçları farklı ve yüksektir. Yıldız ve Aygün (2021), Van'da kırkım işlerini mayıs-eylül ayları arasında yaptıklarını belirlemişlerdir. Keçi ve tekelere kırkım yapılması durumunda bunu %12.5 oranı ile Haziran ayında yaptıkları belirtilmiştir. Bu literatür sonuçları çalışmamız değerinden daha düşük, kırkım mevsimleri ise farklıdır. Dellal ve ark. (2002) göre keçilerin kırkımlarının %60.6 oranı ile Mayıs ve Temmuz aylarında yapıldığı ve alet ve ekipman olarak %99.6 oranı ile kırkım makasının kullanıldığı bildirmişlerdir. Bu literatüre ait kırkım yapanlar oranı, çalışmamız sonuçlarından oldukça düşük, kırkım mevsimi ise yaklaşık benzerdir.

Bu çalışmada, Pandemi (COVID-19 salgın) hakkında bilgi düzeyiniz? sorusuna %68.6 oranında iyi cevabını verdikleri; COVID-19 salgınının hayvancılığı olumsuz yönde etkilediği fikrine katılıyor musunuz? sorusuna %44.3 oranında katılıyorum, %42.9 oranında ise kesinlikle katılıyorum cevabını verdikleri; COVID -19 salgını öncesi ve sonrası arasında kazancınız olumsuz etkilendi mi? sorusuna %57.1 ile kesinlikle katılıyorum cevabı verdikleri; Salgının uzun yıllar sürmesi halinde oluşacak gıda talebinin karşılanmasında hayvan yetiştiriciliğine talep artar fikrine katılıyor musunuz? sorusuna katılımcıların %47.1'i katılıyorum,%27.1'i ise kararsızım cevabını verdikleri ve Pandeminin (COVID-19 Salgını) daha uzun yıllar sürmesi halinde keçi yetiştiriciliğinin biteceği fikrine katılıyor musunuz? sorusuna ise yetiştiricilerin %34.3'ü

katılmıyorum,%31.4'ü kararsızım ve %24.3'ü ise katılıyorum cevabını verdikleri tespit edilmiştir.

Konya ilinde, COVID-19 salgınında işletmelerin %65'i faaliyetlerinde herhangi bir değişiklik yapmadığı, %30'u COVID-19 salgınıyla birlikte yem fiyatlarındaki yüksek artış ve süt satış fiyatlarının düşük olmasından dolayı hayvan sayılarını azalttıklarını söylemişlerdir. Aynı çalışmada, tarım işletmelerinin COVID-19 salgınından dolayı bundan sonraki üretimleri için herhangi bir planlama yapmadıkları mevcut stratejilerine devam ettikleri belirlemişlerdir. Konya ilinde COVID-19 salgınından dolayı %63.90'ında toplam gelirde bir azalma olmadığı %36.10'unda azalma olduğu ve bu azalmaya karşı bazı telafi yollarına gittikleri belirlendiği bildirilmiştir. Alınan telafi yöntemlerinin başında borç erteleme ve gerek tarımsal gerekse tarım dışı masraflarının azaltılması gelirken bunu kredi çekme, komşu-akrabadan borç alma, sosyal yardım alma ve tarım dışı mevsimlik işçi olarak çalışma takip etmektedir. %9.96'sı ise herhangi bir telafi yoluna gitmemiş mevcut geliri ile devam etmiş olduklarını tespit etmişlerdir (BDUTAEM 2021).

Sonuç olarak; Antalya ili ve ilçeleri coğrafi ve iklimsel özellikleri açısından keçi yetiştiriciliğine uygun bir ortama sahiptir. Araştırmanın yapıldığı Manavgat İlçesindeki keçi yetiştiriciliği faaliyeti, işletmelerin en önemli gelir kaynağını oluşturmaktadır. Ayrıca keçi yetiştiriciliğinin geleneksel Anadolu yörük kültürü içindeki önemi ve kuşaklara aktarımı da olabildiğince korunmaktadır. Antalya ili ve Manavgat ilçesi genel olarak küçükbaş yetiştiriciliği için elverişli bir coğrafi konum ile doğal bir ekosisteme sahiptir. Bölgedeki mevcut çayır-meralar, yaygın maki ve ormanlık alanları ile özellikle keçi yetiştiriciliğine uygun ortam sağlamaktadır. Çalışma kapsamında Manavgat ilçesinde en fazla Kıl keçisi ve Kıl keçisi melezi yetiştiriciliğinin hâkim olduğu saptanmıştır. Keçi yetiştiriciliğinin üretimsel boyutu ile hem insanların beslenmesinde hem de pek çok sektörde stratejik bir öneme sahip olduğu bilinmektedir. Dolayısı ile Türkiye'de yetiştirilen farklı genetik varyasyonlara sahip keçi ırklarının ve özelliklede yerli gen kaynaklarımızın korunmasına yönelik politikaların geliştirilmesi, yöreye özgü daha verimli ve kaliteli ırkların tespit edilmesi, yaygınlaştırılması, örgütlenme ile pazar sorununun çözümü gibi konular üzerine daha fazla odaklanılmalıdır. Kaliteli ve güvenli gıdanın temini açısından; bölgesel ve ulusal keçi yetiştiriciliği yapan tüm sektör paydaşlarının durumunun iyileştirilmesi, geliştirilmesi ve desteklenmesi oldukça önemlidir.

ÇIKAR ÇATIŞMASI

Yazarlar bu çalışma için herhangi bir çıkar çatışması olmadığını beyan ederler.

TEŞEKKÜR VE BİLGİLENDİRME

Bu çalışma, Zeki Emre TAVALI isimli yazarın yüksek lisans tezinden özetlenmiştir.

Bu çalışma, Ahi Evran 2nd International Conference on Scientific Research, Kırşehir, Türkiye, 21-23 Ekim 2022, ss. 409-410 isimli kongrede sözlü olarak sunulmuş, kongre kitabında ise özet metin olarak basılmıştır.

YAZAR KATKILARI

Fikir/Kavram: ZET, BÇ
Denetleme/Danışmanlık: ZET, BÇ
Veri Toplama ve/veya İşleme: ZET, BÇ
Analiz ve/veya Yorum: ZET, BÇ
Makalenin Yazımı: ZET, BÇ
Eleştirel İnceleme: ZET, BÇ

KAYNAKLAR

Acar M (2010). Isparta İli Damızlık Koyun Keçi Yetiştiricileri Birliği Üyesi Keçicilik İşletmelerinin Mevcut Durumu ve Teknik Sorunları Üzerine Bir Araştırma. Yüksek Lisans Tezi, Süleyman Demirel Üniversitesi, Isparta.

Bilginturan S (2008). Burdur İli Damızlık Koyun ve Keçi Yetiştiriciler Birliği Üyesi İşletmelerin Yapısal Özellikleri ve Sorunları Üzerine Bir Araştırma. Yüksek Lisans Tezi, Süleyman Demirel Üniversitesi, Isparta.

Budak F, Korkmaz Ş (2020). COVID-19 Pandemi Sürecine Yönelik Genel Bir Değerlendirme: Türkiye Örneği. *SAYOD*, (1), 62-79.

BDUTAEM (2021). Covid-19 Salgınının Konya İlinde Tarım İşletmelerine Etkisinin İncelenmesi, Küçükçongar M., Özdemir F., Karakurt C ve ark., (2021) BDUTAEM, Konya/Türkiye. https://arastirma.tarimorman.gov.tr/bahridagdas/Haber/236/Covid-19-Salginin-Tarim-Isletmelerine-Etkisi-Arastirildi.

Ceyhan A, Ünalan A, Çınar M ve ark (2015). Niğde İli Keçi Yetiştiriciliğinin Yapısal Özellikleri ve Sorunları Üzerine Bir Araştırma. *TURJFAS*, 3 (2), 74-79.

Dellal G, Eliçin A, Tekel N, Dellal İ (2002). GAP Bölgesinde küçükbaş hayvan yetiştiriciliğin yapısal özellikleri. *Tarımsal Ekonomi Araştırma Enstitüsü*. Ankara: TKB. Rapor No: 2002–1

Erdem M (2019). Uşak İli Keçi Yetiştiriciliğinin Mevcut Durumu, Sorunları ve Çözüm Önerileri. Yüksek Lisans Tezi, Uşak Üniversitesi, Uşak.

FAO (2022). Crops and livestock products. Erişim Tarihi: 28 Nisan 2022. Erişim adresi: https://www.fao.org/faostat/en/#data/QCL/visualize.

Günlü A, Alaşahan S (2010). Türkiye'de Keçi Yetiştiriciliği ve Geleceği Üzerine Bazı Değerlendirmeler. *Vet Hekim Der Derg*, 81 (2), 15-20.

Kandemir Ç, Alkan İ, Yılmaz Hİ ve ark. (2015). İzmir Yöresinde Küçükbaş Hayvancılık İşletmelerinin Coğrafik Konumlarına Göre Genel Durumu ve Geliştirilme Olanakları. *Hayvansal Üretim Derg*, 56 (1), 1-17.

Karakuş F, Akkol S (2013). Van ili küçükbaş hayvancılık işletmelerinin mevcut durumu ve verimliliği etkileyen sorunların tespiti üzerine bir araştırma. *Yüzüncü Yıl Üniv Fen Bilimleri Enstitüsü Derg*, 18 (1-2), 9-16.

Kaymakçı M, Aşkın Y (1997). Keçi Yetiştiriciliği. Ege Üniversitesi Ziraat Fakültesi Yayınları. İzmir.

Koyuncu E, Pala A, Savaş T ve ark. (2006). Çanakkale Koyun ve Keçi Yetiştiricileri Birliği Üyesi Keçicilik İşletmelerinde Teknik Sorunların Belirlenmesi Üzerine Bir Araştırma. *Hayvansal Üretim*, 47 (1), 21-27.

SPSS. (2021). SPSS Lnc. Chicago, Illinois, USA.

Şentürklü S, Arslanbaş E (2010). Entansif Keçi Yetiştiriciliği. Ulusal Keçicilik Kongresi, Çanakkale.

Şimşek G (2019). Kırşehir İlinde Küçükbaş Hayvan Yetiştiriciliği Yapan İşletmelerin Teknik ve Ekonomik Yapılarının Belirlenmesi. Yüksek Lisans Tezi, Ahi Evran Üniversitesi, Kırşehir.

Yıldız A, Aygün T (2021). Van İli Merkez İlçede Küçükbaş Hayvancılık Faaliyetleri ve Genel Sorunlar: II. İşletmelerde Yetiştirme İşleri. *JASP*, 4 (1), 37-53.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Çatalkaya E, Karacan Sever N (2023).** Ocular Bacterial and Fungal Flora in Clinically Healthy Horses in Diyarbakır. *Van Vet J*, 34(2), 109-112. DOI: https://doi.org/10.36483/vanvetj.1245071

ISSN: 2149-3359 **Araştırma Makalesi** e-ISSN: 2149-8644

Diyarbakır'da Klinik Olarak Sağlıklı Atlarda Oküler Bakteriyel ve Fungal Flora

Emine ÇATALKAYA[1,*] Nurdan KARACAN SEVER[2]

[1] *Dicle Üniversitesi, Veteriner Fakültesi, Cerrahi Anabilim Dalı, 21200, Diyarbakır, Türkiye*
[2] *Dicle Üniversitesi, Veteriner Fakültesi, Mikrobiyoloji Anabilim Dalı, 21200, Diyarbakır, Türkiye*

Gönderim Tarihi: 31.01.2023 Kabul Tarihi: 25.04.2023

ÖZ

Oküler flora atın yaşadığı ortam, iklim, coğrafya vb. birçok etkenden etkilenebilir. Bu çalışmanın amacını Diyarbakır Hipodromunda yarış koşan ve hipodroma yakın çiftliklerde yarış koşmayan sağlıklı yarış atlarında bakteriyel ve fungal oküler florayı belirlemek ve belirlenen bakteri ve mantar türlerini tanımlamak amaçlandı. Çalışma grubunu değişik yaş ve cinsiyette Diyarbakır Hipodromunda bulunan yarış koşan 28 ve hipodrom yakınlarındaki çiftliklerde yarış koşmayan 28 safkan Arap ve İngiliz atına ait 112 sağlıklı göz oluşturdu. Sağlıklı gözlerin medial kantusundan steril swaplarla sürüntü alınarak soğuk zincirde Dicle Üniversitesi Veteriner Fakültesi Mikrobiyoloji Laboratuvarına ulaştırılarak konjunktival florada bulunan bakteri ve fungal etkenlerin izolasyonu ve identifikasyonu yapıldı. Hipodrom grubundaki atlarda bakteriyolojik üreme oranının %94.64, fungal üremenin ise %28.57 olduğu, çiftlik grubundaki atlarda bakteriyolojik üremenin %100, fungal üremenin ise %14.29 olduğu tespit edildi. Değerlendirilen konjunktival svap örneklerinin izole ve identifiye edilen bakterilerin hipodrom grubunda %87.79'unun Gram pozitif, %12.21'inin Gram negatif, çiftlik grubunda ise %82.56'sının Gram pozitif, %17.43'ünün de Gram negatif olduğu belirlendi. Her iki grupta da mikrofloranın büyük çoğunluğunu *Staphylococcus* spp. tarafından oluştuğu görüldü. Sonuç olarak, atlarda belirli aralıklarla konjunktival floranın belirlenmesinin olası bir kornea veya göz hasarında izlenecek tedavinin belirlenmesine yardımcı olacağı; ayrıca erken müdahale ile kornea hasarına bağlı görme kayıplarının önüne geçilebileceği düşünülmektedir.

Anahtar Kelimeler: *At, Bakteriyel flora, Fungal flora, Gram pozitif bakteri, Gram negatif bakteri.*

ABSTRACT

Ocular Bacterial and Fungal Flora in Clinically Healthy Horses in Diyarbakır

The ocular flora can be affected by many factors such as the environment in which the horse lives, climate, geography, etc. The aim of this study was to determine the bacterial and fungal ocular flora and to define the determined bacterial and fungal species in healthy racehorses that race at Diyarbakir Hippodrome and do not race in farms close to the Hippodrome. The study group consisted of 112 healthy eyes of 28 Thoroughbred Arabian and British horses of different ages and genders, which raced in the Diyarbakir Hippodrome and 28 did not race in the farms near the Hippodrome. Bacterial and fungal agents in the conjunctival flora were isolated and identified by swabbing with sterile swaps from the medial canthus of healthy eyes and transported to Dicle University Veterinary Faculty Microbiology Laboratory in a cold chain. Bacteriological growth was 94.64%, fungal growth was 28.57% in the hippodrome group, bacteriological growth was 100% in the farm group, and fungal growth (14.29%) was detected. It was determined that of the evaluated conjunctival swab samples, 87.79% of isolated and identified bacteria were Gram-positive in the hippodrome group, 12.21% were Gram-negative, 82.56% were Gram-positive and 17.43% were Gram-negative in the farm group. *Staphylococcus* spp. formed the majority of the microflora in both groups. As a result, it is thought that it will help to determine the conjunctival flora at regular intervals and to determine the treatment to be followed in case of possible corneal or eye damage, and vision loss due to corneal damage can be prevented with early intervention.

Keywords: *Bacterial flora, Fungal flora, Gram-positive bacteria, Gram negative bacteria, Horse.*

GİRİŞ

Atlar, diğer türlere kıyasla kornea lezyonlarına daha duyarlı türlerdir. Çünkü fiziksel aktiviteleri ve yaşadıkları çevre (toz, saman, kir vs.) ile ilişkili travmaya duyarlı büyük gözlere sahiptirler (Laus ve ark. 2016; Ferraira ve ark. 2017; Santibáñez ve ark. 2022). Hayvan ve insan gözü mikroflorası, konakçının bağışıklık sistemi ile dengede kalan mantar ve bakteri türleri ile oluşur. Atlarda konjunktival florayı birçok bakteri ve mantar oluşturmaktadır (Baran ve ark. 2015; Laus ve ark. 2016; Fernández-Garayzábal ve ark. 2022; Fraczkowska ve ark.

✉ *Corresponding author: eminecatalkaya21@gmail.com

2022). Bu mikrobiyal flora, antibakteriyel maddeler üreterek, diğer mikroorganizmaların büyüme yüzeyini sınırlar. Kornea ve konjunktival epitel yüzeyindeki besin içeriğini azaltarak mikrobiyal patojenlere karşı koruma sağlar (Andrew ve ark. 2003; Baran ve ark. 2015; Laus ve ark. 2016; Zak ve ark. 2018; Fernández-Garayzábal ve ark. 2022). Yerleşik veya patojenik mikroorganizmalar tarafından enfekte olan kornea hasarı, tedavisi zor olabilen ve görme kaybı ile sonuçlanabilen keratit veya enfekte kornea ülserlerine yol açabilir (Andrew ve ark. 2003; Ferraira ve ark. 2017; Fraczkowska ve ark. 2022; Santibáñez ve ark. 2022). Bu durum performans atlarında yarış hayatının sonlanmasına sebep olabilen önemli bir hastalıktır. Oküler mikroflora birçok faktör tarafından etkilenebilmektedir (Andrew ve ark. 2003). Mevsim, coğrafya, altlık yapısı, habitat ve hayvancılık, normal ve hastalıklı at gözlerinde mikrobiyal yükü etkileyen potansiyel değişkenler olarak öne sürülmüştür (Andrew ve ark. 2003; Baran ve ark. 2015; Laus ve ark. 2016). Sıcaklık ve nem gibi mevsimsel etkilerin de at korneasında etkili olduğu belirlenmiştir. Oküler floradaki geçici mikroorganizma popülasyonları çevresel kontaminasyondan oluşur ve bu mikroorganizmalar kalıcı mikroorganizmalarla temas halindedir (Andrew ve ark. 2003). Atlarda konjuntival fungal floranın çevresel faktörlerden etkilendiği düşünülmektedir. Bu nedenle mevsimsel değişikliklerin de konjunktival fungal florayı etkileyebileceği ifade edilmektedir. Ahır ortamında bakılan atlarda konjunktival mantar yükünün önemli ölçüde daha yüksek olduğu bildirilmiştir (Andrew ve ark. 2003; Zafarnaderi ve Araghi-Sooreh 2017).

Bu çalışmada, Diyarbakır Hipodromunda yarış koşan ve hipodroma yakın çiftliklerde yarış koşmayan sağlıklı yarış atlarında bakteriyel ve fungal oküler florayı belirlemek ve belirlenen bakteri ve mantar türlerini tanımlamak amaçlandı.

MATERYAL VE METOT

Bu çalışmanın hayvan materyalini Diyarbakır Hipodromu çevresinde bulunan at çiftliklerinde yarış koşmayan (n=28) ve hipodromda bulunan (n=28) yarış koşan toplam 56 Safkan İngiliz ve Arap atına ait 112 göz oluşturdu. Çalışma, Dicle Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun 21.01.2023 tarih ve E-35582840-020-436707 sayılı izni ile yapılmıştır. Çalışmaya alınan atlar 2-10 yaş aralığındaydı. Çiftlik seçimi yapılırken rakım, sıcaklık gibi çevresel faktörleri minimanize etmek için hipodroma yakın olmasına özen gösterildi.

Çalışmaya dahil edilen atların ilgililerinden ata yakın tarihte herhangi bir enfeksiyona bağlı antibiyotik kullanılıp kullanılmadığına yönelik bilgiler alındı. Svap alımına yakın bir tarihte enfeksiyon geçiren veya antibiyotik tedavisi yapılan atlar çalışma kapsamına alınmadı. Çalışmaya dahil edilen atların sağlıklı olmasına özen gösterildi.

Svab alımından önce, herhangi bir oküler patolojinin olup olmadığının belirlenmesi için her iki göz oftalmoskop ile (Gowllands, Halogen otoskop/oftalmoskop seti, İngiltere) muayene edildi. Sadece normal eklentileri ve ön segmenti olan ve klinik oküler ağrı, oküler akıntı veya konjunktivit belirtileri olmayan atlar çalışmaya dahil edildi. Üçüncü göz kapağının ön yüzeyinden ve alt göz kapağından topikal anestezi olmaksızın sürüntüler, üçüncü göz kapağının ön yüzeyini ortaya çıkarmak için küre üst göz kapağından retropulse edilerek ve alt göz kapağı ters çevrilerek toplandı. Alınan örnekler soğuk zincirde Dicle Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı Laboratuvarına ulaştırıldı.

Bakteriyolojik izolasyon için göz svapları tamponlanmış peptonlu su (Merck, 107228, Darmstadt, Germany) içerisinde 37 °C'de 18-24 saat aerobik koşullarda inkübe edildi. Sıvı kültürlerden alınan yaklaşık 10 µL (bir öze dolusu) kültür %5-7 koyun kanı içeren kanlı agara (Merck, 110886, Darmstadt, Germany) MacConkey agara (Merck, 05465, Darmstadt, Germany) ve eosin methylene blue agara (EMB agar) (Merck, 103858, Darmstadt, Germany) pasajlanarak 37 °C'de 18-24 saat aerobik olarak inkübe edildi (Markey ve ark. 2013). İnkübasyon sonrası oluşan karışık kültürler nutrient agar (Condalab, 1060, Madrid, Spain) ve %5-7 koyun kanlı agarda (Merck, 110886, Darmstadt, Germany) aynı koşullarda saflaştırıldı. İzole edilen suşlar Matris Destekli Lazer Desorpsiyon/İyonizasyon Uçuş Süresi Kütle Spektrometresi (VITEK MS MALDI-TOF-BioMerieux, Marcy l'Etoile, France) cihazına yüklendi ve elde edilen spektrumlar VITEK MS veri tabanında analiz edilerek identifiye edildi.

Mikolojik izolasyon için alınan svaplar kloramfenikol supplement [(0.05 mg/ml) Oxoid, SR0078, UK] eklenmiş sabouraoud dekstroz agara (SDA) (Oxoid, CM0041, UK) ekildi ve 25 °C'de 21 gün inkübe edildi. Besiyerleri üreme yönünden inkübasyon süresince her gün kontrol edildi. İnkübasyon süresi ve sonunda oluşan koloniler şekil, pigmentasyon, üreme süresi gibi özellikler yönünden değerlendirildi. Makroskobik inceleme sonrası kültürler selofan bant yöntemi ile mikroskobik olarak incelendi ve mikolojik suşların cins ve tür düzeyinde identifikasyonları tamamlandı (Arda 2000; Markey ve ark. 2013).

BULGULAR

Çalışma materyalini değişik yaş ve cinsiyette hipodromda yarış koşan ve at çiftliklerinde yarış koşmayan 56 (n=28 hipodrom, n=28 çiftlik) safkan İngiliz (n=11 hipodrom, n=9 çiftlik) ve Arap (n=17 hipodrom, n=19 çiftlik) atına ait 112 göz oluşturdu. Çalışma kapsamına alınan atlar 2-12 yaş aralığında, 32'si dişi ve 24'ü ise erkekti.

Çiftlikte bulunan yarış koşmayan 28 ata ait 56 göz florasının tamamında bakteriyolojik üreme (%100), 8 göz florasında ise ayrıca fungal üreme (%14.29) tespit edildi. Bu grupta toplam 109 bakteri, 8 fungal etken izole ve identifiye edildi. Çiftlikten alınan göz svaplarından elde edilen bakteriyel ve fungal etkenlerin dağılımı tablo 1'de gösterilmiştir.

Tablo 1: Çiftlik atlarından alınan svap örneklerinden izole ve identifiye edilen bakteriyel (n=109) ve fungal (n=8) etkenlerin dağılımı.

Table 1: Distribution of bacterial (n=109) and fungal (n=8) agents isolated and identified from swab samples taken from farm horses.

Bakteriyolojik ve Mikolojik Etken Adı	n	%
Bacillus cereus	23	21.10
Staphylococcus equorum	17	15.60
Enterococcus faecium	14	12.84
Enterobacter cloacae complex	13	11.93
Staphylococcus lentus	11	10.10
Staphylococcus xylosus	9	8.26
Kocuria kristinae	6	5.50
Enterococcus casseliflavus	5	4.49
Acinetobacter haemolyticus	4	3.67
Bacillus licheniformis	3	2.75
Bacillus pumilus	2	1.83
Klebsiella oxytoco	2	1.83
Aspergillus niger	6	75
Penicillium spp.	2	25

Hipodromda bulunan ve yarış koşan 28 ata ait 53 göz florasında bakteriyolojik üreme (%94.64), 15 göz florasında ise ayrıca fungal üreme (%28.57) tespit edildi. Bu grupta toplam 213 bakteri, 16 fungal etken identifiye ve izole edildi. Üç (%5.36) gözde herhangi bir bakteriyolojik ve fungal üreme belirlenmedi. Hipodromda bulunan atlardan alınan göz svaplarından elde edilen bakteriyel ve fungal etkenlerin dağılımı tablo 2'de özetlenmiştir.

Tablo 2: Hipodromda bulunan atlardan alınan svap örneklerinden izole ve identifiye edilen bakteriyel (n=213) ve fungal (n=16) etkenlerin dağılımı.
Table 2: Distribution of bacterial (n=213) and fungal (n=16) agents isolated and identified from swab samples taken from horses in the Hippodrome.

Bakteriyolojik ve Mikolojik Etken Adı	n	%
Staphylococuc vitulinus	46	21.60
Staphylococcus sciuri	46	21.60
Staphylococcus epidermidis	39	18.31
Staphylococcus lentus	35	16.43
Kocuria kristinae	18	8.45
Klebsiella pneumoniae	11	5.16
Rhizobium radiobacter	4	1.88
Bacillus thurigiensis	3	1.41
Alcaligenes faecalis	3	1.41
Acinetobacter haemolyticus	3	1.41
Escherichia coli	3	1.41
Sphingobacterium spiritivorum	2	0.94
Penicillium spp.	14	87.50
Rhizomucor spp.	1	6.25
Aspergillus niger	1	6.25

Hipodrom grubunun konjunktival svap örneklerinden izole ve identifiye edilen bakterilerin %87.79'unun (n=187) Gram pozitif, %12.21'inin (n=26) Gram negatif olduğu; çiftlik grubunda ise bakterilerin %82.56'sının (n=90) Gram pozitif, %17.43'ünün de (n=19) Gram negatif olduğu belirlendi.

TARTIŞMA VE SONUÇ

Yarış atlarında oküler hastalıkların ve komplikasyonlarının, yarış hayatını etkilediği kadar atların yaşam kalitesi ve değeri üzerinde büyük etkisi vardır. Atın refahını bozabilecek bu durumlarla ilişkili ağrıya ek olarak, kornea lezyonları görmeyi kısmi veya tamamen etkileyebilir. Bu durum atın yarış hayatının sonu olabilir ve binici için potansiyel risk oluşturabilir (Paschalis-Trela ve ark. 2017). Atlarda gözlerin anatomik yapısı, konumu ve çok belirgin olması nedeniyle kornea hasarına yatkındır. At yuvarlanırken, koşarken, yarışırken göze giren kum, küçük taş parçacıkları, toprak, saman gibi yabancı cisimler korneada yüzeysel veya derin hasarlar oluşturabilir. Bunun gibi ve/veya bağışıklık sisteminin zayıfladığı durumlarda konjunktival florada bulunan ve çevreden gelen mikroorganizmalar veya fungal etkenler tarafından enfekte olan kornea hasarı, tedavisi zor olabilen ve görme kaybına neden olabilen keratit, keratomikoz veya enfekte kornea ülserlerine yol açmaktadır (Andrew ve ark. 2003; Ferraira ve ark. 2017; Santibáñez ve ark. 2022). Atlarda akut aşamada ülseratif keratitin hem bakteri hem de mantar etkenlerden oluşan normal konjunktival floradan kaynaklandığı ifade edilmektedir (Johns ve ark. 2011; Hampson ve ark. 2019; Scott ve ark. 2019). Bu sebeple çalışmada konjunktival floranın etkilenmeyeceği şekilde oftalmik muayenede patoloji saptanmayan sağlıklı gözler ve son üç ayda herhangi bir antibiyotik tedavisi görmeyen sağlıklı atlar çalışma kapsamına alındı.

Atlarda konjunktival mikrofloranın detaylandırıldığı birçok çalışmada Gram pozitif bakterilerin fazla olduğu bildirilmiştir. (Andrew ve ark. 2003; Dass ve ark. 2013; Baran ve ark. 2015; Ferreira ve ark. 2017; Scott ve ark. 2019). *Staphylococcus spp.* ve *Bacillus spp.* konjunktival mikrofloranın bir parçasını oluşturan Gram pozitif mikroorganizmalar ve göz problemi olmayan atların oküler konjunktivalarından en sık izole edilen bakterilerdir (Baran ve ark. 2015; Ferraria ve ark. 2017; Soimala ve ark. 2018). Andrew ve ark. (2003) yaptıkları bir çalışmada *Staphylococcus spp.* ve *Bacillus spp.* belirlediklerini, ancak en fazla *Corynebacterium spp.*'u izole ettiklerini bildirmişledir. Bu çalışmada toplamda 21 farklı bakteri ve 3 farklı mantar türü tespit edildi. Her iki gruptan elde edilen izolatlarda da çoğunlukla Gram pozitif (%82.56 çiftlik grubu, %87.79 hipodrom grubu) bakterilerin ürediği gözlendi. Çiftlik grubunda en fazla *Staphylococcus spp.* ve *Bacillus spp.* türleri izole edilirken; hipodrom grubunda *Staphylococcus spp.* ve *Kocuria kristinae* bakterisi izole edildi. Tablo 1 ve Tablo 2'de belirtildiği gibi her iki grupta da belirlenen bakteri çeşitleri birbirinden farklıydı. Bu durumun atın barındığı ortam ile ilişkili veya hipodromda yarış koşmasına bağlı göze girebilecek toz, kum gibi yabancı cisimlere bağlı olduğu ifade edilebilir.

Konjunktivada Gram pozitif ve Gram negatif bakteriler gibi mantarların da mikrofloranın bir parçası olduğu düşünülmektedir. Atlarda fungal konjunktival floranın bilinmesi, keratomikoz gibi fungal oküler hastalıkların belirlenmesi açısından önem arz etmektedir (Khosravi ve ark. 2014; Zafarnaderi ve Araghi-Sooreh 2017; Hampson ve ark. 2019; Walsh ve ark. 2021). Jhons ve ark. (2011) İngiltere'de sağlıklı atlarda yaptıkları bir çalışmada, çalışma kapsamına alınan atların %13'ünde (n=8) *Mucor, Absidia* ve *Aspergillus spp.* türlerinin izole ettiklerini ifade etmişlerdir. İsviçre'de yapılan bir araştırmada (Voelter-Ratson ve ark. 2014) çalışma kapsamına alınan sağlıklı at gözlerinin %92'sinde fungal üreme belirlendiği ve en yaygın olarak *Alternaria, Eurotium, Rhizopus, Cladosporium, Aspergillus* ve *Penicillium spp.* izole edildiği öne sürülmektedir. Khosravi ve ark.'ları (2014) ise çalışmaya dahil ettikleri atların %77'sinde mikolojik üreme tespit ettiklerini ve en fazla *Aspergillus* (%19.9), *Rhizopus* (%15.9) *ve Penicillium spp.* (%15.1) izole ettiklerini bildirmişlerdir. Bu çalışmada hipodrom grubunda değerlendirmeye alınan gözlerin %14.29'unda (n=8), çiftlik grubunda ise %26.79'unda (n=16) mikolojik üreme belirlendi. Hipodrom grubunda *Penicillium spp.* (n=14, %87.5), *Rhizomucor spp.* (n=1, %6.25), *Aspergillus niger* (n=1, %6.25), çiftlik grubunda ise *Aspergillus niger* (n=6, %75), *Penicillium spp.* (n=2, %25) izole edildi. Khosravi ve ark. (2014) farklı at ırklarında yaptıkları bir çalışmada belirledikleri fungal izolatların birbirine benzer olduğunu ve bu durumun hayvan bakımı, iklim, yem vb. benzer çevresel faktörlerden kaynaklanabileceğini vurgulamışlardır. Bu çalışmada da belirlenen fungal izolatlar bibirine yakın belirlendi. Çalışmamızda rakım ve iklim gibi çevresel faktörler arasındaki fark minimize edilmeye çalışıldı. Ancak atların barındıkları ahırların ve yarış koşmanın nispeten oküler florayı etkilediği düşünülebilir.

Konjunktival flora coğrafi konum, iklim, mevsim, ahırın altlığının tipi, yaşadığı çevre, yem ve hayvancılık gibi birçok faktörden etkilenmektedir (Andrew ve ark. 2003; Jhons ve ark. 2011; Bonelli ve ark. 2014; Baran ve ark. 2015). Örneğin ılıman iklimlerde sağlıklı gözlerde

konjuntival mantar varlığının sıcak nemli iklimlerde bulunanlardan daha az olduğu bildirilmiştir (Stoppini ve ark. 2003; Barsotti ve ark. 2006). Barsotti ve ark. (2006) İtalya'da yaptıkları bir çalışmada Stoppini ve ark. (2003) yaptıkları çalışmaya atıf yaparak nemin en yüksek olduğu yaz aylarında çalışmalarını yapmış ve çalışmalarında *Aspergillus, Cladosporium, Mucor, Paecylomyces, Fusarium, Trichoderma, Drechslera, Alternaria, Candida spp.* ve *Cryptococcus sp*. izole ettiklerini bildirmişlerdir. Bu çalışmada oküler floranın iklim ve coğrafi konum gibi çevresel faktörlerden etkilenmemesi için çiftlik grubundaki atlar hipodroma yakın çiftliklerden seçildi. Ancak yaşadıkları ortam ve bakım şartlarının (çiftliklerde bulunan atların açık manejda birarada olması, hipodromlardaki atların diğer atlardan ari olmaları ve baş bölgesi ve tüm vücudun aynı havlu ve malzemelerle temizlenmesi) ve yarışın (yarış esnasında göze giren kum, toz vs.) göz florasındaki bakteri ve fungal çeşitliliğine etkisinin olduğu söylenebilir.

Sonuç olarak, sağlıklı atlarda gözün anatomik yapısı ve konumu yaralanma ve enfeksiyona predispozisyon oluşturmaktadır. Ayrıca sağlıklı atlarda konjunktival floranın birçok çevresel faktörden etkilendiği ve göz florasında bulunan gram pozitif, gram negatif ve fungal etkenlerin vücut direnci düştüğünde veya gözün anatomik yapılarında meydana gelebilecek lezyonlarda ciddi enfeksiyon ve hasarlar oluşturabileceği unutulmamalıdır. Gözün mikolojik ve bakteriyolojik mikroflorasının belirlenmesinin atlarda göz enfeksiyonlarının (konjunktivit, keratit vb) tedavisinin planlanması, enfeksiyonun kontrolünün sağlanması ve kornea defektlerine bağlı görme kayıplarının önlenmesinde önemli olacağı görüşündeyiz.

ÇIKAR ÇATIŞMASI

Yazarlar bu çalışma için herhangi bir çıkar çatışması olmadığını beyan ederler.

TEŞEKKÜR VE BİLGİLENDİRME

Diyarbakır Hipodromu At Hastanesi çalışanlarına yardımlarından dolayı teşekkür ederiz.

YAZAR KATKILARI

Fikir/Kavram: EÇ
Denetleme/Danışmanlık: EÇ, NKS
Veri Toplama ve/veya İşleme: EÇ, NKS
Analiz ve/veya Yorum: EÇ, NKS
Makalenin Yazımı: EÇ
Eleştirel İnceleme: EÇ, NKS

KAYNAKLAR

Andrew SE, Nguyen A, Jones GL, Brooks DE (2003). Seasonal effects on the aerobic bacterial and fungal conjunctival flora of normal thoroughbred brood mares in Florida. *Vet Ophthalmo,* 6 (1), 45-50.

Arda M (2000). Mantarların genel karakterleri. Arda M (Ed). Temel Mikrobiyoloji (s. 315-367). Medisan Yayınevi, Ankara.

Baran V, Özaydın İ, Genç O et al. (2015) The Effects of High and Low Altitudes on Conjunctival Flora in Sport and Work Horses: A Field Study in the Northeast Anatolia Region of Turkey (Kars and Iğdır). *Kafkas Univ Vet Fak Derg,* 21 (4), 477-481.

Barsotti G, Sgorbini M, Nardoni S, Corazza M, Mancianti F (2006). Occurrence of Fungi from Conjunctiva of Healthy Horses in Tuscany, Italy. *Vet Res Commun*, 30 (8), 903-906.

Bonelli F, Barsotti G, Attili AR et al. (2014). Conjunctival bacterial and fungal flora in Clinically normal sheep. *Vet Rec Open,* 1 (1), 1-5.

Dass SP, Neelam S, Kumar GR, Parul S (2013). Aerobic bacterial flora of the normal conjunctiva at high altitude area of Shimla Hills in India: a hospital study. *Int J Ophthalmol,* 6 (5), 723-726.

Ferreira ARA, Santana AF, Almeida ACVR et al. (2017) Bacterial culture and antibiotic sensitivity from the ocular conjunctiva of horses. *Ciência Rural, Santa Maria,* 47 (6), e20160753.

Fernández-Garayzábal JF, LaFrentz S, Casamayor A et al. (2022). Corynebacterium conjunctivae: A New Corynebacterium Species Isolated from the Ocular Surface of Healthy Horses. *Anim,* 12 (14), 1827.

Fraczkowska K, Zak-Bochenek A, Siwinska N, Rypula K, Ploneczka-Janeczko K (2022). Aerobic Commensal Conjunctival Microflora in Healthy Donkeys. *Anim,* 12 (6), 756.

Hampson ECGM, Gibson JS, Barot M, Shapter FM, Greer RM (2019). Identification of bacteria and fungi sampled from the conjunctivalsurface of normal horses in South-East Queensland, Australia. *Vet Ophthalmol*, 22 (3), 265-275.

J**ohns IC, Baxter K, Booler H, Hicks C, Menzies GN (2011)**. Conjunctival bacterial and fungal flora in healthy horses in the UK. *Vet Ophthalmol,* 14 (3), 195-199.

Khosravi AR, Nikaein D, Sharifzadeh A, Gharagozlou F (2014). Ocular fungal flora from healthy horses in Iran. *J Mycol Méd*, 24 (1), 29-33.

Laus F, Faillace V, Attili V et al. (2016) Conjunctival bacterial and fungal flora in healthy donkeys in Central Italy. *Large Animal Review*, 22 (3), 137-142.

Markey B, Leonard F, Archambault M, Cullinane A, Maguire D (2013). Clinical Veterinary Microbiology. 2th. Edition. Missouri, Mosby Elsevier, USA.

Paschalis-Trela K, Cywińska A, Trela J et al. (2017). The prevalence of ocular diseases in polish Arabian horses. *BMC Veterinary Research,* 13 (1), 319.

Santibáñez R, Lara F, Barros TM et al. (2022). Ocular Microbiome in a Group of Clinically Healthy Horses. *Anim,* 12 (8), 943.

Scott EM, Arnold C, Dowell S, Suchodolski JS (2019). Evaluation of the bacterial ocular surface microbiome in clinically normal horses before and after treatment with topical neomycin-polymyxin-bacitracin. *Plos One*, 14 (4), e0214877.

Soimala T, Lübke-Becker A, Schwarz S et al. (2018). Occurrence and molecular composition of methicillin-resistant *Staphylococcus* aureus isolated from ocular surfaces of horses presented with ophthalmologic disease. *Vet Microbiol*, 222, 1–6.

Stoppini R, Barbasso E, Peruccio C, Ratto A, Gallo G (2003). Cheratomicosi equina in Italia Settentrionale: 13 casi clinici (1998–2002). *Ippologia,* 4, 13–28.

Walsh ML, Meason-Smith C, Arnold C, Suchodolski JS, Scottl EM (2021) Evaluation of the ocular surface mycobiota in clinically normal horses. *Plos One*, 16 (2), e0246537.

Voelter-Ratson K, Unger L, Spiess BM, Simon A (2014). Evaluation of the conjunctival fungal flora and its susceptibility to antifungal agents in healthy horses in Switzerland. *Vet Ophthalmol,* 17 (1), 31-36.

Zafarnaderi S, Araghi-Sooreh A (2017). Ocular fungal flora in healthy donkeys in Iran. *Veterinary Journal of Equine Sciences,* 1 (1), 1-6.

Zak A, Siwinska N, Slowikowska M et al. (2018). Conjunctival aerobic bacterial flora in healthy Silesian foals and adult horses in Poland. *BMC Veterinary Research,* 14 (1), 261.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Kara E, Kara H (2023).** Study of the Effects of Modified Colostrum Feeding Method on Passive Transfer Success in New-born Calves and Comparison with the Classical Method. *Van Vet J*, 34 (2), 113-118. DOI: https://doi.org/10.36483/vanvetj.1247020

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Study of the Effects of Modified Colostrum Feeding Method on Passive Transfer Success in New-born Calves and Comparison with the Classical Method

Erdal KARA[1,*] Halime KARA[2]

[1] *Kırıkkale University, Faculty of Veterinary Medicine, Department of Internal Medicine, 71450, Kırıkkale, Türkiye*
[2] *Ankara Yıldırım Beyazıt University, Health Services Vocational School, Laboratory and Veterinary Health Program, 06760, Ankara, Türkiye*

Received: 03.02.2023 Accepted: 18.05.2023

ABSTRACT In this study, it was aimed to evaluate a new colostrum feeding protocol in terms of passive transfer success. In the study, 70 calves each from two different farms with similar characteristics except colostrum feeding protocols were used. According to the modified method, new-born calves were given as much colostrum as the calf could drink, once every 3 hours, a total of 5 times in the first 12 hours. After 12 hours, as in the classical method, 2.5 liters of colostrum was given to the calves in the morning and evening, and then the calves were fed with milk. The amount of colostrum consumed by the calves fed with the modified method at each meal was recorded, and the blood IgG levels were measured using the ELISA method. In the first 5 feedings made in the modified method, the calves drank an average of 5.51 L colostrum in the first 12-hour period, 2.52 L in the first feeding, 0.86 L in the second feeding, 0.52 L in the third feeding, 0.98 L in the fourth feeding, and 0.63 L in the fifth feeding, respectively. While the mean blood IgG level of the calves in the modified colostral feeding group was 37.33 mg/ml, it was measured as 31.04 mg/ml in the classical colostral feeding group. As a result, with this difference made in the colostrum feeding method, the blood IgG levels of the calves reached a significantly higher level compared to the classical method.

Keywords: *Calf, Colostrum, Cow, IgG, Passive immune transfer.*

ÖZ

Yeni Doğan Buzağılarda Modifiye Kolostrum Besleme Yönteminin Pasif Transfer Başarısına Etkilerinin Araştırılması ve Klasik Metot ile Karşılaştırılması

Bu çalışmada, yeni bir kolostrum besleme protokolünün pasif transfer başarısı yönünden değerlendirilmesi amaçlanmıştır. Çalışmada kolostrum besleme protokolleri dışındaki özellikleri benzer olan iki farklı çiftlikten 70' er buzağı kullanılmıştır. Modifiye metotta, yenidoğan buzağılara her 3 saatte bir, ilk 12 saatte toplam 5 kez buzağının içebildiği kadar kolostrum içirilmiştir. 12 saat sonraki beslemelerde klasik metotta olduğu gibi sabah ve akşam 2.5 litre kolostrum ve devamında sütle besleme yapılmıştır. Modifiye metotla beslenen buzağıların her öğünde içtikleri kolostrum miktarları kaydedilmiş, kan IgG seviyeleri ise ELISA metodu kullanılarak ölçülmüştür. Modifiye metotta yapılan ilk 5 beslemede sırasıyla; birinci beslemede 2.52 L, ikinci beslemede 0.86 L, üçüncü beslemede 0.52 L, dördüncü beslemede 0.98 L ve beşinci beslemede 0.63 L olmak üzere ilk 12 saatlik periyotta toplamda ortalama olarak 5.51 L kolostrum içmişlerdir. Modifiye kolostral besleme grubundaki buzağıların ortalama kan IgG seviyesi 37.33 mg/ml olarak ölçülürken, klasik kolostral besleme grubunda ise 31.04 mg/ml olarak ölçülmüştür. Sonuç olarak kolostrum besleme yönteminde yapılan bu farklılık ile klasik metoda göre buzağıların kan IgG seviyelerinin önemli ölçüde yüksek seviyeye ulaşmıştır.

Anahtar Kelimeler: *Buzağı, İnek, IgG, Kolostrum, Pasif immun transfer.*

INTRODUCTION

In a successful colostral passive immune transfer, the calf's serum IgG concentration is expected to rise above 10 g/L between 32-48 hours (Godson et al. 2003). In order for passive immunity to be successful, it is recommended to pay attention to the conditions called 3Q (Quality, Quantity, Quickly) in colostrum intake (Azkur and Aksoy 2018). The success of passive transfer achieved through colostrum can be affected by various factors, including the quality and quantity of colostrum used in feeding, the method of feeding, and the timing of feeding (Kara and Ceylan 2021).

The quality of colostrum is determined mainly by the level of IgG, which constitutes 85% of colostral Ig, and more specifically, of colostral Ig alone (Godden 2008). The quality of colostrum should be assessed after each birth, ensuring that only quality colostrums are used for feeding and cryopreservation (Kaygısız and Köse 2007). The

✉ *Corresponding author: erdalkara@kku.edu.tr

timing of colostrum administration is of great importance in the success of passive transfer. Since intestinal epithelial cells do not mature in the first few hours of life in new-born calves and have a vesicular/vacuole structure, Ig's and other macromolecules are absorbed with maximum efficiency within the first 4 hours without changing (Chigerwe et al. 2009). While it decreases to 12 hours due to structural and chemical changes, it decreases to a minimum in 24 hours (Quigley et al. 2002; Godson et al. 2003; Gökçe and Erdoğan 2013). Another reason for the rapid delivery of colostrum is that the quality of colostrum decreases over time. Structural differences separating colostrum from milk disappear and it turns into normal milk formation (Quigley et al. 2013). In colostrum collected 6 hours, 10 and 14 hours after birth, compared to colostrum collected at 0 hours, there was a 17%, 27% and 33% decrease in Ig levels, respectively (Moore et al. 2005). At the same time, while Ig absorption capacity is close to 100% immediately after birth, it decreases to 50% after 6 hours, to 33% after 8 hours, and comes to a standstill after 24 hours (Cortese 2009). The method of administration of colostrum is important in terms of passage to the intestines and the amount of administration. Calf can be fed by colostrum directly from its mother, or it can be given with a bottle or through esophageal tube (Gökçe and Erdoğan 2013). Natural absorption is known to cause passive transfer failure. Calves cannot get enough colostrum and the first sucking period is delayed for various reasons, such as weak maternal instinct and structural defects in the udder (Weaver et al. 2000; Godson et al. 2003; Bilal 2007). The esophageal tube is a great convenience for those who do not want to suck, but it prolongs the passage to the intestines as it prevents the formation of the sulcus esophagicus (Lateur-Rowet and Breukink 1983). In bottle feeding, the sucking reflex is stimulated and colostrum reaches the abomasum directly thanks to the sulcus oesophagicus, the first sucking time is shortened and the amount of delivery can be determined exactly (Godden et al. 2009a).

Situations, where colostral passive immune transfer fails, are called passive transfer failure (PTF) (Şentürk 2012). The reference value in passive transfer failure is that the IgG concentration in the blood serum of the calf remains below 10 mg/ml in the 24-48 hours postpartum (Godden 2008; Çakıroğlu et al. 2010). PTF is not a disease, it is a condition that predisposes calves to the development of diseases (Weaver et al. 2000). PTF causes an increase in morbidity and mortality rates in the first 6 months of age, especially in the neonatal period. It is a management problem that cannot be ignored in terms of farm productivity and profitability, as it can lead to many consequences such as decreased feed efficiency and daily live weight gains, prolongation of the first calving age, decreased milk yield in the first two lactations and accordingly increased cattle removal from the herd. (Weaver et al. 2000; Godden 2008).

Every stage of herd management in professional enterprises is carried out within the framework of pre-formed protocols. However, it is not possible for veterinarians to follow every practice in large-scale farms, which can only be managed with a large number of workers. For this reason, in the creation of preferred protocols for herd management, the protocol should be applicable and auditable as well as being intended to provide maximum benefit. In addition, it should not be forgotten that the risks posed by the applications and the extra stress load may have adverse effects on health and passive transfer success. In this study, it was aimed to investigate new colostrum feeding strategies in order to minimize the cases of passive transfer failure due to delay in emptying of the abomasum due to human error, extra stress during calf forcing and colostrum feeding processes and excessive fullness in large-scale farms.

MATERIAL AND METHODS

This study was carried out with the permission of the Kırıkkale University Animal Experiments Local Ethics Committee at the meeting numbered 2021/01 on 21.01.2021, decision numbered 01.

Animal Material

The material of the study consisted of cows fed in two different dairy farms in Ankara. The classical feeding and modified feeding groups that are the subject of the study are also the routine feeding protocols of different farms. The calves included in the study from the first farm formed the “classical colostrum feeding group”. In the classical colostrum feeding group, calves were given 10% of their body weight in colostrum within the first 4 hours after birth, and then 2.5 L milk was fed twice a day in the morning and evening. Bottle feeding was normally used, and calves that did not receive the targeted amount of colostrum were fed with an esophageal tube. In the modified colostrum feeding group, colostrum was given as much as the calf could drink voluntarily at each feeding. In the second farm, a different colostrum feeding protocol was created and a "modified feeding" trial was carried out. According to this protocol, calves were fed with colostrum within the first 1 hour of birth, and in the following period, colostrum was given every 3 hours (at 3, 6, 9, and 12 hours) in the first 12 hours, a total of 5 times and in quantities that the calf could drink. The aforementioned modified feeding protocol is a protocol created by the farm against passive transfer failures due to worker errors and aiming the calf to receive maximum colostrum voluntarily. In both groups, 2.5 L (1 bottle) of milk was fed twice daily, in the morning and evening, after the 12th hour. In both groups, calves who could not stand alone were weak, had no sucking reflex, had a difficult birth, and were born by cesarean were excluded from the study.

Work on both farms started on the same date. It was aimed to minimize the effect of climatic conditions on the work with the study that started simultaneously in the farms located in the same geographical region. Both farms were large farms with more than 500 dairy cows. The dry period and new-born protocols of these farms are similar. Holstein cows were used in both farms included in our study. In both farms, animals are taken to the dry period 2 months before birth. In order to protect newborns from calf diarrhea, cows are vaccinated against calf diarrhea agents twice during the dry period. Cows are taken to the birth chambers 1 week before the birth. After birth, the mother is allowed to dry the calf by licking it, then they are fed for 24 hours in a separate area in the delivery room. After 24 hours, the calves are separated and placed in the calf huts. Colostrum feedings in both farms, keeping records and notifying the veterinarians of the calves to be administered esophageal tubes were made by the workers working in the delivery room. 70 calves from both farms were included in the study.

Collection of samples and data

Blood samples were collected from V. Jugularis using an 18-gauge needle (Beybi®, Istanbul, Turkey) in the 32-48 hour age range of the calves and were taken into red-capped serum tubes (Ayset®, Adana, Turkey). The

collected blood was centrifuged at 3000 rpm for 10 minutes to obtain serum and stored in – 20 °C freezers. The amount of colostrum taken by the calves during the first 12 hours in the farm where the modified colostrum was fed was recorded by the delivery room personnel. In the case of using esophageal tube in the farm where conventional feeding was done, the calves were recorded.

The amount of colostrum used in the feeding of the calves fed with the classical method was not recorded, and the amount to be given to the farm personnel was informed and no intervention was made afterward.

IgG Analysis

IgG concentrations of serum samples were determined using a commercial ELISA kit (Bovine IgG ELISA Kit, Biox, Belgium). Analyzes were performed in Diagen Diagnostic laboratories according to the application principles of the commercial kit.

Statistical Analysis

Statistical analyses were performed with SPSS (Version 17.0; Chicago, IL). For continuous data normality distribution and variance homogeneity assumptions, Kolmogorov Smirnov test was performed. Descriptive statistics were calculated as mean and standard deviation minimum and maximum. Independent samples t-test (Indipendent Samples Test) was applied to the parameters to analyze the difference between the groups since the data were in accordance with the normal distribution. $p<0.05$ was accepted as the limit of significance.

Before statistical analysis, normality analyses were performed to see if the distribution of the data was normal, and the Independent Sample T-Test was applied to the data showing normal distribution.

RESULTS

Within the study, two different colostral feeding methods were compared. The first feeding after birth was taken as the 1st feeding, and feeding was made every 3 hours after the birth, forming the 2nd, 3rd, 4th, and 5th feedings. In the first 12-hour period, the modified colostrum feeding group was fed 5 times, while the classical colostrum feeding group was fed 2 times, at the 0th and 12th hours. In the modified colostrum feeding group, the amount of colostrum that the calf can drink voluntarily was given at each feeding; They drank an average of 5.51 L colostrum in the first 12-hour period, 2.52 L in the first feeding, 0.86 L in the second feeding, 0.52 L in the third feeding, 0.98 L in the fourth feeding, and 0.63 L in the fifth feeding (Table 1).

Within the study, 70 calves from both groups were evaluated. While the mean blood IgG level of the calves in the modified colostral feeding group was 37.33 mg/ml, it was measured as 31.04 mg/ml in the classical colostral feeding group. With this difference made in the colostrum feeding method, the blood IgG levels of the calves reached a significantly higher level compared to the classical method ($p<0.05$) (Table 2).

It was observed that blood IgG levels of all calves in both groups were above 10 mg/ml, which is the threshold value for passive transfer failure (PTF).

Table 1: Average amount of colostrum consumed in the first 12 hours in calves in the modified colostrum feeding (MCF) group.

Group	1. Feeding	2. Feeding	3. Feeding	4. Feeding	5. Feeding	Total
MCF	2.52	0.86	0.52	0.98	0.63	5.51

Table 2: Comparison of IgG levels between groups.

	Group	N	Mean	SD	SE	p
Serum IgG	MCF	70	37.33	13.68	1.63	**0.003**
	CCF	70	31.04	10.71	1.28	

*MCF: Modified colostral feeding, CCF: Classical colostral feeding.

DISCUSSION AND CONCLUSION

Successful immunity is provided by colostrum in calves born with agammaglobulinemic (Dewell et al. 2006; Godden 2008; Gökçe and Erdoğan 2013). In a successful colostral passive immune transfer, the calf's serum IgG concentration is expected to rise above 10 g/L between 32-48 hours (Godson et al. 2003). In this study, the serum IgG concentration of all calves in the modified colostrum feeding and classical colostrum feeding groups was well above 10g/L and it was determined that a successful passive transfer was achieved. This is an indication that the newborn protocols on both farms were successful.

In order for passive immunity to be successful, it is recommended that care should be taken to ensure the Quality, Quantity and Quickly conditions, which are called the 3Q formula, in colostrum intake (Azkur and Aksoy 2018). It is known that in the first 4 hours of life, Ig's are absorbed unchanged in relation to the vesicular/vacuolated structure of the intestines (Chigerwe et al. 2009). It has been determined that the absorption of Ig decreases over time due to the development of the intestines, gaining digestive properties, increased protease activity and increased acidity in the abomasum. For this reason, while the absorption of Ig is at its maximum level in the first 4 hours, it decreases in the 12th hour (Quigley 2007). In a study, it was reported that the intake of equal amounts of colostrum at 1, 2, 3 and 4 hours did not cause a change in the serum IgG ratio (Chigerwe et al. 2009). It was determined that it decreased to a minimum level in the first 24 hours (Quigley 2002; Godson et al. 2003; Gökçe and Erdoğan 2013). In another study, the amount of IgG needed by the calf for successful passive transfer was found to be 150-200gr at the 2nd hour, 164-226g at the 6th hour, and 185-309gr at the 12th hour after birth (Chigerwe et al. 2008). In this study, serum IgG ratio was measured as 37.33 mg/L in the modified model. In the classical colostrum feeding method, the average IgG value was measured as 31.04 mg/L (Table 2). When the models were compared, the ability of the modified model to transfer more IgG to the serum was found to be significantly higher than the classical model ($p<0.05$).

Frequent feeding during periods of higher intestinal absorption in modified feeding with a significantly higher serum IgG ratio is thought to be effective.

There is an accepted view that increasing the amount of colostrum to be given to the calf will also increase the total amount of Ig reaching the intestines, regardless of its quality, and will affect the success of its passive transfer. (Gökçe and Erdoğan 2013). However, it has been reported that the increase of colostrum above a certain rate will cause a negative effect, and the amount given after a certain amount of colostrum has a negative correlation with the absorption ability (Conneely et al. 2014). It is thought that reaching saturation is effective in the transport of macromolecules (Stott et al. 1979). In a study, when the colostrum IgG concentration was above 20g/L, it was observed that 1 liter of colostrum increased the serum IgG ratio more than the equivalent 2L colostrum in terms of IgG (Stott and Fellah 1983). In the study by Conneely et al. 7%, 8.5% and 10% of live weight of colostrum was given to all groups by tube within the first 2 hours after birth. It was determined that the IgG concentration measured at the 24th hour in calves was significantly higher in those who ingested 8.5% of the live weight colostrum. In this study, calves in the modified group received an average of 6.3% of their weight in colostrum within the first hour. The second feeding was given after 3 hours, that is, within 4 hours of birth. With the second feeding, an average of 8.45% of the total body weight of colostrum was given within the first 4 hours. In the first 4 hours, calves fed with the modified method received 8.45% of their weight, and an estimated 10% of their weight in the classical method, and the IgG level was found to be higher in the group that received 8.45% colostrum in line with Conneely 2014.

In studies on colostrum quality, it has been determined that the Ig concentration in colostrum is at its highest level after birth and begins to decrease gradually after birth (Baumrucker et al. 2010; Conneely et al. 2013). In a study, it was reported that the IgG concentration in colostrum decreased by 3.7% every hour after birth (Morin et al. 2010). In another study, it was reported that delaying colostrum expression for 6, 10, and 14 hours after delivery caused a decrease in the amount of IgG in colostrum by 17%, 27%, and 33%, respectively (Moore et al. 2005). In the present study, the calves fed with the modified model were fed every 3 hours, but the mothers were not milked every 3 hours, the colostrum obtained at the first milking was pooled, and the first milking colostrum of each newly born mother was included in the feeding. For this reason, although the feedings were done every 3 hours, in fact, during the first 12 hours, the first milking was fed with colostrums with the highest IgG level in five feedings. Therefore, it was not affected by the colostrum quality, which is expected to decrease with the next hour.

In addition to the rate, quantity and quality of colostrum delivery, the methods of delivery of colostrum are also of great importance in the success of passive transfer. The method of administration directly affects the success of passive immune transfer, as it determines the time of colostrum reaching the intestines and the amount of colostrum consumed (Godden 2008). Colostrum can be given to the calf directly from mother by sucking the udder, bottle or with an esophageal tube (Gökçe and Erdoğan 2013). It is not recommended for the calf to take colostrum from its mother by natural sucking method, as it causes a high rate of passive immune transfer failure (Weaver et al. 2000). In studies, it has been determined that passive transfer failure is seen at high rates such as 22.3-61% in the natural breastfeeding method (Besser et al. 1991; Filteau et al. 2003; Trotz-Williams et al. 2008; Morrill et al. 2012; Quigley et al. 2013). Although feeding using the esophageal tube is seen as a convenience in the feeding of calves that do not want to suckle, milk goes to the rumen because it does not stimulate the closure of the sulcus esophagus. It will be delayed by 2-4 hours for the milk that goes to the rumen first to pass into the intestines (Lateur-Rowet and Breukink 1983). In one study, 1.5L colostrum substituted calves fed with an esophageal tube and bottle showed an increase of 27.6% and 26% in serum IgG concentration and colostrum absorption ability in bottle fed calves, respectively (Godden et al. 2009b). In addition, it has been stated that the possibility of adverse events such as esophageal and pharyngeal trauma, aspiration pneumonia and stress is high during esophageal tube applications (Chigerwe et al. 2012). Various studies have been conducted on whether hyperadrenalemia caused by various stresses in newborns may suppress colostral immunoglobulin absorption or terminate intestinal permeability (Stott 1980). While colostrum consumption can be reduced by 74% in the first 12 hours in calves with fetal stress (Vermorel et al. 1989), it has been found that severe acidosis reduces colostrum uptake by 52% and serum IgG concentration by 35% (Vermorel et al. 1989; Drewry et al. 1999). Heat stress in calves has also been associated with low serum IgG concentrations through reduced calf viability or reduced intestinal permeability (Stott et al. 1976; Stott 1980; Donovan et al. 1986). In this study, it was thought that feeding with an esophageal tube may also create a stress factor. In addition, due to the use of dirty environmental equipment, it can colonize my digestive system and cause the closure of the intestines (Quigley et al. 1998). Although the use of esophageal tube was not required in any of the calves fed with the classical method in the studies, the stress factor was partially reduced, but forcing the calf to receive the targeted amount in the first feeding is a source of stress on the calf. Considering that stress and increase in cortisol levels negatively affect Ig absorption, it can be thought that modified feeding reduces stress factors. In this study, it is thought that calves fed with the modified method are effective in significantly ($p<0.05$) high serum IgG concentrations.

The biggest advantages of giving colostrum with a bottle are; since the sucking reflex is stimulated, colostrum can be directly transferred to the abomasum by forming the sulcus esophagicus as in natural sucking, the amount of colostrum given can be controlled, the calf is not waited for colostrum sucking, it can be applied as soon as it is born (Godden et al. 2009a). In the first 4 hours of maximum absorption, a calf with a birth weight of 43 kg should receive 100 g of IgG in order to achieve a sufficient level of serum IgG by successful passive immune transfer. In order to achieve this value, an average of 4 liters of colostrum with a concentration of 25 g/L should be taken, while it would be sufficient to take 2 liters from a colostrum containing 50 g/L IgG and only 1 liter of colostrum with a concentration of 100 g/L IgG (Gökçe and Erdoğan 2013). It has been reported that the second application within the first 12 hours following the administration of colostrum in newborn calves causes an increase in the serum IgG ratio (Morin et al. 1997). In one study, it was shared that ≥3 liters of colostrum in the first 4 hours and 1 more liter of colostrum within 12 hours would be sufficient for a successful passive transfer (Chigerwe et al. 2009).

A decrease in the absorption efficiency of IgG is seen when calves are fed large volumes of colostrum in a single feed

(Mokhber-Dezfooli et al. 2012). This is due to mechanical swelling of the abomasum and other anterior stomachs, resulting in a reduction in emptying of the abomasum (Mokhber-Dezfooli et al. 2012). Abomasal emptying rate is a factor affecting colostral IgG absorption (Mokhber-Dezfooli et al. 2012). Slower abomasum emptying may result in decreased absorption of colostral components (Sakai et al. 2012). Chigerwe et al. (2008) reported that calves fed 4 L colostrum had a reduced serum IgG concentration at 48 h compared to calves fed 3 L colostrum. Jaster et al. (2005) found that the serum IgG concentration at the 48th hour was 38.6 g/L and 45.6 g/L, respectively, in two groups given 4 liters of colostrum immediately after birth, 2 liters of colostrum immediately after birth, and 2 liters of colostrum at the 12th hour. In this study, 4 liters of colostrum in the classical model was drunk with the help of a bottle. It has been understood that the amount of colostrum given in the classical method delays the emptying of the abomasum and prolongs the transit time to the intestines due to the administration of more colostrum than the volume of the abomasum. For this reason, it is thought that the serum IgG ratio in the modified method is higher.

In the classical colostrum feeding method, which is effectively applied today, calves given 10% of their weight in colostrum make their next feeding 12 hours later. Colostrum can be affected by factors such as the quality and quantity of colostrum used in feeding, the method of administration and the time of administration for passive immune transfer success. This study was conducted on two different farms with similar newborn protocols except for the colostrum feeding method. As a result of the study, it was seen that giving as much milk as the calf can drink every 3 hours caused the serum IgG concentration to be significantly higher ($p<0.05$) compared to the classical method. Considering the results of this study, it was seen that the quality of colostrum milked for the second feeding in the classical method in the classical method decreased after 12 hours, the calf's colostrum absorption decreased, the attempts to make the calf drink colostrum involuntarily in the first feeding disrupted the absorption by causing stress, and giving a high amount of colostrum at one time delayed the emptying of the abomasum. However, it should be noted that this study is not an experimental study, the amount of colostrum used in each feeding is not recorded in the classical method, and the colostrum quality and total IgG mass given are not measured. In addition, the modified feeding method can be applied practically in large-scale farms with a large number of animals, where fresh colostrum can be reached continuously, who employ personnel whose sole duty is the care and feeding of calves, and its practical application in smaller-scale farms such as extra personnel, labor and continuous colostrum thawing. It should be noted that it is not suitable as it will require processes. This method is designed to minimize worker errors, eliminate extra stress on calves and ensure constant control of calves in farms with a large number of animals and difficult to control new-born protocols. More precise results can be obtained by conducting an experimentally controlled trial of this method, which has been tested as a field study.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENT

We would like to thank the technical staff of Bala Agriculture and Has Başak Farm for collecting the samples, Prof. Dr. Mikail ASLAN for statistical analysis and lecturer Mustafa GÜVEN for his contribution to the compilation of the data and translation of the manuscript.

AUTHOR CONTRIBUTIONS

Idea / Concept: EK, HK
Supervision / Consultancy: EK, HK
Data Collection and / or Processing: EK, HK
Analysis and / or Interpretation: EK, HK
Writing the Article: EK, HK
Critical Review: EK, HK

REFERENCES

Azkur AK, Aksoy E (2018). Buzağı Hastalıklarında Koruyucu Önlemler. *Lalahan Hay Araşt Enst Derg*, 58 (3), 56-63.

Baumrucker CR, Burkett AM, Magliaro-Macrina AL, Dechow CD (2010). Colostrogenesis: Mass transfer of immunoglobulin G1 into colostrum. *J Dairy Sci,* 93 (7), 3031-3038.

Besser TE, Gay CC, Pritchett L (1991). Comparison of three methods of feeding colostrum to dairy calves. *JAVMA,* 198 (3), 419-422.

Bilal T (2007). Yeni Doğanların İç Hastalıkları. I. Baskı. İstanbul Üniversitesi Basım ve Yayın Evi Müdürlüğü, İstanbul.

Chigerwe M, Tyler JW, Schultz LG et al. (2008). Effect of colostrum administration by use of oroesophageal intubation on serum IgG concentrations in Holstein bull calves. *AVMA,* 69 (9), 1158-1163.

Chigerwe M, Tyler JW, Summers MK et al. (2009). Evaluation of factors affecting serum IgG concentrations in bottle-fed calves. *JAVMA,* 234 (6), 785-789.

Chigerwe M, Coons DM, Hagey JV (2012). Comparison of colostrum feeding by nipple bottle versus oroesophageal tubing in Holstein dairy bull calves. *JAVMA,* 241 (1), 104-109.

Conneely M, Berry DP, Sayers R et al. (2013). Factors associated with the concentration of immunoglobulin G in the colostrum of dairy cows. *Animal*, 7 (11), 1824-1832.

Conneely M, Berry DP, Murphy JP et al. (2014). Effect of feeding colostrum at different volumes and subsequent number of transition milk feeds on the serum immunoglobulin G concentration and health status of dairy calves. *J Dairy sci,* 97 (11), 6991-7000.

Cortese VS (2009). Neonatal Immunology. *Vet Clin North Am Food Anim Pract*, 25 (1), 221-227.

Çakıroğlu D, Meral Y, Pekmezci D, Onuk EE, Gökalp G (2010). Yeni doğan buzağılarda çeşitli hematolojik ve biyokimyasal parametreler ile kolostral immun globulinler arasındaki ilişkinin belirlenmesi. *F.Ü.Sağ.Bil.Vet.Derg.*, 24 (1), 43-46.

Dewell RD, Hungerford LL, Keen JE et al. (2006). Association of neonatal serum immunoglobulin G1 concentration with health and performance in beef calves. *JAVMA,* 228 (6), 914-921.

Donovan GA, Badinga L, Collier RJ, Wilcox CJ, Braun RK (1986). Factors influencing passive transfer in dairy calves. *J Dairy Sci,* 69 (3), 754-759.

Drewry JJ, Quigley 3rd JD, Geiser DR, Welborn MG (1999). Effect of high arterial carbon dioxide tension on efficiency of immunoglobulin G absorption in calves. *AVMA,* 60 (5), 609-614.

Filteau V, Bouchard É, Fecteau G, Dutil L, DuTremblay D (2003). Health status and risk factors associated with failure of passive transfer of immunity in newborn beef calves in Quebec. *CVMA*, 44 (11), 907.

Godden S (2008). Colostrum management for dairy calves. *Vet Clin North Am Food Anim Pract,* 24 (1), 19-39.

Godden SM, Haines DM, Hagman D (2009a). Improving passive transfer of immunoglobulins in calves. I: Dose effect of feeding a commercial colostrum replacer. *J Dairy Sci,* 92 (4), 1750-1757.

Godden SM, Haines DM, Konkol K, Peterson J (2009b). Improving passive transfer of immunoglobulins in calves. II: Interaction between feeding method and volume of colostrum fed. *J Dairy Sci,* 92 (4), 1758-1764.

Godson DL, Acres SD, Haines DM (2003). Failure of passive transfer and effective colostrum management in calves. *Large Animal Veterinary Rounds*, 3 (10), 1-6.

Gökçe E, Erdoğan HM (2013). Neonatal buzağılarda kolostral immunoglobulinlerin pasif transferi. *Turkiye Klinikleri J Vet Sci*, 4 (1), 18-46.

Jaster EH (2005). Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G1 absorption in Jersey calves. *J Dairy Sci,* 88 (1), 296-302.

Kara E, Ceylan E (2021). Failure of passive transfer in neonatal calves in dairy farms in Ankara region. *Turkish J Vet Anim*, 45 (3), 556-565.

Kaygisiz A, Köse M (2007). The quality of colostrum and its effects on calves growth characteristics in holstein cattle. *J Agric Sci*, 13 (04), 321-325.

Lateur-Rowet HJM, Breukink HJ (1983). The failure of the oesophageal groove reflex, when fluids are given with an oesophageal feeder to newborn and young calves. *Vet Q,* 5 (2), 68-74.

Mokhber-Dezfooli MR, Nouri M, Rasekh M, Constable PD (2012). Effect of abomasal emptying rate on the apparent efficiency of colostral immunoglobulin G absorption in neonatal Holstein-Friesian calves. *J Dairy Sci,* 95 (11), 6740-6749.

Moore M, Tyler JW, Chigerwe M, Dawes ME, Middleton JR (2005). Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. *JAVMA*, 226 (8), 1375-1377.

Morin DE, McCoy GC, Hurley WL (1997). Effects of quality, quantity, and timing of colostrum feeding and addition of a dried colostrum supplement on immunoglobulin G1 absorption in Holstein bull calves. *J Dairy Sci,* 80 (4), 747-753.

Morin DE, Nelson SV, Reid ED et al. (2010). Effect of colostral volume, interval between calving and first milking, and photoperiod on colostral IgG concentrations in dairy cows. *JAVMA*, 237 (4), 420-428.

Morrill KM, Conrad E, Lago A et al. (2012). Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. *J Dairy Sci,* 95 (7), 3997-4005.

Quigley III JD, Drewry JJ (1998). Nutrient and immunity transfer from cow to calf pre-and postcalving. *J Dairy Sci,* 81 (10), 2779-2790.

Quigley JD, Lago A, Chapman C, Erickson P, Polo J (2013). Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum. *J Dairy Sci,* 96 (2), 1148-1155.

Quigley J, Hammer CJ, Russel LE, Polo J (2002). Passive immunity in newborn calves. *Advances in dairy technology*, 14, 273-292.

Sakai RR, Coons DM, Chigerwe M (2012). Effect of single oroesophageal feeding of 3 L versus 4 L of colostrum on absorption of colostral IgG in Holstein bull calves. *Livest Sci,* 148 (3), 296-299.

Stott GH (1980). Immunoglobulin absorption in calf neonates with special considerations of stress. *J Dairy Sci, 63* (4), 681-688.

Stott GH, Fellah A (1983). Colostral immunoglobulin absorption linearly related to concentration for calves. *J Dairy Sci,* 66 (6), 1319-1328.

Stott GH, Marx DB, Menefee BE, Nightengale GT (1979). Colostral immunoglobulin transfer in calves. III. Amount of absorption. *J Dairy Sci,* 62 (12), 1902-1907.

Stott GH, Wiersma F, Menefee BE, Radwanski FR (1976). Influence of environment on passive immunity in calves. *J Dairy Sci*, 59 (7), 1306-1311.

Şentürk S (2012). Buzağıların İç Hastalıkları. II. Baskı. F Özsan Matbacılık, Bursa.

Trotz-Williams LA, Leslie KE, Peregrine AS (2008). Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. *J Dairy Sci,* 91 (10), 3840-3849.

Vermorel M, Vernet J, Saido Dardillat C, Demigne C, Davicco MJ (1989). Energy metabolism and thermoregulation in the newborn calf; effect of calving conditions. *Can J Anim Sci,* 69 (1), 113-122.

Weaver DM, Tyler JW, VanMetre DC, Hostetler DE, Barrington GM (2000). Passive transfer of colostral immunoglobulins in calves. *ACVIM*, 14 (6), 569-577.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Belhan S, Çibuk S, Kömüroğlu AF, Altındağ F, Değer Y (2023).** Evaluation of the Effect of Ferula Rigidula Extract on Sperm Parameters, Antioxidant Parameters and Testicular Structure in Male Rats in Experimental Diabetic Condition. *Van Vet J*, 34 (2), 119-125. DOI: https://doi.org/10.36483/vanvetj.1255832

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Evaluation of the Effect of Ferula Rigidula Extract on Sperm Parameters, Antioxidant Parameters and Testicular Structure in Male Rats in Experimental Diabetic Condition

Saadet BELHAN[1,*] Salih ÇİBUK[2] Ahmet Ufuk KÖMÜROĞLU[2] Fikret ALTINDAĞ[3] Yeter DEĞER[4]

[1] *Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, 65080, Van, Türkiye*
[2] *Van Yuzuncu Yil University, Vocational School of Health Services, 65080, Van, Türkiye*
[3] *Van Yuzuncu Yil University, Faculty of Medicine, Department of Histology and Embryology, 65080, Van, Türkiye*
[4] *Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Biochemistry, 65080, Van, Türkiye*

Received: 24.02.2023 Accepted: 25.04.2023

ABSTRACT The study was conducted to investigate how Ferula rigidula extract affected sperm profile, antioxidant parameters, and stereological profile in experimental diabetic rats. Performed on forty-nine male rats. The rats were randomly assigned to control group, diabetic group, diabetic + Ferula rigidula group 1, diabetic + Ferula rigidula group 2, diabetic + glibenclamide group, Ferula rigidula group 1, and Ferula rigidula group 2. While sperm count, motility, antioxidant parameters, testosterone hormone, germinal epithelial volume, and germinal epithelial height decreased in the diabetic group, abnormal sperm count, malondialdehyde level, and lumen volume increased. When Ferula rigidula (extract) was given to diabetic rats, it brought the stereological findings to the same level as the control group. In addition, it was determined that there were improvements in biochemical parameters, approaching the values of the control group. Specifically, when Ferula rigidula extract was administered alone, testosterone levels and stereological findings improved in group 1. In addition, it was determined that there were significant improvements in sperm parameters. However, it was determined that the positive effect of Ferula rigidula extract was very significant at low doses (250 mg/kg) and decreased at high doses (500 mg/kg). As a result, Ferula rigidula extract has an antioxidant role and can be used to alleviate the problems caused by diabetes in the male reproductive system.

Keywords: *Diabetes, Ferula, Rat, Sperm.*

ÖZ

Deneysel Diyabetik Durumda Erkek Sıçanlarda Ferula Rigidula Ekstraktının Sperm Parametreleri, Antioksidan Parametreler ve Testis Yapısı Üzerine Etkisinin Değerlendirilmesi

Çalışma, deneysel diyabetik sıçanlarda Ferula rigidula ekstraktının sperm profilini, antioksidan parametreleri ve stereolojik profili nasıl etkilediğini araştırmak için yapıldı. Kırk dokuz erkek sıçan üzerinde gerçekleştirildi. Sıçanlar kontrol grubu, diyabetik grup, diyabetik + Ferula rigidula grup 1, diyabetik + Ferula rigidula grup 2, Diyabetik + glibenklamid grubu, Ferula rigidula grup 1 ve Ferula rigidula grup 2 olarak rastgele ayrıldı. Diyabetik grupta sperm sayısı, motilite, antioksidan parametreler, testosteron hormonu, germinal epitel hacmi ve germinal epitel yüksekliği azalırken, anormal sperm sayısı, malondialdehit düzeyi ve lümen hacmi arttı. Diyabetik ratlara Ferula rigidula (ekstrakt) verildiğinde stereolojik bulguları kontrol grubu ile aynı düzeye getirdi. Ayrıca biyokimyasal parametrelerde kontrol grubu değerlerine yaklaşacak şekilde iyileşmelerin olduğu tespit edildi. Özellikle, Ferula rigidula ekstresi tek başına uygulandığında, 1. grupta testosteron düzeylerinde ve stereolojik bulgularda iyileşme oldu. Bunun yanı sıra sperm parametrelerinde anlamlı olarak düzelmelerin olduğu belirlendi. Ancak, Ferula rigidula ekstraktının olumlu etkisinin düşük dozda (250 mg/kg) çok belirgin olduğu, yüksek dozlarda (500 mg/kg) ise azaldığı tespit edildi. Sonuç olarak, Ferula rigidula ekstresi antioksidan role sahiptir ve erkek üreme sisteminde diyabetin neden olduğu sorunları hafifletmek için kullanılabilir.

Anahtar Kelimeler: *Diyabet, Ferula, Sıçan, Sperm.*

✉ *Corresponding author: saadetbelhan@yyu.edu.tr

INTRODUCTION

Diabetes mellitus is a metabolic disease that is characterized by hyperglycemia in particular and affects the male reproductive system at various levels (Vlad and Popa 2016). It is known that prolonged hyperglycemia causes deterioration in libido and spermatogenesis (Mulholland et al. 2011; Ghasemi et al. 2016). Diabetic condition causes excessive oxygen radical production in many tissues such as testis and epididymis (Jain and Jangir 2014). The resulting oxidative stress leads to spermatogenic dysfunction, testicular dysfunction and hypogonadism (Feyli et al. 2017; Shi et al. 2017).

Research results have indicated that diabetes reduces sperm count, motility and viability and causes significant increases in abnormal sperm count and malondialdehyde (MDA) level (Jiao et al. 2020; Sahu et al. 2020). In addition, diabetic people suffer from decreases in follicle stimulating hormone, luteinizing hormone and testosterone levels as well as body and testicular weight (Jiao et al. 2020).

Although many chemicals have been synthesized for therapeutic purposes, herbal medicines have always maintained their importance. Turkey has a great tradition of folk medicine due to its diverse flora (Tufan et al. 2018). Some Ferula species (Ferula caspica Bieb: Stomach pain, gynecological diseases and diabetes; Ferula elaeochytris Korovin: Aphrodisiac; Ferula rigidula DC: Diabetes, hypercholesterolemia) are used in the traditional medicine especially in Eastern Anatolia (Altundağ 2011). Bagheri et al. (2015) found that administration of Ferula assa foetida increased sperm count, motility, morphology and viability in rats.

Glibenclamide, which works by stimulating insulin secretion, is an antidiabetic agent (Serrano-Martín et al. 2006). Although it is preferred by diabetics, there is no study reporting that glibenclamide has antioxidant properties. It is known that the formation of reactive oxygen species in diabetes induced by streptozotocin administration exceeds physiological levels (Bolzan and Bianchi 2002). Therefore, the combined use of antioxidant agents and antihyperglycemic agents may be a correct approach to minimize the possible problems of free oxygen radicals that occur at pathological levels in diabetes.

Ferula rigidula, which is preferred in traditional medicine, is consumed by diabetics, especially in the eastern Turkey. However, there is no scientific report in the literature to support this traditional use. The effect of Ferula rigidula on sperm profile, antioxidant parameters, testosterone level and stereological profile in diabetic rats was evaluated. This study will help close the gap in this field.

MATERIAL AND METHODS

Collection of Plant material and Preparation of the extract

Ferula rigidula plant was collected from districts of Van Province between May-June of the year as a result of taxonomic analysis. Its stem and leaf parts were washed with distilled water. They were then dried in a shade and dry environment. After they were dried and pulverized with a grinder, the extract was prepared by following the steps in the method described by (Farkhad et al. 2012).

Animal Material

Forty nine rats weighing 200-300 g were supplied from Van Yuzuncu Yıl University Experimental Research Center. They were weighed and clinically examined. Appropriate conditions for temperature, humidity and light were provided and daily observations were made on them. There were given feed and drinking water ad libitum. Experimental studies were carried out in the same center. The study procedures were approved by the ethics committee (Van Yuzuncu Yıl University Animal Experiments Local Ethics Committee, Van, Turkey, Approval number: 2020/08-05), and the principles of Care and Use of Laboratory Animals were followed in all applications.

Diabetes Induction

Basal glucose levels were determined before administration of streptozotocin. Later, 45 mg/kg streptozotocin was administered intraperitoneally (i.p) to the rats which were fasted the night before. Blood glucose level was measured 72 hours after administration of streptozotocin. Those who showed a glucose level above 200 mg/dL in the measurement were considered as diabetic (Naghibi et al. 2022).

Experimental Planning

Experimental groups in this study were 7 groups including an equal number of rats in each group.

Control group (n=7): No specific application was made except for giving feed and drinking water.

Diabetic group (n=7): Streptozotocin 45 mg/kg i.p. administered and diabetes was induced.

Diabetic+Ferula rigidula group 1 (n=7): Ferula rigidula was administered to rats with diabetes induction at a dose of 250 mg/kg via gastric gavage.

Diabetic+Ferula rigidula group 2 (n=7): Ferula rigidula was administered at a dose of 500 mg/kg by gastric tube to diabetes-induced rats.

Diabetic+glibenclamide group (n=7): 5 mg/kg glibenclamide was administered by gastric tube to diabetes-induced rats (Andrade-Cetto 2011).

Ferula rigidula group 1 (n=7): Ferula rigidula was administered via gastric gavage at a dose of 250 mg/kg.

Ferula rigidula group 2 (n=7): Ferula rigidula was administered at a dose of 500 mg/kg via gastric gavage.

All rats were anesthetized 24 hours after the last application, and after blood collection and testicular tissue collection, the experimental application was terminated upon sacrifice of the rats.

Spermatological Evaluation

While the rats were anesthetized, motility examination was performed from the cauda epididymis of the testis removed from the scrotal sac (before the body and testicles were cooled). Spermatological evaluation was performed as in our previous study (Belhan et al. 2020).

Biochemical Evaluation

While testosterone was measured in serum, antioxidant parameters were examined in testicular homogenate. First, the mass of the removed testicular tissue was determined and phosphate buffer (pH: 7.4) was added. It was then homogenized using a homogenizer (Samarghandian et al. 2015). Glutathione (GSH), MDA, Catalase (CAT) and Superoxide dismutase (SOD) were measured using spectrometric method according to the methods given, respectively (Beutler et al. 1963; Aebi 1984; Sun et al. 1988; Dubovskiy et al. 2008). A commercial kit from Abbott was used to measure testosterone. The measurement was made through the ARCHITECT c1616200 autoanalyzer.

Stereological Evaluation

Randomly isotropic identical sections were taken from testicular tissue. The orientation method was used to take the sections. 8-10 tissue samples were taken from each rat and embedded in paraffin blocks. 10-15 consecutive sections were obtained from these blocks. The sections were then stained by using Masson's trichrome dye and evaluated in a microscopic environment (light microscope).

Estimation of Volume density and Germinal epithelial height

The volume density of the germinal epithelium, lumen and interstitial tissue was calculated using the dot grid (Figure 1A) and the following formula (Mayhew and Gundersen 1996).

$$\text{The volume density} = \frac{V_{(\text{Structure})}}{V_{(\text{Testis})}} \times 100$$

Germinal epithelium height was estimated according to previously described methods (Gundersen et al. 1988; Noorafshan 2014). Attention was paid to associate each test line with a point (Figure 1B).

Histopathological Evaluation

Samples followed for tissue were embedded in paraffin blocks. Later, sections (4 μm) were stained through Masson trichrome dye and evaluated microscopically (Light microscope-Nikon Y-IM 7551012, Japan). The findings was assessed semi-quantitatively.

Statistical Analysis

Statistical analysis was carried out by using the package program (SPSS 21.0). ANOVA (One Way Analysis of Variance) and Post-Hoc Tukey test were applied for sperm profile and histopathological parameters. For biochemical parameters, ANOVA and Duncan's test were used. Data were expressed as mean±standard deviation (SD). Data with $p \leq 0.05$ were considered significant.

RESULTS

Sperm Findings

Table 1 shows findings related to sperm profile. While the diabetic group had lower sperm motility and density, the abnormal sperm count was higher ($p<0.05$). Sperm motility and density increased significantly in rats treated with Ferula rigidula (250 mg/kg), while the abnormal sperm count decreased significantly. However, motility and sperm density were significantly lower in rats treated with Ferula rigidula of 500 mg/kg than the controls ($p<0.05$). No significant difference was detected between diabetic, diabetic + Ferula rigidula and diabetic + glibenclamide groups in terms of sperm motility, sperm density and abnormal sperm count ($p>0.05$).

Table 1: Effects of glibenclamide and Ferula rigidula on sperm profile in diabetic rats.

Groups	Motility (%)	Density (x10⁶)	Abnormal sperm rate (%)		
			Head	Tail	Total
Control	73.57±2.43^{b}	111.14±1.21^{b}	5.71±0.75^{c}	5.00±0.57^{c}	10.71±0.75^{c}
Diabetic	27.85±2.67^{d}	33.14±0.69^{d}	25.00±1.29^{a}	23.42±0.78^{a}	48.42±1.27^{a}
Diabetic+Ferula rigidula (250)	28.57±2.43^{d}	33.57±0.53^{d}	23.85±1.06^{a}	23.85±1.34^{a}	47.71±1.25^{a}
Diabetic+Ferula rigidula (500)	26.42±2.43^{d}	34.00±0.81^{d}	24.57±0.97^{a}	23.71±0.75^{a}	48.28±1.11^{a}
Diabetic+Glibenclamide	25.71±1.88^{d}	32.42±0.53^{d}	24.71±0.95^{a}	24.42±0.97^{a}	49.14±0.69^{a}
Ferula rigidula (250)	81.42±3.77^{a}	130.00±1.52^{a}	4.00±0.57^{d}	4.42±0.78^{c}	8.42±0.53^{d}
Ferula rigidula (500)	58.57±2.43^{c}	91.14±0.89^{c}	8.00±0.57^{b}	8.00±0.81^{b}	16.00±1.00^{b}

The letters a, b, c, d denote the statistical difference between different groups in the same column ($p<0.05$).

Biochemical Findings

Table 2 shows findings on biochemical parameters. In the present study, the diabetic group had significantly lower level of testosterone ($p<0.05$). Testosterone value of Ferula rigidula group 1 (250 mg/kg) was significantly higher when compared to the value of the control group ($p<0.05$). The diabetic + Ferula rigidula group had higher testosterone value when compared to the diabetic + glibenclamide group ($p>0.05$) however it was not statistically significant. GSH, CAT, SOD values increased and MDA levels decreased in diabetic + Ferula rigidula group compared to the diabetic group.

Stereological Findings

As seen in Table 3, germinal epithelial thickness significantly decreased in the diabetic group and the diabetic + glibenclamide group ($p<0.05$). On the other hand, these parameters significantly increased in in the diabetic + Ferula rigidula (250 mg/kg) and diabetic + Ferula rigidula (500 mg/kg) groups than the diabetic group ($p<0.05$). No significant difference was detected among the groups.in terms of the interstitial space volume ratio. The volume density of the germinal epithelium, lumen, and interstitial tissue and the height of the germinal epithelium are presented in Figure 1.

Histopathological Findings

Control group had a normal histological structure. All spermatogenic cells and Sertoli cells were observed in the germinal epithelium. Normal connective tissue cells were observed along with Leydig cells in the interstitial area (Figure 2NC). Upon comparison made between the diabetic and diabetic + glibenclamide groups and the control group, it was found that the number of germinal epithelial layers decreased, seminiferous tubules had degeneration and atrophy, and the basal lamina got thickened and the area between sertoli and spermatogenic cells opened up (Figure 2DC, DG). Diabetic + Ferula rigidula (250 mg/kg) (Figure 2DFR1), diabetic + Ferula rigidula (500mg/kg) (Figure 2DFR2), Ferula rigidula (250 mg/kg) (Figure 2FR1) and Ferula rigidula (500 mg/kg) (Figure 2FR2) groups were histopathologically similar to the control group.

Table 2: Effects of glibenclamide and Ferula rigidula on biochemical profile in diabetic rats.

Groups	GSH (µmol/g)	CAT (U/g)	MDA (nmol/g)	SOD (U/g protein)	Testosterone (nmol/L)
Control	6.82±0.05^{a}	271.88±3.55^{a}	23.77±1.18^{c}	605.87±10.79^{a}	2.14±0.39^{b}
Diabetic	4.66±0.27^{e}	161.55±5.28^{d}	42.83±1.75^{a}	426.24±10.09^{f}	0.31±0.02^{d}
Diabetic+Ferula rigidula (250)	5.51±0.19^{b,c,d}	187.47±4.09^{b,c}	33.29±1.33^{b}	471.68±8.07^{d,e}	0.43±0.02^{d}
Diabetic+Ferula rigidula (500)	5.13±0.15^{d,e}	169.63±5.54^{c,d}	40.74±0.98^{a}	451.52±7.27^{e,f}	0.32±0.01^{d}
Diabetic+Glibenclamide	5.68±0.23^{b,c}	191.86±6.23^{b}	31.25±0.89^{b}	511.09±3.18^{c,d}	0.24±0.02^{d}
Ferula rigidula (250)	6.03±0.13^{b}	271.75±10.20^{a}	24.63±0.60^{c}	557.65±23.52^{b}	3.01±0.20^{a}
Ferula rigidula (500)	5.43±0.09^{c,d}	206.88±7.73^{b}	26.11±0.64^{c}	537.17±22.64^{b,c}	1.14±0.18^{c}

The letters a, b, c, d, e denote the statistical difference between different groups in the same column ($p<0.05$). **GSH:** Glutathione, **CAT:** Catalase, **MDA:** Malondialdehyde, **SOD:** Superoxide dismutase.

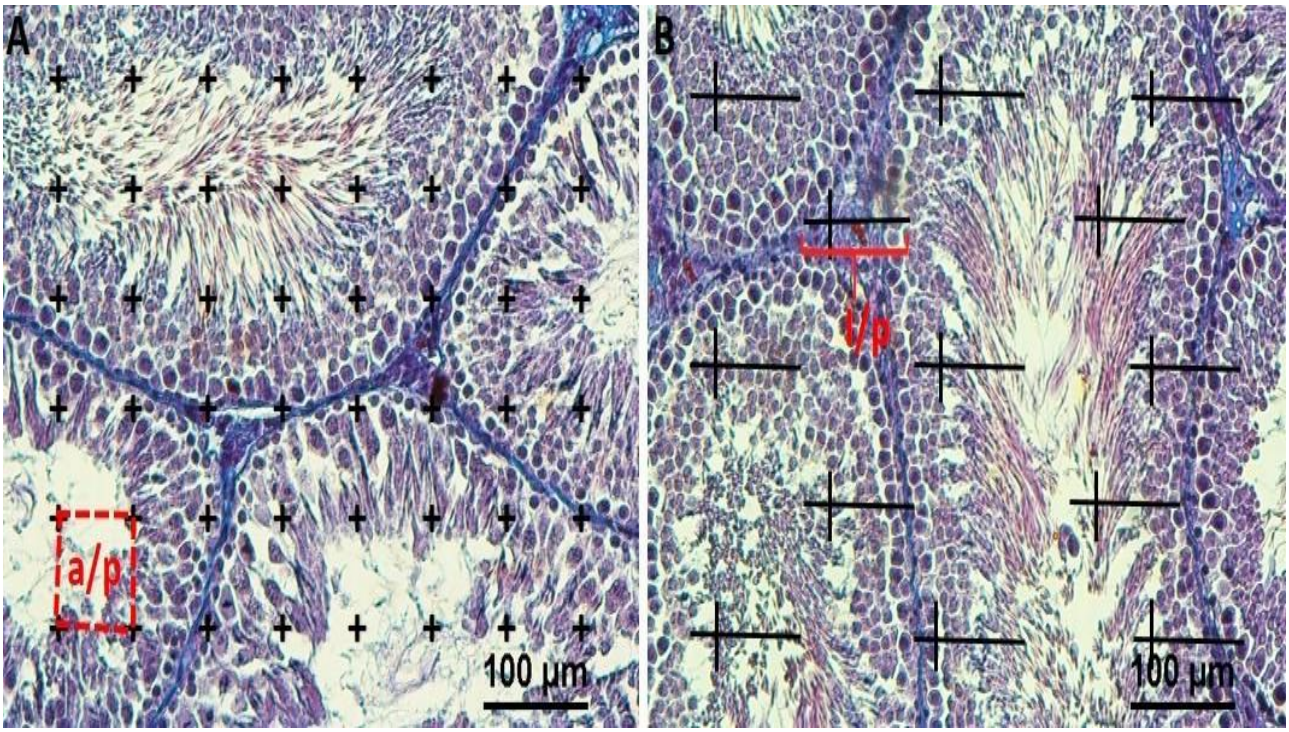


Figure 1: A. The superimposed of a point grid on the image of section. **B**. The superimposed of a grid of test lines on the image of section.

Table 3: Effects of glibenclamide and Ferula rigidula on stereological profile in diabetic rats.

Groups	Germinal epithelial volume (mm3)	Interstitial tissue volume (mm^3)	Lumen volume (mm^3)	Germinal epithelial height (µm)
Control	58.23±2.00^b	16.43±3.24	25.33±2.45^b	61.19±2.77^b
Diabetic	52.83±7.33^a	15,36±5.31	31.79±3.52^a	51.04±2.08^a
Diabetic+Ferula rigidula (250)	59.71±7.87^b	14.58±3.97	25.72±4.67^b	58.89±3.05^b
Diabetic+Ferula rigidula (500)	59.67±7.81^b	14.47±3.82	27.18±1.14^b	59.67±2.89^b
Diabetic+Glibenclamide	51.76±1.87^a	14.79±1.30	32.44±1.71^a	53.01±3.59^a
Ferula rigidula (250)	60.05±5.41^b	15.14±6.25	24.80±1.08^b	62.05±3.64^b
Ferula rigidula (500)	59.69±5.59^b	14.32±4.82	26.14±1.40^b	63.46±3.76^b

The letters a, b denote the statistical difference between different groups in the same column ($p<0.05$).

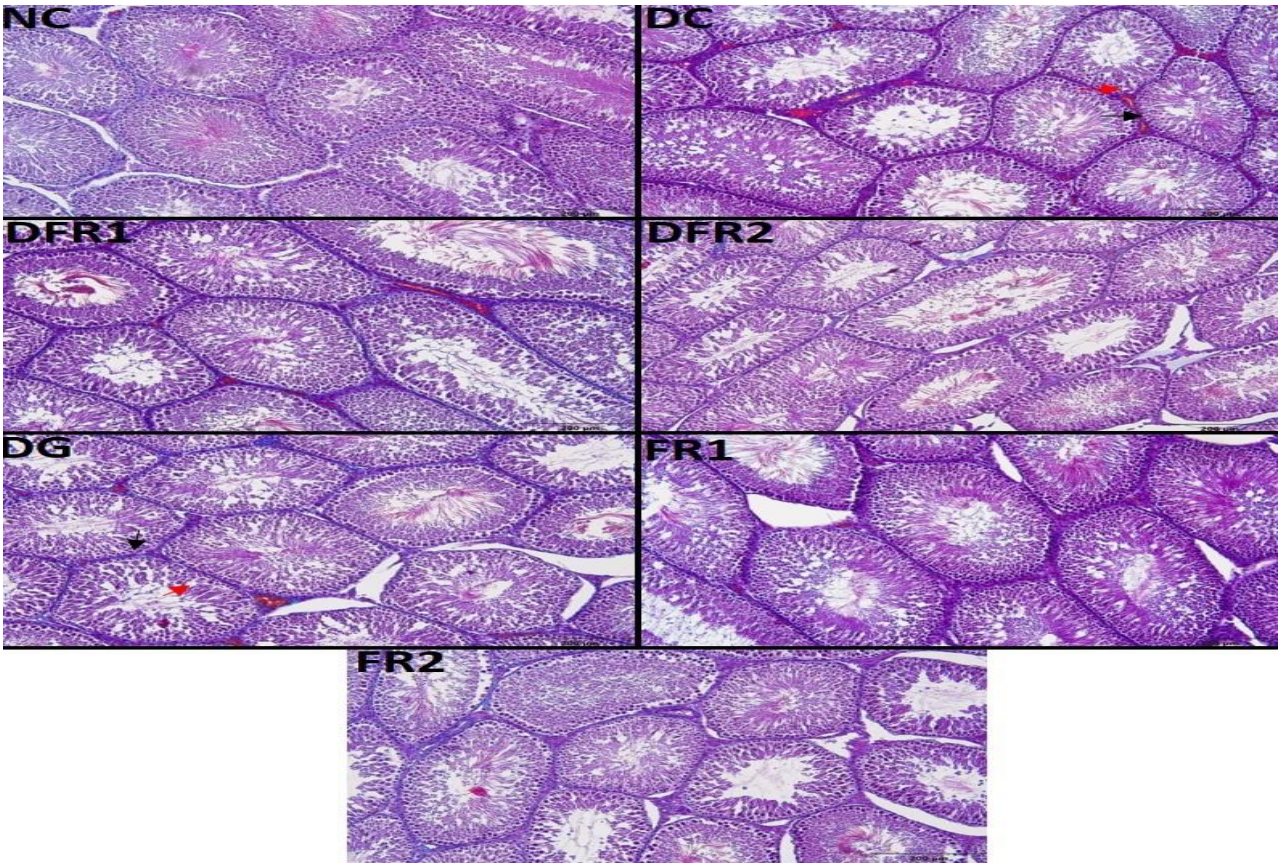


Figure 2: Photomicrographs of testicular sections. NC; in normal histological appearance. DC; there was a decrease in the number of epithelial layer, opening in the lumen, degeneration and atrophy in the tubules. D+FR1, D+FR2 and D+G; there was a decrease in the number of epithelial layer, opening in the lumen, degeneration and atrophy in the tubules. FR1; a slight increase in the number of epithelial layer compared to the control group. FR2; moderate increase in the number of epithelial layer compared to the control group. (**NC;** normal control, **DC;** Diabetic control, **D+FR1;** Diabetic + Ferula Rigidula 250 mg / kg , **D+FR2;** Diabetic + Ferula Rigidula 500 mg / kg , **D+G;** Diabetic + Glibenclamide, **FR1;** Ferula Rigidula 250 mg / kg, **FR2;** Ferula Rigidula 500 mg / kg).

DISCUSSION AND CONCLUSION

Many natural resources have a biological activity (Mohammed et al. 2020; Sevindik 2020). Today, many people increasingly tend to use phytotherapy due to a number of difficulties they experience in modern medicine (Dahl 2001). However, scientific research needs to show how people here in Turkey - just as in other parts of the world - use herbal products for therapeutic purposes and how that impacts their health (Sucaklı et al. 2014). Although testicular dysfunction caused by diabetes is not a life-threatening factor, associated psychological and emotional problems are of great importance (Zhang et al. 2020). This is because sexual dysfunction seriously affects the quality of life of men.

When the sperm profile was evaluated, it was found that the diabetic group had lower sperm motility and density compared to the controls, and the abnormal sperm count was higher. This result is compatible with the diabetes studies (Jiang et al. 2020; Jiao et al. 2020; Sahu et al. 2020). The undesirable result obtained regarding sperm parameters in the diabetic group can be explained by the disrupted activities of the hypothalamic -pituitary-gonadal axis (Brüning et al. 2020). It was determined that the motility obtained by administrating the glibenclamide in the diabetic group was lower than the motility value of the diabetic group, although it was not significant. The decrease in sperm motility in rats treated with glibenclamide may be a result of oxidative stress because streptozotocin damages the DNA of pancreatic beta cells and causes the formation of reactive oxygen species (Bolzan and Bianchi 2002). It is an expected result that glibenclamide, which does not have anti-oxidant properties, cannot protect diabetic rats from oxidative stress (Odo et al. 2018). Sperm motility value was found to be significantly higher in ferula rigidula group 1 (250 mg/kg) than the control group. This situation can be associated with the antioxidant properties of Ferula rigidula (Köse and Ocak 2018). However, Ferula rigidula decreased the motility significantly in ferula rigidula group 2 (500 mg/kg) than the control group. This result reveals the importance of the dose. When examining sperm density, it was determined that while 250 mg/kg dose of Ferula rigidula significantly increased sperm count compared to the control group, 500 mg/kg dose significantly decreased sperm count. This indicated the importance of administration dose of Ferula rigidula. Bagheri et al. (2015) found that Asafoetida derived from some Ferula species (F. assa - foetida and Ferula foetida, Ferula rubricaulis, Ferula rigidula, Ferula alliacea) increased sperm count at all doses (25, 50, 100, 200). In the present study, the effect of Ferula rigidula at the dose of 250 mg/kg on sperm morphology was remarkable. However, the same dose did not have any positive effect on diabetic rats. Sperm morphology was highly impaired in the diabetic group, which is compatible with previous studies (Jiang et al. 2020; Sahu et al. 2020).

In the present study, the testosterone levels of the diabetic group were low, which is compatible with previous studies (Jiao et al. 2020; Sahu et al. 2020). Low testosterone level is likely to be associated with the change in proliferation and differentiation of Leydig cells due to diabetes. It was found in the present study that the Ferula rigidula (250 mg/kg) group 1 had significantly higher testosterone level compared to the control group. The decrease in testosterone level when Ferula rigidula was administered at a dose of 500 mg/kg is consistent with the testosterone result that Ayoubi et al. (2013) received when they administrated asafoetida at high doses (300 mg/kg). Both studies reported lower testosterone levels at high doses. This situation may be associated with vacuuming of Leydig cells (Bagheri et al. 2015). The testosterone value of the diabetic + Ferula rigidula group was higher than the value of the diabetic + glibenclamide group but it was not significant. This may be due to the fact that while Ferula rigidula has antioxidant properties, glibenclamide does not have antioxidant properties (Köse and Ocak 2018; Odo et al. 2018).

In the present study, it was found that while both doses of Ferula rigidula administrated to diabetic rats increased GSH, CAT, SOD values compared to the diabetic group, it decreased the MDA level. It is possible to see the expected effect of ferula rigidula in diabetic rats particularly at a dose of 250 mg/kg. As a matter of fact, the GSH, CAT, SOD values in the diabetic + Ferula rigidula (250 mg/kg) group 1 were significantly higher than the diabetic group, while the MDA value was significantly lower. This result is compatible with the previous study (Yusufoglu et al. 2015). In the ferula rigidula group 1 (250 mg/kg), the CAT, MDA, GSH and SOD values were similar to values of the control group, which show us the antioxidant power of the relevant dose. As a matter of fact, it has been reported that Ferula rigidula has antioxidant properties (Köse and Ocak 2018).

When examining stereological findings in diabetic and control groups, it was found that the germinal epithelial height and germinal epithelial volume decreased in the diabetic group, which is compatible with other studies (Kianifard et al. 2011; Keyhanmanesh et al. 2018; Gholizadeh et al. 2019; Hosseinipour et al. 2019). It has been reported that apoptosis occurring during the course of spermatogenesis is effective in this decrease (Hosseinipour et al. 2019), resulting in suppression of the activity of spermatogenic cells (Kianifard et al. 2011). Interstitial tissue volume did not change in the present study, which is compatible with other studies (Gholizadeh et al. 2019; Hosseinipour et al. 2019). It was found that the lumen volume increased in the diabetic group than the control group, and this result supports a previous study (Keyhanmanesh et al. 2018).

The results of this study showed that negative changes on sperm parameters in streptozotocin induced diabetic rats were significantly restored by administration of Ferula rigidula extract (250 mg/kg). It is especially important that its effect on sperm parameters has been studied for the first time. Positive changes in antioxidant enzyme levels suggested that the extract may have antioxidant properties. As a result of the findings of the present study, it is of great importance in terms of revealing the scientific validity of the use of Ferula rigidula extract among the public and shedding light on the studies to be conducted. However, it can be asserted that the therapeutic effect of Ferula rigidula extract depends on dose.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENT

We would like thank Assoc. Dr. Mesut Pınar for his taxonomic evaluation of Ferula rigidula.

AUTHOR CONTRIBUTIONS

Idea / Concept: SB, UO
Supervision / Consultancy: SB
Data Collection and / or Processing: SB
Analysis and / or Interpretation: SB, UO, AUK, FA
Writing the Article: SB
Critical Review: SB, UO, AUK, FA, YD

REFERENCES

Aebi H (1984). Catalase in vitro. *Meth Enzymol,* 105, 121-126.

Altundag E, Ozturk M (2011). Ethnomedicinal studies on the plant resources of East Anatolia, Turkey. *Proc Soc Behavi Sci,* 19, 756-777.

Andrade-Cetto A (2011). Hypoglycemic effect of Smilax moranensis root on N5-STZdiabetic rats. *PhOL*, 1, 111-115.

Ayoubi A, Arshami J, Valizadeh R, Mousavi Z, Mousaie A (2013). The Effect of asafetida Gum Extract on Blood Parameters and Histopathology of Testes in Male Wistar Rat. *Iran J Anim Sci Res,* 4 (4), 310‑315.

Bagheri SM, Yadegari M, Porentezari M et al. (2015). Effect of Ferula assa-foetida oleo gum resin on spermatic parameters and testicular histopathology in male wistar rats. *J Ayurveda Integr Med,* 6 (3), 175‑180.

Belhan S, Özkaraca M, Özdek U, Kömüroğlu AU (2020). Protective role of chrysin on doxorubicin induced oxidative stress and DNA damage in rat testes. *Andrologia,* 52 (9), e13747.

Beutler E, Duron O, Kelly BM (1963). Improved method for the determination of blood glutathione. *J Lab Clin Med,* 61, 882-888.

Bolzan AD, Bianchi MS (2002). Genotoxicity of streptozotocin. *Mutat Res,* 512 (2-3), 121-134.

Brüning JC, Gautam D, Burks DJ et al. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science,* 289 (5487), 2122-2125.

Dahl NV (2001). Herbs and supplements in dialysis patients: panacea or poison? *Semin Dial,* 14 (3), 186-192.

Dubovskiy IM, Martemyanov VV, Vorontsova YL et al. (2008). Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of Galleria mellonella L. larvae (Lepidoptera, Pyralidae). *Comp Biochem Physiol C Toxicol Pharmacol,* 148 (1), 1-5.

Farkhad NK, Farokhi F, Tukmacki A, Band KS (2012). Hydro-alcoholic extract of the root of Prangos ferulacea (L.) Lindl can improve serum glucose and lipids in alloxan-induced diabetic rats. *Avicenna J Phytomed,* 2 (4), 179-87.

Feyli SA, Ghanbari A, Keshtmand Z (2017). Therapeutic effect of pentoxifylline on reproductive parameters in diabetic male mice. *Andrologia,* 49 (1), 1-12.

Ghasemi H, Karimi J, Goodarzi MT et al. (2016). Seminal plasma zinc and magnesium levels and their relation to spermatozoa parameters in semen of diabetic men. *Int J Diabetes Develop Countr,* 36 (1), 34-39.

Gholizadeh F, Dastghaib S, Koohpeyma F et al. (2019). The protective effect of Stevia rebaudiana Bertoni on serum hormone levels, key steroidogenesis enzymes, and testicular damage in testes of diabetic rats. *Acta Histochemica,* 121 (7), 833-840.

Gundersen HJ, Bagger P, Bendtsen TF et al. (1988). The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. Apmis, 96 (10), 857-881.

Hosseinipour M, Goodarzi N, Bakhtiari M (2019). Protective efficiency of Ashrasi date palm hydroalcoholic extract against diabetes-induced testicular toxicity: A biochemical and stereological study. *Andrologia,* 51 (11), e13420.

Jain GC, Jangir RN (2014). Modulation of diabetes-mellitus-induced male reproductive dysfunctions in experimental animal models with medicinal plants. *Pharmacogn Rev,* 8 (16), 113-121.

Jiang Y, Ye R, Yang J et al. (2020). Protective effects of Salidroside on spermatogenesis in streptozotocin induced type-1 diabetic male mice by inhibiting oxidative stress mediated blood-testis barrier damage. *Chem Biol Interact,* 315, e108869.

Jiao N, Chen Y, Zhu Y et al. (2020). Protective effects of catalpol on diabetes mellitus-induced male reproductive damage via suppression of the AGEs/RAGE/Nox4 signaling pathway. *Life Sci,* 256, e116736.

Keyhanmanesh R, Hamidian G, Alipour MR, Ranjbar M, Oghbaei H (2018). Protective effects of sodium nitrate against testicular apoptosis and spermatogenesis impairments in streptozotocin-induced diabetic male rats. *Life Sci,* 211, 63-73.

Kianifard D, Sadrkhanlou RA, Hasanzadeh S (2011). The Histological, Histomorphometrical and Histochemical Changes of Testicular Tissue in the Metformin Treated and Untreated Streptozotocin-Induced Adult Diabetic Rats. *Vet Res Forum,* 2 (1), 13-24.

Köse Ş, Ocak E (2018). Antimicrobial and antioxidant properties of sırmo (allium vineale l.), mendi (chaerophyllum macropodum boiss.) and sıyabo (ferula rigidula dc.). *J Food,* 43 (2), 294-302.

Mayhew TM, Gundersen HJ (1996). If you assume, you can make an ass out of u and me: a decade of the disector for stereological counting of particles in 3D space. *J Anat,* 188 (Pt 1), 1-15.

Mohammed FS, Şabik AE, Sevindik E, Pehlivan M, Sevindik M (2020). Determination of Antioxidant and Oxidant Potentials of Thymbra spicata Collected from Duhok-Iraq. *TURJAF,* 8(5), 1171-1173.

Mulholland J, Mallidis C, Agbaje I, McClure N (2011). Male diabetes mellitus and assisted reproduction treatment outcome. *Reprod Biomed Online,* 22 (2), 215-219.

Naghibi M, Nasrabadi HT, Rad JS, Farashah MSG, Mohammadnejad D (2022). The effects of metformin and forskolin on sperm quality parameters and sexual hormones in type II diabetic male rats. *Andrologia,* 54 (7), 1605-1617.

Noorafshan A (2014). Stereology as a valuable tool in the toolbox of testicular research. *Ann Anat,* 196 (1), 57-66.

Odo RI, Ugwu NE, Akusu S (2018). The protective effects of Vitamin E and glibenclamide on spermatogenic and haematological changes in alloxan-induced diabetic male rats. *Afr J Pharm Pharmacol,* 12 (28), 431-435.

Sahu C, Dwivedi DK, Jena GB (2020). Zinc and selenium combination treatment protected diabetes-induced testicular and epididymal damage in rat. *Hum Exp Toxicol,* 39(9), 1235-1256.

Samarghandian S, Azimi-Nezhad M, Samini F, Farkhondeh T (2015). Chrysin treatment improves diabetes and its complications in liver, brain, and pancreas in streptozotocin-induced diabetic rats. *Can J Physiol Pharmacol,* 94 (4), 388-393.

Serrano-Martín X, Payares G, Mendoza-León A (2006). Glibenclamide, a blocker of K+(ATP) channels, shows antileishmanial activity in experimental murine cutaneous leishmaniasis. *Antimicrob Agents Chemother,* 50 (12), 4214-4216.

Sevindik M (2020). Antioxidant and antimicrobial capacity of Lactifluus rugatus and its antiproliferative activity on A549 cells. Indian J Trad Know, 19 (2), 423-427.

Shi GJ, Zheng J, Wu J, Qiao HQ, Chang Q (2017). Beneficial effects of Lycium barbarum polysaccharide on spermatogenesis by improving antioxidant activity and inhibiting apoptosis in streptozotocin-induced diabetic male mice. *Food Func,* 8 (3), 1215-1226.

Sucaklı MH, Ölmez S, Keten HS et al. (2014). Herbal Products Usage Among University Students. *Med Sci,* 3 (3), 1352-1360.

Sun Y, Oberley LW, Li Y (1988). A simple method for clinical assay of superoxide dismutase. *Clin Chem,* 34 (3), 497-500.

Tufan S, Toplan GG, Mat A (2018). Ethnobotanical usage of plants as aphrodisiac agents in Anatolian folk medicine. *Marmara Pharm,* 22 (2), 142-151.

Vlad I, Popa AR (2012). Epidemiology of diabetes mellitus: a current review. *Roman J Diab Nutr Metab Dis,* 19 (4), 433-440.

Yusufoglu HS, Soliman GA, Abdel-Rahman RF et al. (2015). Antihyperglycemic and antihyperlipidemic effects of Ferula duranii in experimental type 2 diabetic rats. *Int J Pharmacol,* 11 (6), 532-541.

Zhang L, Yang Z, Zhao Y et al. (2020). Renoprotective effects of Gushen Jiedu capsule on diabetic nephropathy in rats. *Sci Rep,* 10 (1), 2040.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Kırbaş Doğan G, Yediel Aras Ş, Karadağ Sari E (2023).** Macro Anatomical and Histological Study of Larynx Cartilage, Trachea, and Lungs in Lynx (*Lynx lynx*). *Van Vet J*, 34 (2), 126-132. DOI: https://doi.org/10.36483/vanvetj.1255862

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Macro Anatomical and Histological Study of Larynx Cartilage, Trachea, and Lungs in Lynx (*Lynx lynx*)

Gülseren KIRBAŞ DOĞAN[1, *] Şükran YEDİEL ARAS[2] Ebru KARADAĞ SARI[3]

[1] *Kafkas University, Faculty of Veterinary Medicine, Department of Anatomy, 36100, Kars, Türkiye*
[2] *Kafkas University, Faculty of Health Sciences, Department of Midwifery, 36100, Kars, Türkiye*
[3] *Kafkas University, Faculty of Veterinary Medicine, Department of Histology and Embryology, 36100, Kars, Türkiye*

Received: 24.02.2023 Accepted: 03.05.2023

ABSTRACT In this study the larynx, trachea, and lungs of one 1.5-year-old adult female lynx (*Lynx lynx*) were examined. The macro anatomical and histological structure of the larynx cartilages, trachea, and lungs were tried to be revealed by dissection and measurements. It was determined that the trachea had 48 cartilage rings (*cartilago trachealis*) up to the bifurcatio trachealis, the diameters of which narrow as they approach the lungs. The total tracheal length was 172.36 mm. It was seen that the cross-section of the lynx trachea resembled a circle. The hyaline cartilage structure surrounding the trachea was determined. A muscle structure called musculus transversus trachea, which closes the open ends of this cartilage and surrounds the cartilage from the outside, was detected. It was determined that the trachea consisted of tunica mucosa, submucosa, and tunica adventitia layers. The pulmo sinister in the lynx lung consisted of two main lobes, lobus cranialis, and lobus caudalis. Lobus cranialis was also divided among itself as pars cranialis and pars caudalis. Pulmo dexter was divided into four main lobes as lobus cranialis, lobus medius, lobus caudalis, and lobus accessorius. It was determined that there was connective tissue capsule surrounding the lung, respiratory bronchioles, alveoli, intraalveolar septum, and abundant blood vessels. In intraalveolar septum; capillaries, erythrocytes were determined. Type I and type II pneumocytes were seen covering the alveolar surface. In addition, it was determined that there were macrophages in the alveolar sacs.

Keywords: *Anatomy, Histology, Larynx, Lung, Lynx, Trachea.*

ÖZ

Vaşak (*Lynx lynx*)'ta Larynx Kıkırdakları, Trachea ve Akciğerler Üzerine Makroanatomik ve Histolojik Bir Çalışma

Bu çalışmada 1 adet erişkin vaşağın (*Lynx lynx*) larynx, trachea ve akciğerleri incelendi. Diseksiyon ve ölçümlerle larynx kıkırdakları, trachea ve akciğerlerin makroanatomik ve histolojik yapısı ortaya konulmaya çalışıldı. Trachea'nın bifurcatio trachealis'e kadar, çapları akciğerlere doğru yaklaştıkça daralan, 48 adet kıkırdak halkaya (cartilago trachealis) sahip olduğu tespit edildi. Toplam trachea uzunluğu 172.36 mm idi. Vaşak trachea'sının enine kesitinin daireye benzediği görüldü. Vaşak akciğerindeki pulmo sinister lobus cranialis ve lobus caudalis adında iki ana loptan oluşuyordu. Pulmo sinister'in lobus cranialis'i de kendi arasında pars cranialis ve pars caudalis olarak bölünmüştü. Pulmo dexter lobus cranialis, lobus medius, lobus accessorius ve lobus caudalis olmak üzere dört ana loptan oluşmaktaydı. Trachea'yı dışarıdan saran hiyalin kıkırdak yapısı belirlendi. Bu kıkırdağın açık olan uçlarını kapatan ve kıkırdağı dışardan saran musculus transversus trachea adlı kas yapısı tespit edildi. Trachea'nın tunika mukoza, submukoza, tunika adventisya katmanlarından oluştuğu belirlendi. Akciğeri dıştan saran bağ doku kapsülü, respiratuvar bronşiol sonları, alveoller, intraalveoler septum ve bol miktarda kan damarlarının bulunduğu tespit edildi. İntraalveolar septumlarda; kapillar damarlar, eritrositler belirlendi. Alveol yüzeyini kaplayan tip I ve tip II pnömositler görüldü. Ayrıca alveoler keselerde makrofajların olduğu belirlendi.

Anahtar Kelimeler: *Anatomi, Akciğer, Gırtlak, Histoloji, Soluk borusu, Vaşak.*

✉ *Corresponding author: glsrn36@gmail.com

INTRODUCTION

Lynx is the common name of medium-sized carnivorous vertebrate and wild animal species that is found in the Lynx genus from the feline (*Felidae*) family (W.W.F. 2016). The fur color is yellowish brown in summer, and gray-white in winter with distinctive black markings. They have long hind legs, and wide paws, and they are mostly active at night. The lynx is a forest cat, so it is seen in wooded and mountainous areas. They shelter in tree hollows, dens, and thick bushes. They have a 15-year lifespan. They feed on roe deer, wild goats, hares, foxes, birds, and squirrel-like rodents. They were seen in various regions in the south, especially in the Western and Eastern Black Sea Regions (Kütükçü 2016). There are four species, namely the Eurasian lynx (*Lynx lynx*), Canadian lynx (*Lynx canadensis*), Iberian lynx (*Lynx pardinus*), and lynx lynx (*Lynx rufus*). It has been reported that the Eurasian lynx subspecies *L. l. Dinniki* live in Turkey, the Caucasus mountains, northern Iran and northern Iraq (WWF 2016) and based on this information, it was thought that our research material is *L. l. Dinniki*.

The respiratory system provides breathing in mammalians. The main organ of this system is the lungs and the auxiliary organs are the larynx, trachea, nasopharynx, nose, and nasal cavity (Demiraslan and Dayan 2021). Larynx is a muscular and cartilaginous organ located at the beginning of the trachea. It is responsible for the formation of sound and the transmission of inspiratory air (Evans and de Lahunta 2013). Larynx was closer to the spatium mandible in non-carnivorous animals, while it shifted towards the neck in carnivores. It connects the pars nasalis pharyngis part of the pharynx with the trachea. It is responsible for the formation of sound as well as transmitting the breathing air. The larynx skeleton is composed of cartilages called cartilagines laryngis (Bahadır and Yıldız 2014). Cartilago thyroidea, the largest larynx cartilage, is formed by the union of two lamina thyroidea. The dorsal edge of the lamina thyroidea extends anteriorly to join the cornu rostrale and posteriorly to the cornu caudale. Cornu caudale articulates with cartilago cricoidea and the fissura thyroidea is between the cornu rostrale and the lamina. The nerve called nervus laryngeus cranialis comes out of this fissura thyroidea. There is incisura thyroidea caudalis, which is shallower in carnivores is caudal of the region where the laminae join ventrally. Nervus laryngeus caudalis is extend in this region. Cartilago cricoidea is ring-shaped. The wide flat part in the dorsal is called lamina cartilaginis cricoidea, and the arch part is called arcus cartilaginis cricoidea. The dorsal projection where the laminae meet is called the crista. Cartilago arytenoidea is comb-shaped and the protruding part on its outer surface is called the processus muscularis. The downward extending part is the processus vocalis. Its protrusion, which emerges from the craniodorsal and leads caudal like a horn, is the cartilago corniculata which is not found in felis. In canis, there is a second projection, called the cartilago cuneiformis, just in front of the cartilago corniculata (Evans and de Lahunta 2013). The epiglottis is the leaf-shaped cartilage of the larynx, located at the most cranial. Its upper end, called apex, is located at cranial in carnivores. There is a projection called the petiolus epiglottidis at the base called the basis (König and Liebich 2015). The trachea extends from the cartilago cricoidea of the larynx to the bifurcatio trachea. The number of tracheal rings is 42-46 in dogs and 38-43 in cats. Cartilago trachealis join with ligamentum annulare. At the same time, the dorsal space of the cartilages is closed with the musculus trachealis (König and Liebich 2015). The lungs are divided into pulmo dexter and pulmo sinister at the level of the bifurcatio trachea. The lungs have four faces: facies costalis, facies mediastinalis, facies diaphragmatica, and facies interlobaris. It has two edges, called margo ventralis (acutus) and margo dorsalis (obtusus) (Dursun 2008). There is a notch called incisura cardiaca at the heart level of margo ventralis (König and Liebich 2015). The part of the lungs opposite the aperture thoracis cranialis is called the apex pulmonis, the part of opposite the diaphragm is called the basis pulmonis (Demiraslan and Dayan 2021). The lung parenchyma has structures that exchange CO_2 and O_2 in the respiratory air. The pulmonary airways begin with the bronchus principalis dexter and sinister at the level of the bifurcatio trachea. Each bronchus principalis is divided into bronchus lobaris, which goes to different lobes of the lungs and is named after the same lobe to name which it goes (König and Liebich 2015). Lung loping in carnivores is as follows; pulmo sinister is divided into two main lobes as lobus cranialis and lobus caudalis. Lobus cranialis is also divided into pars cranialis and pars caudalis. Pulmo dexter is divided into four main lobes as lobus cranialis, lobus medius, lobus caudalis, and lobus accessorius (Bahadır and Yıldız 2014).

In the literature review, it was seen that there were studies on the respiratory system in different animal species (Düzler et al. 2005; Perez et al. 2006; Cano and Perez 2009; Gezer İnce and Pazvant 2010; Wysocki et al. 2010; Onuk et al. 2013; Özkadif et al. 2016; Fonseca et al. 2017; Gündemir et al. 2017; Abbasabadi et al. 2021; Halıgür and Özkadif 2021). However, no anatomical and histological studies were found on the larynx, trachea, and lungs of the lynx. This study was designed in line with this perceived shortcoming. We believe that this presented study will support scientific studies on similar subjects, tracheal collapse, corneal operation, and respiratory system diseases.

MATERIAL AND METHODS

Conditional permission was obtained from the Animal Experiments Local Ethics Committee of Kafkas University (KAÜ-HADYEK/2021-175) to conduct this study.

A 1.5-year-old female Eurasian lynx (*Lynx lynx*), which was brought to the Kafkas University Wildlife Rescue and Rehabilitation Center injured but could not be saved despite all the interventions, constituted our study material. The dissection process started with the larynx located at the beginning of the neck of the lynx cadaver, which was brought to the anatomy laboratory for anatomical examination. Larynx cartilages and trachea were exposed. The total trachea length, the width of the trachea at the beginning, middle and end, and the length and width of the lung lobes were measured with the help of a digital caliper (stainless steel 1- to 150-mm). For the nomenclature of anatomical terms, N.A.V. (2017) used. After the macro anatomical findings were obtained, tissue samples were taken from the head of the trachea and the tip of the lung for histological examinations and fixed in 10% formaldehyde solution. Afterwards, the routine tissue procedure was applied and it was blocked in paraffin. 5µm thick sections were taken from paraffin blocks and Crosman's triple staining and Periodic acid–Schiff (PAS) staining were performed to examine the general structure of trachea and lung tissue. The prepared sections were evaluated under the light microscope and photographed (Olympus BX43, JAPAN).

RESULTS

Macro Anatomical Results

The macro anatomical view of the larynx, trachea, and lungs of the lynx is shown in Figure 1. It was observed that the cartilages of the larynx were composed of double cartilago arythenoidea and single cartilago epiglottis, cartilago thyroidea, cartilago cricoidea. The largest cartilage in size was the thyroidea. The cornu rostrale and cornu caudale of the cartilago thyroidea were clearly identified. Incisura thyroidea caudalis, where the nervus laryngeus caudalis passes, was determined in the caudal of the region where the laminae of the cartilago thyroidea converge in the ventral. The processus muscularis of cartilago arytenoidea extending dorsally was detected. Processus vocalis was seen extending towards the cavum laryngis ventral of the same cartilage. Cartilago corniculata was not seen clearly. It was observed that the apex of the epiglottis was located. Trachea was consisted of 48 cartilago trachealis and the cross-section of the cartilago trachealis was circular in shape. Total tracheal length was 172.36 mm. Tracheal ring width was measured 12.40 mm at the beginning, 9.98 mm in the middle, and 9.23 mm at the end. The pulmo sinister in the lynx lung was divided into two main lobes as lobus cranialis and lobus caudalis. The lobus cranialis was also divided among itself as pars cranialis and pars caudalis. The pulmo dexter was divided into four main lobes as lobus cranialis, lobus medius, lobus caudalis, and lobus accessorius (Figure 1). The pulmo sinister in the lynx lung consisted of two main lobes, lobus cranialis and lobus caudalis. The lobus cranialis of the pulmo sinister was also divided among itself as pars cranialis and pars caudalis. Pars cranialis length was measured 65.75 mm, width 45.42 mm; pars caudalis as 72.61 mm in length and 54.12 mm in width. The lobus caudalis length of the pulmo sinister was 40.28 mm and the width was 34.95 mm. The pulmo dexter consisted of four main lobes: lobus cranialis, lobus medius, lobus accessorius, and lobus caudalis. Pulmo dexter's lobus cranialis was 84.04 mm long, 58.70 mm wide, lobus medius length 59.95 mm wide 30.99 mm, lobus accessorius length 36.41 mm wide 41.51 mm lobus caudalis 47.35 mm wide 38.44 mm.

Histological Results

Histological Structure of Trachea

It was determined that the C-shaped hyaline cartilage structure surrounding the trachea. It was observed that the hyaline cartilage was surrounded by the perichondrium and there were chondroblast cells just below the perichondrium. There were chondrocytes in the lacunae as we went towards the inner part of the cartilage. In addition, it was determined that the musculus transversus trachea, which consists of smooth muscle cells that close the open ends of the cartilage, surrounds the cartilage from the outside (Figure 2). It was determined that the trachea was composed of tunica mucosa, submucosa, and tunica adventitia. Lamina epithelialis was consisted of ciliated pseudostratified columnar epithelial cells and goblet cells. Lamina propria and submucosa were composed of connective tissue and in the submucosa; serous, mucous, and seromucous tracheal glands were determined. Tunica adventitia surrounded the trachea externally and had a loose connective tissue structure (Figure 3).

The Lung

The connective tissue capsule was surrounding the lung, respiratory bronchioles, alveoli, and abundant blood vessels. The connective tissue was penetrated into the lungs and the respiratory bronchioles were surrounded by the smooth muscle layer. Intraalveolar septa were separating adjacent alveoli from each other. In intraalveolar septum; capillaries, erythrocytes, type I pneumocytes which are squamous epithelial cells covering the alveolar surface, and type II pneumocytes which are less numerous and larger than type I pneumocytes, were observed. In addition, it was determined that there were macrophages (dust cells) in the alveolar sacs (Figure 4-6).

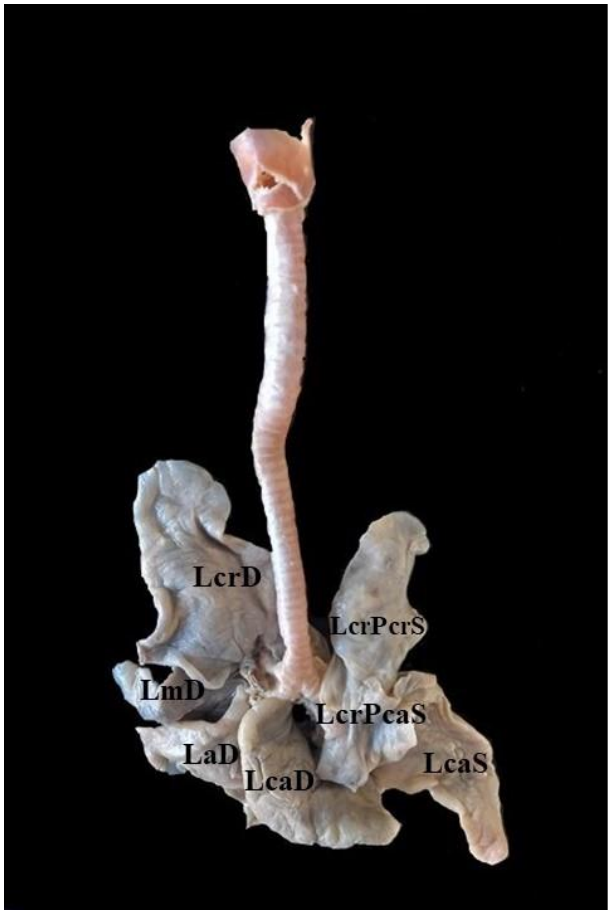


Figure 1: Larynx, trachea and lung in lynx (LcrD: Lobus cranialis of pulmo dexter, LmD: Lobus medius of pulmo dexter, LcaD: Lobus caudalis of pulmo dexter, LaD: Lobus accessorius of pulmo dexter, LcrPcS: Pars cranialis of lobus cranialis of pulmo sinister, LcrPcaS: Pars caudalis of lobus cranialis of pulmo sinister, LcaS: Lobus caudalis of pulmo sinister.

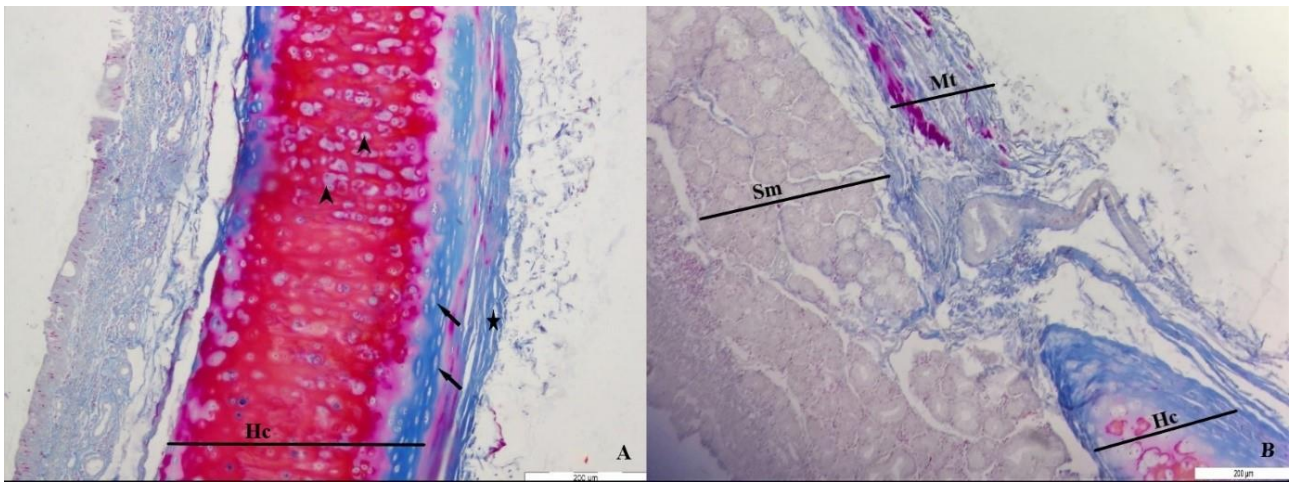


Figure 2: Lynx trachea (A, B). Hc: hyaline cartilage, Sm: Submucosa, Mt: musculus transversus, Chondroblasts (arrows), chondrocytes (arrowheads), and perichondrium (stars). Triple staining.

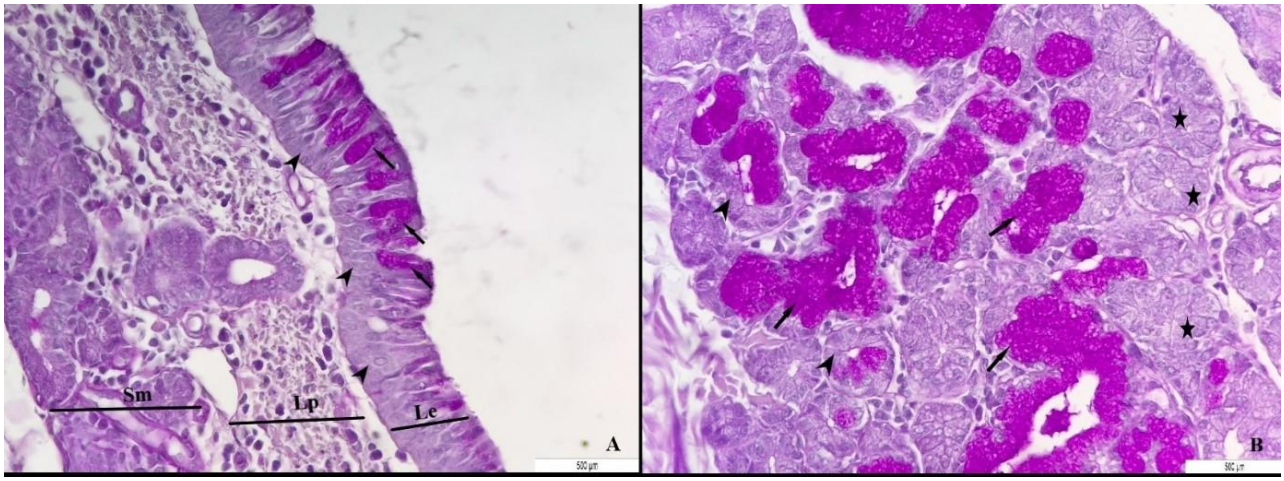


Figure 3: Lynx trachea (A, B). Le: Lamina epithelialis, Lp: Lamina propria, Sm: Submucosa. A: Epithelial cells (arrowheads) and goblet cells (arrows). B: Serous glands (stars), mucous glands (arrows), and seromucous (arrowheads) glands in the submucosa. PAS staining.

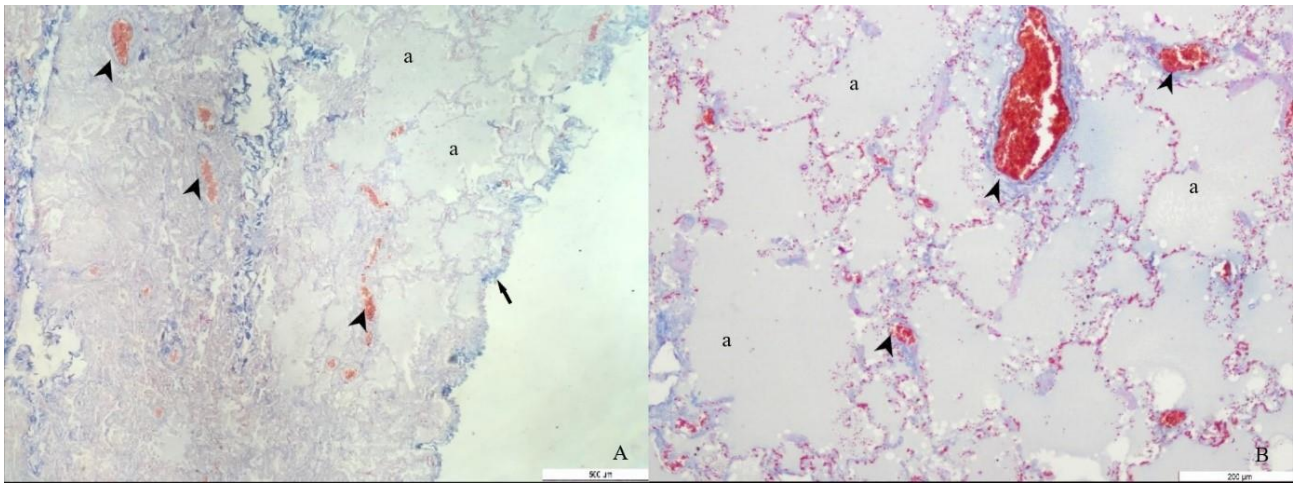


Figure 4: Lynx lung (A, B). Capsule (arrow), blood vessel (arrowhead), alveoli (a). Triple staining.

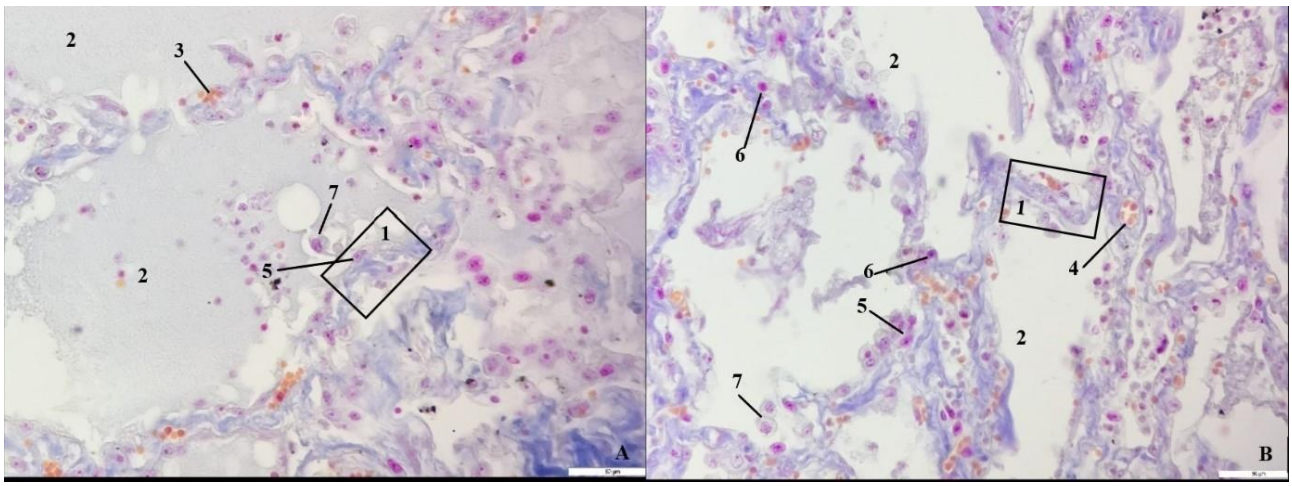


Figure 5: Lynx lung (A, B). Alveolar septum (1), alveoli (2), erythrocytes (3), capillary (4), Type 1 pneumocyte (5), Type 2 pneumocyte (6), macrophage (7). Triple staining.

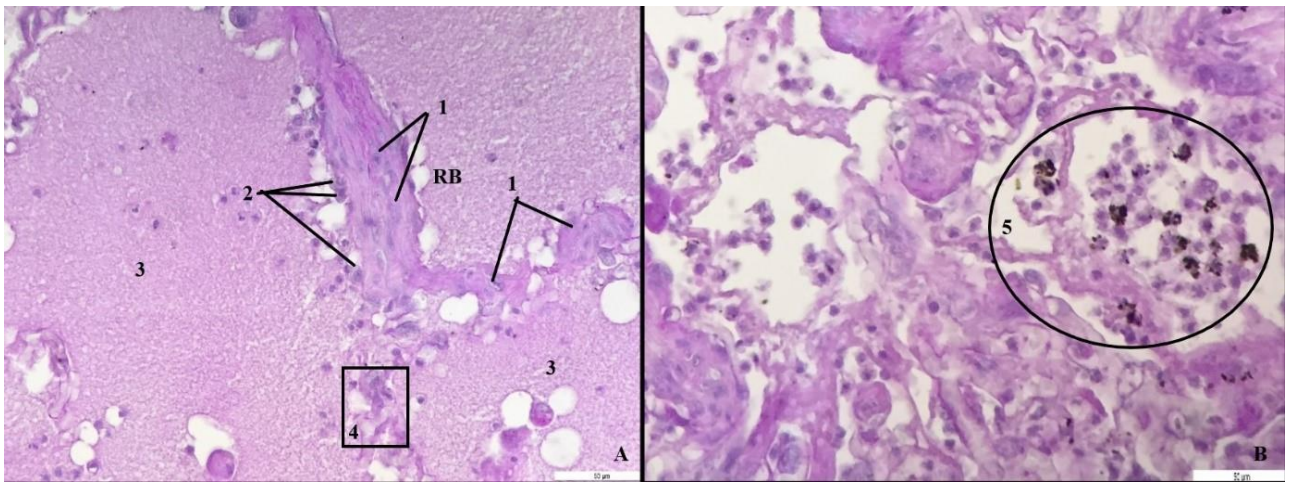


Figure 6: Lynx lung (A, B). Respiratory bronchiole ends (RB), smooth muscle layer (1), clara cells (2), alveoli (3), alveolar septum (4), dust cells (5). PAS staining.

DISCUSSION AND CONCLUSION

The respiratory system is tasked with exchanging oxygen and carbon dioxide and delivering oxygen to all tissues of the body. This system consists of two parts. These are the part where the air is transmitted and respiration, and the part where gas exchange, takes place (Gartner and Hiatt 2014). The trachea, the part through which air is conducted, is a tubular organ that extends from the larynx to the primary extrapulmonary bronchi (Banks 1993). Gartner and Hiatt (2014) reported that 15-20 horseshoe-shaped (C-ring) rings formed the trachea, Halıgür and Özkadif (2021) found that trachea rings were 42-48 in foxes, Dabanoğlu et al. (2001) found as 36-45 in dog. In the present study, it was determined that the trachea consisted of 48 rings. When we compare the number of cartilage rings detected with the literature, it is seen that it is closer to the fox. They (Halıgür and Özkadif 2021) reported the trachea length as 154.16± 0.60 mm in male foxes and 137.28± 0.62 mm in female foxes. The total tracheal length was 172.36 mm in our study so; we can say that the lynx trachea is longer than the fox trachea.

Haligür and Özkadif (2021) was measured the diameter of the cartilago trachealis as 13.37±0.38 mm in male foxes and 12.28±0.23 mm in female foxes. In the present study, the tracheal ring width in the female lynx was measured as 12.40 mm at the beginning, 9.98 mm in the middle, and 9.23 mm in the last part. Initial values of tracheal ring width were close to Haligür and Özkadif (2021). The open ends of the C-rings are connected posteriorly by smooth muscle (*musculus trachealis*). With the contraction of this muscle, the lumen of the trachea narrows, thereby increasing the speed of airflow (Gartner and Hiatt 2014). Musculus trachealis was located inside the cavity of the tracheal cartilages in horses, pigs, and ruminants but in cats and dogs outside the cavity (Bacha and Bacha 2012). Tracheal lumen is lined by pseudostratified ciliary columnar epithelium known as respiratory epithelium. This epithelium also contains DNES cells, about 30% of goblet cells. Goblet cells are single-celled glands that produce mucinogen, a mucus substance that is released onto the surface of the wet epithelium (Gartner and Hiatt 2014). The lamina propria and submucosa which are not clearly separated from each other, are located under the epithelium (Bacha and Bacha 2012). The lamina propria and lamina muscularis consist of prominent elastic fibers believed to replace the mucosa (Banks 1993). In the submucosa, there are tubuloacinar seromucous glands that open into the lumen via channels lined with ciliary cells, mucus-secreting cells, and various intermediate cells. The tubular parts of the tracheal glands are lined with mucus-secreting cells, while the acinar parts are lined with serous secretory cells. These glands are abundant in the proximal

parts of the trachea of almost all domestic mammal species (Eurell and Frappier 2006). It was observed that the C-shaped non-continuous cartilage rings provided the opening of the lumen, the cartilage was thicker in the anterior than in the posterior, and was continuous with the perichondrium in the monkey trachea. The lamina propria is thin, the submucosa is thick, and there are mucous and seromucous glands. The perichondrium of the cartilage fuses with the connective tissue in the submucosa and there are plenty of blood vessels (Gartner and Hiatt 2014). In the pig trachea, the ends of the cartilage rings are not joined and are interconnected by connective tissue. The musculus trachealis is inside the cartilage. The epithelial layer of the mucosa is typically composed of ciliated epithelium, including goblet cells (Banks 1993). Carnivorous trachea is lined by goblet cell, ciliated, and pseudostratified columnar epithelium. The lamina propria is in the form of a longitudinal band of elastic fibers. The musculus trachealis (with a smooth structure) is located outside the C-shaped cartilaginous cavity (Bacha and Bacha 2012). C-shaped hyaline cartilage surrounding the trachea externally, musculus transversus trachea, outside the cartilage rings; lamina epithelialis, ciliated pseudostratified columnar epithelial cells, and goblet cells, loose connective tissue in the lamina propria. Serous, mucous, and seromucous glands in the submucosa and loose connective tissue adventitia surrounding the trachea were determined in lynx.

The lungs, located in the rib cage, serve as the main organ of the respiratory system. The visceral leaf of the capsule-shaped pleura surrounds the lungs externally. This connective tissue capsule enters the organ and divides the lungs into lobes and lobules (Girgin et al. 2010). The pulmo sinister of carnivores is divided into two main lobes, lobus cranialis and lobus caudalis. Lobus cranialis is also divided among as pars cranialis and pars caudalis. Pulmo dexter is divided into four main lobes as lobus cranialis, lobus medius, lobus caudalis, and lobus accessorius (Hazıroğlu and Çakır 2018; Demiraslan and Dayan 2021). In a study of lung typing (Voyevoda et al. 1992), the right lung lobe mostly (70%) consists of four lobes in arctic foxes: cranial, medial, caudal, and infracardiac. In our study, the right lung consisted of four lobes. Only the lobus accessorius of pulmo dexter was named lobus infracardiaca in the other study. Voyevoda et al. (1992) stated that the left lung lobe in dogs mostly consists of three lobes as cranial, medial, and caudal. In the lynx, the left lung lobe consisted of three lobes in total. But the nomenclature was different. In our study, while the lobus cranialis of the left lung lobe was divided into pars cranialis and pars caudalis, Voyevoda et al. (1992) named the left lung lobe in the dog as cranial, caudal, and medial. We think that this difference is due to the different branching of the bronchi.

The structure of the lungs is generally examined in two parts as air-conducting pipes and respiratory tissue. The airways are the bronchi and bronchioles. It begins where the trachea splits in the thoracic cavity and continues to the respiratory tissue (Girgin et al. 2010). Bronchioles are air-conducting ducts of 1 mm or less in diameter. Larger bronchioles represent branches of segmental bronchi. These ducts branch repeatedly to form terminal bronchioles. Terminal bronchioles also branch to form respiratory bronchioles. Respiratory bronchioles form a transition zone in the respiratory system and are involved in air conduction and gas exchange. They have a narrow diameter. The first sections contain ciliated cubic epithelium and clara cells. Clara cells predominate in its distal. Rarely brush cells and dense nucleated granule cells are also present along the length of the respiratory bronchiole (Ross and Pawlina 2016). Respiratory bronchioles are well developed in cats and dogs (Bacha and Bacha 2012). The alveoli are surrounded by capillary networks. Capillaries are attenuated, non-perforated, continuous endothelial cells, very close to type I pneumocytes. The alveoli are separated from each other by walls of varying thickness known as interalveolar septa. Macrophages, known as dust cells, are usually seen in the interalveolar septa. Dust cells differentiate from monocytes and enter the lungs through the bloodstream (Gartner and Hiatt 2014). Each alveolar duct divides into three or more alveolar sacs. There are no smooth muscles in the sacs. Thin squamous epithelial cells (type I alveolar cells) are abundant in the alveoli, while surfactant-producing type II alveolar cells are rare (Bacha and Bacha 2012). Type I pneumocytes are not capable of dividing. They are fairly thin squamous cells and occupy most (95%) of the alveolar surface. Cells, also called type II pneumocytes or septal cells, are cubic in shape. It is found interspersed between type I pneumocytes and tends to collect at septal junctions. Type II pneumocytes occupy only 5% of the alveolar air surface (Ross and Pawlina 2016). In the monkey, the two alveoli are separated by an interalveolar septum. There are capillaries containing abundant erythrocytes in the septum. The entire alveolar surface consists of an abundance of type I pneumocytes and a smaller number of type II pneumocytes. The interalveolar septa are thick, containing blood vessels and connective tissue elements, including macrophages known as dust cells. At the entrance to the alveoli are smooth muscle cells that look like knobs in the dog, the alveoli are composed of highly attenuated endothelial cells, type I pneumocytes, and an intervening basal lamina. Cytoplasm is scarce in both cell types. The air space of the alveoli is empty, while the capillary lumen contains red blood cells (Gartner and Hiatt 2014). In the lynx lung; it was determined that the connective tissue capsule surrounding the lung from the outside entered the inside of the organ and divided the organ into lobes. It was observed that the respiratory bronchiole endings were surrounded by the smooth muscle layer. In addition, intraalveolar septums separating adjacent alveoli from each other were detected. In intraalveolar septum; capillary vessel nuclei, erythrocytes, type I pneumocytes with squamous epithelial cells and covering the entire alveolar surface were seen interspersed with type II pneumocytes. Also, it was determined that there were macrophages (dust cells) interspersed in the alveolar sacs and lung tissue.

In conclusion, Lynxes are endangered wild animals. Therefore, very few studies have been found on lynxes (Arı et al. 2018; Arı and Uslu 2021). The larynx, trachea and lungs of the wild animal lynx were analyzed anatomically and histologically. We believe that this presented study will support scientific studies on similar subjects (Akgün et al. 2018; Osorio-Echeverri et al. 2019) cornage operation with tracheal collapse, and approach to respiratory system diseases (Masseaua and Reinero 2019).

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest for this study.

ACKNOWLEDGMENT

We thank 'Kafkas University Wildlife Protection and Rescue Center'.

AUTHOR CONTRIBUTIONS

Idea / Concept: GKD
Supervision / Consultancy: EKS
Data Collection and / or Processing: ŞYA, GKD
Analysis and / or Interpretation: ŞYA, GKD
Writing the Article: ŞYA, GKD, EKS
Critical Review: ŞYA, GKD, EKS

REFERENCES

Abbasabadi BM, Moradi HR, Aferi S, Kyıllar M (2021). Histomorphology of the lower respiratory tract in the Indian crested porcupine (*Hystrix indica*). *Anat Histol Embryol*, 50 (3), 534-542.

Akgün RO, Bakici C, Ekim O, Bumin A, Orhan İÖ (2018). Sectional evaluation of anatomic structures in cat (*felis catus*) thoracic cavity by computed tomography imaging and silicone plastination methods. *Int J Morphol*, 36 (4), 1246-1251.

Arı HH, Kuru N, Uslu S, Özdemir Ö (2018). Morphological and histological study on the foot pads of the Anatolian bobcat (*Lynx lynx*). *Anat Rec*, 301, 932–938.

Arı HH, Uslu S (2021). Morphology and histology of the Eurasian lynx (*lynx lynx*) planum nasale. *Slov Vet Res,* 58 (4), 147-153.

Bacha WJ, Bacha LM (2012). Color Atlas of Veterinary Histology. 3th ed. John Wiley & Sons, Ltd., New Jersey.

Bahadır A, Yıldız H (2014). Veteriner Anatomi, Hareket Sistemi & İç Organlar, extended 5th ed., Ezgi bookstore, Bursa.

Banks WJ (1993). Applied Veterinary Histology. 3th ed. St. Louis, Missouri.

Cano I, Perez W (2009). Quantitative anatomy of the trachea of the giraffe (*Giraffa camelopardalis rothschildi*). *Int J Morphol,* 27 (3), 905-908.

Dabanoğlu I, Öcal MK, Kara ME (2001). A quantitavie study on the trachea of the dog. *Anat Histol Embryol*, 30 (1), 57-59.

Demiraslan Y, Dayan MO (2021). Veteriner Sistematik Anatomi, 1th ed., Atlas bookstore, Konya.

Dursun N (2008). Veteriner Anatomi II, 12th ed., Medisan publication, Ankara.

Düzler A, Nur İH, Çirli Ş (2005). Ceylanda larynx kıkırdakları ve trachea üzerinde makro-anatomik bir araştırma. *Erciyes Üniv Vet Fak Derg*, 2(1), 23-28.

Eurell JA, Frappier BL (2006). Textbook of Veterinary Histology. 6th ed. Blackwell Publishing Ltd., Iowa, USA.

Evans HE, de Lahunta A (2013). Miller's Anatomy of the Dog. 4th ed., WB Sunders Company, Philadelphia.

Fonseca CMB, da Silva ABS, Cavalcante MMADS et al. (2017). Morphology of laryngeal cartilage of the nine-banded armadillo (*Dasypus novemcinctus*) Linnaeus, 1758. *Microsc Res Tech*, 80 (10), 1089-1095.

Gartner LP, Hiatt JL (2014). Color Atlas and Text of Histology. 6th ed., Lippincott Williams & Wilkins, a Wolters Kluwer business, Philadelphia.

Gezer İnce N, Pazvant N (2010). Macro-anatomic study on larynx and trachea in sea gulls. *Acta Vet Eurasia*, 36, 1-6.

Gündemir O, Esener OBB, Alpak H (2017). A macroanatomic study on larynx cranialis of turkeys in thrace region, Turkey. *Acta Vet Eurasia*, 43, 89-91.

Girgin A, Alabay B, Liman N et al. (2010). Veteriner Özel Histoloji. 2th ed., Nobel publication, Ankara.

Halıgür A, Özkadif S (2021). Kızıl tilki (*Vulpes vulpes*)'de larynx kıkırdak'larının ve trachea'nın morfolojik çalışması. *MAE Vet Fak Derg*, 6 (3), 109- 114.

Hazıroğlu M, Çakır A (2018). Veteriner Anatomi Konu Anlatımı ve Atlas. Güneş bookstore, 4th ed. in: Dyce KM, Sack WO, Wensing CJC. Textbook of Veterinary Anatomy, 2nd ed. Philadelphia, London, New York, St. Louis, Sydney, Toronto: W.B. Saunders Company.

König HE, Liebich HG (2015). Veteriner Anatomi (Evcil Memeli Hayvanlar), 6th ed., Medipres, Germany.

Kütükçü AE. (2016). Türkiye'deki Memeli Hayvanların İz Rehberi. WWF.

Masseaua I, Reinero CR (2019). Thoracic computed tomographic interpretation for clinicians to aid in the diagnosis of dogs and cats with respiratory disease. *Vet J,* 253, 105388.

N.A.V. (2017). International Committee on Veterinary Gross Anatomical Nomenclature. Nomina Anatomica Veterinaria (NAV). 6th ed. World Association of Veterinary Anatomists, Hanover (Germany), Ghent (Belgium), Columbia, MO (U.S.A.), Rio de Janeiro (Brazil).

Onuk B, Tütüncü Ş, Kabak M (2013). The morphological study of the larynx cranialis in stork (*Ciconia ciconia l.*). *Acta Vet Eurasia*, 39, 148-154.

Osorio-Echeverri JS, Orrego-Metaute DA, Murillo-Escobar JP, Tamayo-Arango L (2019). Three-dimensional cat virtual anatomy: development of an interactive virtual anatomical software. *J Morphol Sci*, 36, 105–114.

Özkadif S, Dayan MO, Demiraslan Y, Aykut M, Özgel Ö (2016). Morphometric properties of larynx and trachea in the New Zealand rabbit. *Eurasian J Vet Sci*, 32 (4), 208-213.

Perez W, Lima M, Cunarro B (2006). Larynx anatomy in a tiger (*Panther tigris, Linnaeus, 1758*). *J Anim Vet Adv*, 5 (12), 1093-1095.

Ross MH, Pawlina W (2016). Histology a Text and Atlas. 7th ed., Wolters Kluwer Health, Netherlands.

W.W.F. (2016)-Türkiye (Doğal Hayatı Koruma Vakfı) Türkiye'deki Memeli Hayvanların İz Rehberi.

Voyevoda TV, Shishkin GS, Valitskaya RI, Umantseva ND (1992). Macrostructure differences of polar fox and dog lungs. *Anat Rec*, 234, 89-92.

Wysocki J, Kielska E, Janiuk I, Charuta A (2010). Analysis of larynx measurements and proportions in young and adult domestic pigs (*Sus scropha domestica*). *Turkish J Vet Anim Sci*, 34 (4), 339-347.

Van Vet J, 2023, 34 (2) 133-139

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Koç Yıldırım E, Kaya M, Dereli Fidan E (2023).** The Effect of Cold Water and Stocking Density on Oxidative Metabolism in Broiler Chickens During Hot Dry Season. *Van Vet J*, 34 (2), 133-139. DOI: https://doi.org/10.36483/vanvetj.1256926

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

The Effect of Cold Water and Stocking Density on Oxidative Metabolism in Broiler Chickens During Hot Dry Season

Ece KOÇ YILDIRIM[1,*] Mehmet KAYA[2] Evrim DERELİ FİDAN[2]

[1] *Department of Physiology, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, 09000, Aydin, Türkiye*
[2] *Department of Animal Science, Faculty of Veterinary Medicine, Aydin Adnan Menderes University, 09000, Aydin, Türkiye*

Received: 28.02.2023 Accepted: 18.05.2023

ABSTRACT The present experiment was conducted to investigate the effect of drinking water temperature and stocking density (SD) on oxidative metabolism in the heart, liver, *bursa fabricius*, and thymus in broiler chickens raised under heat stress. The experiment comprised of 360 one-day-old Ross 308 male broiler chickens randomly divided to 6 experimental groups with 4 replicates in each group. Experimental treatments included three different SD (low = 12 birds/m^2, medium = 15 birds/m^2 or high = 18 birds/m^2) and two different drinking water temperature (10 ˚C or 31 ˚C) in a 3 x 2 factorial arrangement. At the end of the experiment (42 days of age), two birds per replicate were euthanized for sample collection. The results indicated high SD increased oxidative damage and caused an increase in MDA formation in the heart, liver and thymus. On the other hand, cold water ameliorated the oxidative damage due to the high SD in the thymus. In the study, the statistically non-significant interaction was generally determined between cage stocking density and cold drinking water on the antioxidant system. Besides, while cold water administration increased CAT activity in heart and thymus tissues, decreased GSH activity. In conclusion, drinking water temperature and stocking density are key environmental factors effecting oxidative metabolism when broilers under high temperature conditions; however, more studies are needed in terms of the interactive effects of water temperature and stocking density on antioxidant enzymes under current conditions.

Keywords: *Chickens, Housing, Hot temperature, Oxidative stress, Water.*

ÖZ

Etlik Piliçlerde Sıcak İklimde Soğuk Su ve Yerleşim Sıklığının Oksidatif Metabolizmaya Etkisi

Bu çalışma, sıcaklık stresi altında yetiştirilen etlik piliçlerde, içme suyu sıcaklığı ve yerleşim sıklığının kalp, karaciğer, *bursa fabricius* ve timus dokularında oksidatif metabolizma üzerindeki etkisini araştırmak için yapıldı. Deney, 360 adet bir günlük Ross 308 erkek etlik civcivin, her grupta 4 tekerrür olacak şekilde rastgele 6 deney grubuna bölünmesiyle oluşturuldu. Deneysel uygulamalar, 3 x 2 faktöriyel düzenlemede üç farklı yerleşim sıklığı (düşük = 12 kanatlı/m^2, orta = 15 kanatlı/m^2 veya yüksek = 18 kanatlı/m^2) ve iki farklı içme suyu sıcaklığını (10 ˚C veya 31 ˚C) içerdi. Deney sonunda örnek alımı için her tekrar grubundan 2 tavuk alınarak ötenazi edildi. Sonuçlar, yüksek yerleşim sıklığının oksidatif hasarda ve kalp, karaciğer ve timusta MDA oluşumunda bir artışa neden olduğunu gösterdi. Buna karşın soğuk su, timustaki yüksek yerleşim sıklığı nedeniyle oluşan oksidatif hasarı iyileştirdi. Çalışmada, antioksidan sistem üzerine yerleşim sıklığı ve soğuk içme suyu arasındaki etkileşimler genel olarak önemsiz bulundu. Bunun yanısıra, soğuk su uygulaması kalp ve timus dokularında CAT aktivitesini artırırken GSH aktivitesini azalttı. Sonuç olarak, içme suyu sıcaklığı ve yerleşim sıklığı yüksek sıcaklıkta yetiştirilen etlik piliçlerde oksidatif metabolizmayı etkileyen anahtar çevresel faktörlerdir; ancak su sıcaklığı ve yerleşim sıklığı interaksiyonunun antioksidan enzimler üzerindeki etkilerinin belirlenmesi açısından günümüz koşullarında daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: *Barınak, Oksidatif stres, Su, Tavuk, Yüksek sıcaklık.*

INTRODUCTION

In today's livestock industry, animals are exposed and raised under certain conditions, such as high temperatures, high density of stocking, diseases, inadequate health services, which adversely affect their reproductive performance, health status and well-being. For this reason, researchers are making efforts to improve the response of animals to stress. However, there is limited information on the physiological mechanisms of stress responses in animals exposed to various stress factors (Goo et al. 2019).

✉ *Corresponding author: vetece04@hotmail.com.tr

One of the biggest problems in livestock farming in many countries is heat stress. Among livestock animals, especially poultry are the most susceptible to heat stress. Poultry lack the inhibition of body heat production due to the fact that their bodies are almost entirely covered with feathers and they have limited sweat glands. In poultry, which are frequently exposed to heat stress, first of all, feed consumption decreases. This leads to loss of body weight and rapid depletion of fat reserves (Quinteiro-Filho et al. 2010). Hormonal balance, immune system and blood values as well as feed consumption are negatively affected by heat stress (Aengwanich, 2007). It induces oxidative stress and causes respiratory alkalosis (Teeter et al. 1985; Altan et al. 2003; Lin et al. 2006). Heat stress can cause an increase in lipid peroxidation products and protein carbonyls in plasma and tissues, whereas the severity and duration of heat stress change the activity of antioxidant enzymes (Akbarian et al. 2016). Thus, the performance and health of the animals are adversely affected.

Stocking density can also be a critical stress factor in intensive poultry farming, as high stocking density is highly associated with problems in poultry health, performance and welfare. Possible causes of these problems are reduced access to feed and water, abnormal behavior, inadequate air and poor soil quality. (Esteyez 2007). In addition, high stocking density may result in increased temperature in the micro-environment surrounding the broilers and reduced heat loss from the body, resulting in moderate heat stress (Cengiz et al. 2015). High stocking density causes increase in heterophile to lymphocyte ratio, blood stress hormones, and oxidative stress but decrease in immune response (Mustafa et al. 2010; Najafi et al. 2015; Astaneh et al. 2018; Nasr et al. 2021). Therefore, high stocking density may induce some pathological events similar to heat stress (Goo et al. 2019).

In order to diminish the possible negative effects of heat stress, some practices such as changing the feed content, feed restriction, intermittent feeding and lighting programs are recommended. Apart from these, giving cold water can be an important strategy. Although water is not a nutritional element on its own, it is very necessary to evaluate the feed taken and to keep the body temperature of the animal constant (Park et al. 2014). It is observed that broilers under heat stress cannot regulate their body temperature when their water consumption decreases, but it is reported that cooling the drinking water positively affects the animals' ability to cope with heat stress (Bruno et al. 2011). In addition, there are studies suggesting that cold water given to poultry animals exposed to heat stress positively affects the development and performance of the animals (Park et al. 2014; Farghly et al. 2018).

In the present study, it was aimed to specify the effects of cold drinking water and different stocking density on oxidative metabolism of selected organs in broiler chickens raised under high temperature.

MATERIAL AND METHODS

Birds, Experimental Design and Management

The present study was approved by Aydin Adnan Menderes University Animal Ethical Committee (ADÜ-HADYEK Approval no: 64583101/2020/065).

The experiment comprised of 360 one-day-old Ross 308 male broiler chickens randomly divided to 6 experimental groups with 4 replicates in each group as a totally random design with 3 x 2 factorial arrangement of the stocking density (SD) [low = 12 birds/m^2 (LSD), medium = 15 birds/m^2 (MSD) or high = 18 birds/m^2 (HSD)] and the drinking water temperature (10 ˚C or 31 ˚C). The birds were housed in coops with a floor area of 1 m^2, excluding the feeder and water areas, and 5-7 cm in height, homogeneously laid with wood shavings. A 23L:1D lighting program was applied up to 7 days and 18L:6D thereafter until day 42. The temperature was sustained at 32°C until day 7 followed by a reduction of 3°C per week until day 21 and a temperature of 24–26°C was sustained afterwards.

Starter feed containing 3000 kcal/kg metabolic energy (ME) and 23% crude protein (CP) in the age period of 0-10 days, grower feed containing 3100 kcal/kg ME and 21.5% CP between days 11-24, and finisher feed containing 3200 kcal/kg ME and 19.5% CP between days 25- 42 was given *ad libitum*.Free access of birds to feed and water was being certain throughout the experiment. The experiment's duration was 42 days.

Sample Collection and Determination of Oxidative Metabolism

At the end of the experiment a total of 48 birds, two birds per replicate, were slaughtered and the heart, liver, *bursa fabricius* and thymus tissue samples were collected to determine the oxidative metabolism. Tissue samples were first diluted 10 times with cold 150 mM PBS (pH 7.4) and homogenized for 1-2 minutes at 2,000 rpm with a tissue homogenizer (IKA WERKE Yellowline OST Basic S2 Analog Overhead Stirrer, Athy, Ireland). Homogenates were centrifuged at 12000 rpm for 10 minutes at +4^{o}C. Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) activities were established in the supernatants obtained after centrifugation. The determination of MDA was made as per the method reported by Ohkawa et al. (1979). In this method, a pink colored pigment was formed when thiobarbituric acid and MDA react in acidic pH and hot environment, and this color was measured at 532 nm and the results were given as nm/mg protein. GSH was determined as described by Tietze (1969). In this procedure, 5,5'-dithiobis,2-nitrobenzoic acid (DTNB) is reacted to yield a product measured at 412 nm within 4 minutes, and the results are expressed as mg/g protein. CAT activity was measured in supernatants according to the method determined by Luck (1965). In this method, the conversion of substrate H_2O_2 to H_2O was observed spectrophotometrically at 240 nm at 20-second intervals and the decrease in absorbance was measured. Enzyme activity was given using k/mg protein. SOD activity was specified as per the method of Sun et al. (1998). In this method, superoxide radicals form formazone dye in the presence of nitro blue tetrazolium. This color intensity was measured spectrophotometrically at 560 nm. The percent inhibition was calculated depending on SOD activity and the results were expressed as U/mg protein.

Statistical Analysis

The data were statistically analyzed using the SPSS software package (version 22.0 SPSS Inc., Chicago, IL, USA). Levene's test was used to confirm the homogeneity of variances. The oxidative stress data (MDA, SOD, CAT, and GSH) were subjected to ANOVA using the General Linear Model (GLM) procedure with cold water and stocking density as the main effects along with their interactions included in the following model:

$x_{ijk} = \mu + M_i + D_j + (MD)_{ij} + e_{ijk}$,

Where, x_{ijk} = analyzed measurement, μ = Overall mean, M_i = cold water (10 ˚C or 31 ˚C), D_j= effect of stocking density (12, 15, and 18 birds/m^2), $(MD)_{ij}$= effect of interaction,

e_{ijk}= residual random error. In analyzes GLM was designed to reveal the effect of cold water and stocking density on oxidative stress parameters. The partial effects of cold water and stocking density for each factor were analyzed with Least Squares Means Test and multiple comparisons were performed with a Duncan Test.

RESULTS

High SD (18 birds/m²) in broilers significantly increased MDA levels of the heart, liver, and thymus compared to low SD (12 birds/m²) (p=0.029) (Table 1). The interaction between SD and drinking water temperature was also significant in regard to thymus MDA level (p=0.026) (Table 1).Cold water led to a reduction in SOD activity in *bursa fabricius*. (p=0.003) (Table 2).

In addition, cold water applied to broilers increased CAT activity in heart and thymus tissues (p=0.040 and p=0.000, respectively), while reduced GSH activity in both tissue (p=0.002 and p=0.045, respectively) compared to normal water administration (Table 3 and 4).

The interaction between SD and drinking water temperature caused a significant reduction only in cardiac GSH activity (p=0.048) (Table 4).

In addition to the antioxidant enzyme activities in the selected tissues of drinking water, the stocking density affected only liver GSH activity. High SD significantly decreased liver GSH activity compared to low SD (p=0.039) (Table 4).

Table 1: Effect of drinking water temperature (WT) and stocking density (SD) on MDA activity of the selected organs in broilers (nmol/mg protein).

Factors	MDA			
	Heart	**Liver**	***Bursa fabricius***	**Thymus**
Water temperature				
Normal	43.97	19.52	8.68	28.08
Cold	43.15	19.33	8.69	26.45
SEM[1]	2.55	0.75	0.27	1.30
Stocking density				
12 birds/m²	38.65[b]	17.42[b]	9.09	23.95[b]
15 birds/m²	41.58[ab]	19.23[ab]	8.34	28.55[ab]
18 birds/m²	50.46[a]	21.63[a]	8.63	29.31[a]
SEM[2]	4.42	1.30	0.48	2.26
WTx SD Interactions				
Normal-12 birds/m²	47.19	20.24	9.40	25.74[ab]
Normal-15 birds/m²	35.44	20.80	7.89	27.09[ab]
Normal-18 birds/m²	49.29	17.52	8.75	31.43[a]
Cold-12 birds/m²	35.98	18.22	8.78	31.36[a]
Cold-15 birds/m²	41.85	22.45	8.80	27.18[ab]
Cold-18 birds/m²	51.63	17.33	8.51	20.80[b]
SEM[3]	1.80	0.53	0.19	0.92
Significance of main effects			**p value**	
Water temperature	0.821	0.862	0.972	0.381
Stocking density	**0.029**	**0.009**	0.306	**0.047**
WTX SD Interaction	0.126	0.379	0.265	**0.026**

[a, b]: Means with different superscript letters in the same column differ (p<0.05), [1, 2]: Standard error of the mean, [3]: Standard error of the mean for interaction effect.

Table 2: Effect of drinking water temperature (WT) and stocking density (SD) on SOD activity of the selected organs in broilers (nmol/mg protein).

Factors	SOD			
	Heart	**Liver**	***Bursa fabricius***	**Thymus**
Water temperature				
Normal	4.38	1.47	1.86[a]	3.05
Cold	3.82	1.64	1.66[b]	2.96
SEM[1]	0.20	0.07	0.04	0.13
Stocking density				
12 birds/m^2	4.06	1.61	1.85	3.27
15 birds/m^2	3.86	1.53	1.74	3.02
18 birds/m^2	4.38	1.52	1.69	2.73
SEM[2]	0.36	0.13	0.07	0.24
WTx SD Interactions				
Normal-12 birds/m^2	4.36	1.56	1.99	3.26
Normal-15 birds/m^2	3.94	1.41	1.84	3.09
Normal-18 birds/m^2	4.83	1.43	1.75	2.80
Cold-12 birds/m^2	3.76	1.67	1.71	3.27
Cold-15 birds/m^2	3.78	1.65	1.63	2.95
Cold-18 birds/m^2	3.92	1.60	1.64	2.67
SEM[3]	0.14	0.05	0.03	0.09
Significance of main effects			**p-value**	
Water temperature	0.065	0.124	**0.003**	0.657
Stocking density	0.360	0.733	0.132	0.097
WTX SD Interaction	0.577	0.879	0.549	0.939

[a, b]: Means with different superscript letters in the same column differ ($p<0.05$), [1, 2]: Standard error of the mean, [3]: Standard error of the mean for interaction effect.

Table 3: Effect of drinking water temperature (WT) and stocking density (SD) on CAT activity of the selected organs in broilers (k/mg protein).

Factors	CAT			
	Heart	**Liver**	***Bursa fabricius***	**Thymus**
Water temperature				
Normal	0.47[b]	2.29	0.11	0.23[b]
Cold	0.71[a]	1.70	0.14	0.60[a]
SEM[1]	0.08	0.21	0.01	0.06
Stocking density				
12 birds/m^2	0.61	2.36	0.11	0.47
15 birds/m^2	0.68	1.71	0.12	0.30
18 birds/m^2	0.48	1.92	0.13	0.47
SEM[2]	0.14	0.37	0.01	0.11
WTx SD Interactions				
Normal-12 birds/m^2	0.62	2.59	0.10	0.19
Normal-15 birds/m^2	0.55	2.14	0.11	0.27
Normal-18 birds/m^2	0.25	2.12	0.12	0.22
Cold-12 birds/m^2	0.61	2.12	0.12	0.75
Cold-15 birds/m^2	0.82	1.27	0.14	0.33
Cold-18 birds/m^2	0.72	1.72	0.15	0.72
SEM[3]	0.05	0.15	0.00	0.04
Significance of main effects			**p-value**	
Water temperature	**0.040**	0.060	0.073	**0.000**
Stocking density	0.364	0.213	0.388	0.218
WTX SD Interaction	0.242	0.789	0.952	0.057

[a, b]: Means with different superscript letters in the same column differ ($p<0.05$), [1, 2]: Standard error of the mean, [3]: Standard error of the mean for interaction effect.

Table 4: Effect of drinking water temperature (WT) and stocking density (SD) on GSH activity of the selected organs in broilers (mg/g protein).

Factors	GSH			
	Heart	**Liver**	***Bursa fabricius***	**Thymus**
Water temperature				
Normal	18.42[a]	16.99	31.20	7.35[a]
Cold	12.13[b]	15.67	30.20	5.50[b]
SEM[1]	1.36	0.62	1.59	0.63
Stocking density				
12 birds/m^2	15.83	17.81[a]	32.03	7.60
15 birds/m^2	14.75	16.26[ab]	29.82	5.14
18 birds/m^2	15.25	14.93[b]	30.24	6.53
SEM[2]	2.36	1.08	2.76	1.09
WTx SD Interactions				
Normal-12 birds/m^2	16.69[ab]	18.67	31.63	8.98
Normal-15 birds/m^2	16.73[ab]	14.75	28.83	4.77
Normal-18 birds/m^2	21.83[a]	17.54	32.93	8.29
Cold-12 birds/m^2	14.96[abc]	16.95	32.24	6.21
Cold-15 birds/m^2	12.77[bc]	15.10	30.80	5.52
Cold-18 birds/m^2	8.67[c]	14.97	27.55	4.77
SEM[3]	0.96	0.44	1.12	0.44
Significance of main effects	**p-value**			
Water temperature	**0.002**	0.147	0.659	**0.045**
Stocking density	0.901	**0.039**	0.698	0.092
WTX SD Interaction	**0.048**	0.393	0.383	0.127

[a, b, c]: Means with different superscript letters in the same column differ ($p<0.05$), [1, 2]: Standard error of the mean, [3]: Standard error of the mean for interaction effect.

DISCUSSION AND CONCLUSION

Heat stress and stocking density induce oxidative stress and damage the immune system and antioxidant system. Lipid peroxidation is a stress indicator of the autocatalytic mechanism that causes oxidative degradation of cellular membranes. MDA is the main final product of lipid peroxidation but a high production of MDA has been reported as an indicator of oxidative stress (Dalle-Donne et al. 2006). High SD (18 birds/m^2) in broilers significantly increased MDA levels of the heart, liver, and thymus. A similar result was reported by Simsek et al. (2009) and it was stated that crowding increases oxidative damage and causes an increase in MDA formation. Cold water application decreased the thymus MDA level in animals raised in high stocking density. Farghly et al. (2018) found that cold water application in Muscovy ducklings decreased serum MDA levels similar to this study. Cold water administration can positively affect performance by causing feed consumption and daily body weight gain in chickens raised under heat stress (Beker and Teeter 1994), as well as may reduce oxidative stress due to high stocking density.

The effect of reactive oxygen species (ROS) is kept in balance by non-enzymatic and enzymatic antioxidants. SOD and CAT are enzymatic, while GSH is part of the non-enzymatic antioxidant system. Hydrogen peroxide (H_2O_2) is the product of the reaction catalyzed by SOD which is the first line of defense of the antioxidant system, the substrate of both CAT and glutathione peroxidase GPx (Irato and Santovito 2021). GSH plays a role as a cofactor for GPx and reacts directly with ROS by its sulfhydryl groups (Michelli et al. 2016). In this study, cold water administration to broilers reared under high ambient temperature increased cardiac and thymus CAT activity, while decreasing GSH activity. In addition, cold water led to a reduction in SOD activity in *bursa fabricius*. Cold water application did not ameliorate the decreased cardiac GSH

activity due to high stocking density. In contrast to our study, Farghly et al. (2018) suggested that cold water increased serum total antioxidant capacity in Muscovy ducklings. Although there is no study on the effect of cold water on different antioxidant enzymes in different tissues in birds raised under heat stress, our results are thought to confirm those of Fadillioglu et al. (2002) that antioxidant enzymes can have complementary roles for each other for the tissue injuries. Finally, in addition to these effects of water temperature, Nasr et al. (2021) reported similar findings that the SD had an effect only on liver GSH activity, and high SD decreased liver GSH activity.

As a result, high stocking density in broilers raised under high ambient temperature increased oxidative stress in heart, liver and thymus tissues. Cold water application was not effective in reducing oxidative stress in tissues, except thymus. The effect of cold water on the antioxidant system is controversial. While it increased CAT activity in heart and thymus tissues, decreased GSH activity. The stocking density and their interactions did not have a significant effect on the antioxidant system.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENT

We gratefully thank Dr. Cemre KOC for correcting the English grammar mistakes of the manuscript.

AUTHOR CONTRIBUTIONS

Idea / Concept: EDF, MK
Supervision / Consultancy: EDF
Data Collection and / or Processing: MK, EKY
Analysis and / or Interpretation: EKY
Writing the Article: EKY
Critical Review: MK

REFERENCES

Aengwanich W (2007). Effects of High Environmental Temperature on Blood Indices of Thai Indigenous Chickens, Thai Indigenous Chickens Crossbred and Broilers. *Int J Poult Sci*, 6 (6), 427-430.

Akbarian A, Michiels J, Degroote J et al. (2016). Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *J Anim Sci Biotechnol*, 7, 37-51.

Altan O, Pabuçcuoğlu A, Altan A, Konyalioğlu S, Bayraktar H (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *Br Poult Sci*, 44 (4), 545-550.

Astaneh IY, Chamani M, Mousavi SN, Sadeghi AA, Afshar MA (2018). Effects of stocking density on performance and immunity in Ross 308 broiler chickens. *Kafkas Univ Vet Fak Derg,* 24 (4), 483-489.

Beker A, Teeter RG (1994). Drinking water temperature and potassium chloride supplementation effects on broiler body temperature and performance during heat stress. *J Appl Poult Res*, 3 (1), 87-92.

Bruno LDG, Maiorka M, Macari M, Furlan RL, Givisiez PEN (2011). Water intake behavior of broiler chickens exposed to heat stress and drinking from bell and nipple drinkers. *Braz J Poult Sci*, 13 (2), 147-152.

Cengiz Ö, Köksal BH, Tatlı O et al. (2015). Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. *Poult Sci*, 94 (10), 2395-2403.

Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A (2006). Biomarkers of oxidative damage in human disease. *Clin Chem.*, *52* (4), 601-623.

Fadillioglu E, Erdogan H, Polat A, Emre MH (2002). Renal antioxidant status in rats with hypertension induced by N sup omega nitro-L-arginine methyl ester. *Kidney Blood Press Res*, 25 (4), 211-216.

Farghly M, Abd El-Hack ME, Alagawany M, Saadeldin IM, Swelum AA (2018). Wet feed and cold water as heat stress modulators in growing Muscovy ducklings. *Poult Sci*, 97 (5), 1588-1594.

Goo D, Kim JH, Park GH, Delos Reyes JB, Kil DY (2019). Effect of Heat Stress and Stocking Density on Growth Performance, Breast Meat Quality, and Intestinal Barrier Function in Broiler Chickens. *Animals (Basel)*, 9 (3), 107-117.

Irato P, Santovito G (2021). Enzymatic and Non-Enzymatic Molecules with Antioxidant Function. *Antioxidants (Basel)*, 10 (4), 579-583.

Lin H, Decuypere E, Buyse J (2006). Acute heat stress induces oxidative stress in broiler chickens. *Comp Biochem Physiol A Mol Integr Physiol*, 144 (1), 11-17.

Luck H (1965). Catalase: In methods of enzymatic analysis. Bergmeyer HU, (Ed), Academic Press New York, USA, 885-894.

Mustafa MY, Muneer MA, Anjum AA, Ahamd M (2010). Influence of stocking density on immune response of broilers against newcastle disease virus. *PJLSS,* 8 (1), 7-10.

Najafi P, Zulkifli I, Jajuli NA (2015). Environmental temperature and stocking density effects on acute phase proteins, heat shock protein 70, circulating corticosterone and performance in broiler chickens. *Int J Biometeorol*, 59 (11), 1577-1583.

Nasr M, Alkhedaide AQ, Ramadan A, Hafez A, Hussein MA (2021). Potential impact of stocking density on growth, carcass traits, indicators of biochemical and oxidative stress and meat quality of different broiler breeds. *Poult Sci*, 100 (11), 101442.

Ohkawa H, Ohishi N, Yogi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95 (2), 351-358.

Park SO, Park BS, Hwangbo J (2015). Effect of cold water and inverse lighting on growth performance of broiler chickens under extreme heat stress. *J Environ Biol*, 36 (4), 865-873.

Quinteiro-Filho WM, Ribeiro A, Ferraz-de-Paula V et al. (2010). Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. *Poult Sci*, 89 (9), 1905-1914.

Simsek U, Dalkilic B, Ciftci M, Yuce A (2009). The Influences of different stocking densities on some welfare indicators, lipid peroxidation (MDA) and antioxidant enzyme activities (GSH, GSH-Px, CAT) in broiler chickens. *JAVA*, 8 (8), 1568-1572.

Sun Y, Oberley LW, Li Y (1998). A simple method for clinical assay of superoxide dismutase. *Clin Chem*, 34 (3), 479-500.

Teeter RG, Smith MO, Owens FN et al. (1985). Chronic heat stress and respiratory alkalosis: occurrence and treatment in broiler chicks. *Poult Sci*, 64 (6), 1060-1064.

Tietze F (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Ann Biochem*, 27 (3), 502-522.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Raheem SK, Kozat S (2023).** Serological Investigation of Peste Des Petits Ruminants in Lambs in Iraq-Kirkuk Region. *Van Vet J*, 34 (2), 140-145. DOI: https://doi.org/10.36483/vanvetj.1269834

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Serological Investigation of Peste Des Petits Ruminants in Lambs in Iraq-Kirkuk Region

Sarwat Khorshed RAHEEM[1] Süleyman KOZAT[2,*]

[1] *Van Yüzüncü Yıl University, Health Sciences Institute, 65080, Van, Türkiye*
[2] *Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Internal Diseases, 65080, Van, Türkiye*

Received: 23.03.2023 Accepted: 20.06.2023

ABSTRACT This study was carried out to determine the prevalence of Peste des Petits Ruminant (PPR) in sheep herds in different areas in the Iraq - Kirkuk region by using the ELISA test technique. According to clinical findings, the presence of seropositive samples by PPR test kit and ELISA method was revealed in the sera obtained from blood samples of lambs suspected of PPR disease. According to the obtained the test results, 47% of the antibodies against the PPR virus of all lambs used in the study were positive. Presence of PPR antibody was detected as 41.5% in 2-6 month old lambs. The disease was detected at the highest level in April with a rate of 56.50%. As a result, it was revealed that PPR is endemic in the Kirkuk-Iraq region. In conclusion; In the fight against Peste des Petits Ruminant (PPR) disease; It was concluded that in addition to preventive vaccine studies, disinfection systems, quarantine, training of sheep breeders and development of a plan for global disease prevention are required.

Keywords: *Diseas, Kirkuk-Iraq, Lamb, PPR.*

ÖZ

Irak-Kerkük Bölgesindeki Kuzularda Küçük Ruminant Vebası Virüsünün Serolojik Araştırılması

Bu çalışma, Irak - Kerkük bölgesindeki farklı alanlardaki koyun sürülerinde Peste des Petits Ruminant (PPR) hastalığının yaygınlığını ELISA test tekniği kullanılarak hastalığın varlığın ortaya koymak amacıyla yapılmıştır. Klinik bulgulara göre PPR hastalığında şüphelinen kuzularda kan numunelerinde elde edilen serumlarda PPR test kiti ile ELISA yöntemi seropozitif olan numunelerin varlığı ortaya konuldu. Elde edilen test sonuçlarınra göre çalışmada kullanılan tüm kuzuların PPR virüsüne karşı antikor varlığının oranın %47'si pozitif tespit edildi. PPR antikor varlığı 2-6 aylık kuzularda %41.5 olarak saptandı. Hastalık Nisan ayında en yüksek düzeyde ve %56.50 oranında tespit edildi. Sonuç olarak, PPR'nin Kerkük- Irak bölgesinde endemik olarak görüldüğü ortaya konuldu. Sonuç olarak; Peste des Petits Ruminant (PPR) hastalığıyla mücadelede; koruyucu aşı çalışmalarının yanı sıra dezenfeksiyon sistemleri, karantina, koyun yetiştiricilerin eğitimi ve küresel boyutta hastalıkta korunmada plan geliştirilmesi gerekli olduğu sonucuna varılmıştır.

Anahtar Kelimeler: *Hastalık, Kerkuk-Irak, Kuzu, PPR.*

INTRODUCTION

Peste des petits ruminants (PPR) is infectious sickness of domestic and small ruminants, which is a highly contagious, infectious, and fatal viral disease. Fever, necrotic mouth, gastroenteritis, and pneumonia are all symptoms (Abdalla et al. 2012). This family of viruses, the Paramyxoviridae (RPV and Canine Distemper) is closely related to the PPRV (Ozkul et al. 2002). According to reports, the disease has been most prevalent in sub-Saharan in Africa, the Middle East, and South Asia (Omani et al. 2019). The Food and Agriculture Organization and the World Organization for Animal Health have suggested a control program for the illness, with 2030 set as the goal year for elimination (FAO and OIE 2016). PPRV is rapidly spreading throughout continents, especially in North and East Africa and Asia, which creates a difficult environment for efforts to eradicate PPRV before 2030. Epidemics that reoccur in North Africa's Maghreb region (Libya, Tunisia, Algeria, Morocco, Mauritania, and the Western Sahara) provide as a good example of these difficulties. PPRV appears to have been eradicated in Morocco as a result of widespread vaccination when it was first discovered there in 2008. Following the relaxation of these vaccination tactics, the World Organisation for Animal Health (OIE) was informed in 2015 of the re-emergence of PPRV in Morocco (Baazizi et al. 2017).

The genetics of the inflaming viral strain, the infectious dosages of the virus, the infection routes, the type and breed of infected animals, and the immunological and

✉ *Corresponding author: skozat@hotmail.com

nutritional status of an infected animal may all influence the severity of the illness (Parida et al. 2015). The research reported that young animals provided higher positive percentages of PPR than older animals (Rahman et al. 2004).

There is evidence that females have greater antibody titers than males in Bangladesh. In terms of PPR, male goats are somewhat more susceptible than females (Abdalla et al. 2012). Despite this, data from Pakistan show no significant difference in susceptibility between males and females (Kozat and Sepehrizadeh 2017). Environmental factors that promote PPR virus persistence and propagation can also have an impact on the seasonal distribution of PPR outbreaks. PPRV outbreaks were most frequent in the first and fourth quarters of the year, with March being the most common, followed by April (Abubakar et al. 2009).

The current study aimed to investigate and confirm the prevalence of PPR illness using a competitive ELISA test, as well as to identify the primary risk factors in Kirkuk governorate, Iraq.

MATERIAL AND METHODS

This research was carried out with the permission numbered 73 on 22.12.2021 from the Republic of Iraq Ministre of Agriculture Veterinary Directorate/Kirkuk Ethics Committee.

Animals

A cross-sectional study was done in Iraq's Kirkuk governorate, which is located at 35.46° north latitude and 44.38° east longitude. It borders Salahiddin province to the north and west, AS-Sulimania province to the east, and Erbil and Nineveh province to the north. Two hunderd male and female local breed lambs between the ages of 1 and 6 months who had a fever, diarrhea, lacrimation, and mouth lesions were given blood samples. Between February and April 2022, samples were collected from several locations within the city of Kirkuk and its surrounding regions (Shwan, Laylan, Daquq, and Rashad).

Clinical Examination

Blood samples were taken from 200 lambs in various parts of Kirkuk province that had fever, oral lesions, and diarrhea from February to June in 2022. These samples came from lambs that were 1 to 6 months old. Following a clinical examination, clinical results were acquired and documented, including the sampling date, address, animal breed, clinical examination (age, body temperature), and clinical signs (mouth lesions, diarrhea, and lacrimation).

Collecting Samples

For this purpose, 5 ml of blood was taken from the jugular vein and kept in the non-anticoagulant tube. Then, blood samples were transported to the Kirkuk veterinary laboratory in cold chain within the hours of collection and the blood was centrifuged at 3000 RPM for 15 min. Each sample's serum was separated into two Eppendorf tubes and kept at -20 °C in a deep freezer. Samples were transferred to (Biological laboratory in the Faculty of Education in the Salahiddin University in Erbil) in order to perform analyses.

Laboratory Analysis

All serum samples were examined using the company's specified methodology. The identification of antibodies against the Peste des Petits Ruminants (PPR) virus in serum from lambs was carried out using a commercial test kit (PPR ELISA Kit Sunlong® Biotech Co. Ltd, China).

Statistical Analysis

The SPSS package program was used in this study to evaluate the statistical analysis. In terms of sex, age, area, season, and clinical findings, ELISA results and percentages of acquired results were computed. The statistical significance level was taken as 5% and SPSS (ver: 26) statistical package program was used for calculations.

RESULTS

Two hundred distinct local breeds of sheep with PPR symptoms had blood samples taken. These animals were of various sexes, ages, and geographical origins. Laboratory testing for PPR disease was done on blood samples using the ELISA technique. The research found that of 200 samples, the percentages of positive, suspected, and negative findings were 94 (47%), 8 (4%) and 98 (49%), respectively (Table 1).

Table 1: Serological results according to ELISA.

Results	Number of samples	Percentage %
Positive	94	47
Suspected	8	4
Negative	98	49

Findings According to Sex

A total of 200 blood samples were collected at random from 114 female and 86 male animals. While 39 of 86 female animal samples (45.3%) were seropositive, 55 of 114 male animal samples (48.2%) were seropositive. Table 2 show the sample variances by gender.

Table 2: ELISA findings based on the sex.

	Sex	
	Male	**Female**
Number of samples	114	86
Positive	55	39
Suspected	4	4
Negative	55	43
Percentages of positive (%)	48.2	45.3

Findings According to Age

In this investigation, 200 serum samples were collected from animals aged 1 to 6 months. According to the findings, the seropositivity ratios of age groups are shown in the table and figure. When the table and figure were analyzed, the disease's seropositivity rate increased with age (Table 3).

Table 3: ELISA findings based on the ages.

	Age (Day)		
	1-60	**61-90**	**91-180**
Number of samples	57	69	74
Positive	18	37	39
Suspected	4	4	0
Negative	35	28	35
Percentages of positive (%)	19.1	39.4	41.5

Findings According to Regions

In this study, 200 blood samples were collected from animals in the Kirkuk city areas of Daquq, Lalan, Rashad, and Shown. Table4 show the number of samples and seropositivity rates by area. When Table 4 were analyzed, Rashad had the greatest seropositivity rate (66%). Other regions' seropositivity rates were 45.8%, 47.2%, and 28.6%, respectively (Table 4).

Findings According to Clinical Symptoms

Clinical symptoms in animals were documented, and blood was drawn following a general examination. Serological data in terms of clinical symptoms such as fever, oral lesion, diarrhea and lacrimation were presented in tabular and figure form (Figure 1 and Figure 2). The percentages of fever, oral lesions, diarrhea and lacrimation were 90.4%, 76.5%, 82.9%, and 44.7%, respectively, according to these data (Table 5).

Figure 1: Diarrhea symptoms caused by PPR in a lamb.

Table 4: ELISA findings based on the location.

Location	Number of samples	Positive	Suspected	Negative	Percentages of positive (%)
Shown	48	22	0	26	45.8%
Laylan	53	25	0	28	47.2%
Daquq	49	24	3	32	28.6%
Rashad	50	33	5	12	66%

Table 5: ELISA findings based on the clinical sings.

Clinical sings	Positive showing clinical sings	Suspected showing clinical sings	Negative showing clinical sings	Percentages of positive (%)
Fever	85/94	2/8	45/98	90.4%
Mouth lesion	72/94	2/8	35/98	76.5%
Diarrhea	78/94	5/8	47/98	82.9%
Red eye membrane and lacrimation	59/94	4/8	69/98	44.7%

Figure 2: Severe lacrimation in a lamb.

Results According to Season

Between February and April 2022, samples were collected from several locations in Kirkuk and surrounding regions. We have seen that the infection rate rises in March and then again in April (Table 6).

Table 6: ELISA findings based on the season.

	Month		
	February	**March**	**April**
Positive	7	35	52
Suspected	3	0	5
Negative	20	43	35
Percentages of positive (%)	23.3%	44.9%	56.5%

DISCUSSION AND CONCLUSION

In Iraq as in many parts of the Middle East, PPRV remains unknown, with outbreaks in Iraq the most often recorded. A virus with significant morbidity but low mortality was identified in Iraq in 2000, and that's when PPRV was originally characterized (Banyard et al. 2010).

According to the OIE and FAO, Iraq reported an epidemic of peste des petits ruminants (PPR) in its northern governorates in September 1998. Despite the fact that this disease had been suspected in the central and northern governorates for some years and was known to exist in neighboring countries, this was the country's first official report of PPR, which prompted tremendous alarm. Iraqi veterinary officials have insufficient resources to deal with this highly infectious illness of small ruminants due to international sanctions imposed on the country. A FAO TCP project was launched in 1999 to help eliminate the disease through targeted vaccination, strengthen laboratory-assisted surveillance, improve field veterinary staff diagnostic capacity, and establish a national network for surveillance and early warning systems against transboundary animal diseases (Alwan and Alsaad 2022).

PPR has been detected clinically in sheep and goats in Irbil and Dahuk Governorates in recent years, and has been suspected clinically in Mosul, As-Sulaimaniyah, and Ta'amim (Kirkuk), all of which are in the country's north (FAO 2000). Between August 2010 and February 2011, the Erbil Governorate in northern Iraq lost approximately 750 wild goats. The participation of the peste des petits ruminants' virus (PPRV) was hypothesized based on the clinical symptoms and post-mortem results. Laboratory testing supported this and revealed a virus with a similar resemblance to a Turkish variant discovered in 2000. There were no cases of illness in domestic animals during the wild goat epidemic (Hoffmann et al. 2011). In then As-Sulaimaniyah Governorate in the north of Iraq, an outbreak of peste despetitst ruminants (PPR) in sheep was investigated between 2012 and 2013. The findings gave the PPRV lineage linked to lethal PPR infections in small ruminants its first molecular characterization. The diagnosis was made using RT-PCR (Babashek et al. 2014).

In 1995, a peste des petits ruminants (PPR) pandemic was found serologically and virologically in Ilam province, Iran, near the Iraqi border. PPR has been identified throughout the country despite all control attempts, costing Iranian sheep and goat owners at least $1.5 million US (Bazarghani et al. 2007).

PPRV infection was first officially documented in neighboring Turkey in southern and eastern Anatolia in 1999. From 1999 to 2018, the OIE documented roughly 1,000 PPR outbreaks in Turkey. These outbreaks peaked in 2007 and 2011, particularly in Turkey's Marmara and Aegean regions (Altan et al. 2019).

All of these reported results reflect a considerable occurrence of PPR. It is necessary to establish a surveillance system, to monitor and manage this illness. Because of the huge economic ramifications of PPR in Iraq, analyzing PPR prevalence and risk factor data is crucial. Concerns concerning PPR epidemiology are crucial in attempts to control, manage, and eliminate the illness effectively.

The current study was conducted to assess and construct an epidemiological history of PPR prevalence in the Kirkuk region of Iraq. Despite the spread of the disease in such a wide way in most of the governorates of Iraq and the neighboring countries of Iraq, there are no adequate studies on the disease in Iraq in general and in Kirkuk governorate in particular, so to understand the epidemiology of this disease in Iraq, it is important to perform this study as an attempt to complete this deficiency.

The utilization of quick, targeted, and responsive diagnostic techniques is required for the effective implementation of PPR control strategies. Small ruminant PPR infection is frequently identified by clinical assessment, gross anatomy, histological findings, and laboratory confirmation. A range of serological and molecular diagnostic assays are used to find PPR virus (Munir et al. 2014). For quick diagnostic and control measures, pen-side trials are particularly tempting. These include chromatographic strip tests, dot ELISA, and others. They may be performed without the need for specific equipment or technical expertise (Balamurugan et al. 2012).

In this study, according to the findings obtained from ELISA (regardless of sex, age and animal's regions), blood samples reflect that positive, suspected and negative

results were determined as 47%, 4% and 49%, respectively of PPR (Table 1). It appears to be very similar as a result of a study conducted in the neighboring governorate (Nineveh) in 2021 which was 47.46% (Hussain 2021).

It appears to be greater than comparable to the prevalence documented in bordering countries. These findings are consistent with previous epidemiological research. PPR was found in 3.1% of sheep in the Kingdom of Saudi Arabia (KSA) using a microtiter neutralization test (AL-Afaleq el at. 2004). The frequency of PPR in sheep was found to be 96% in Syria, much higher than in Jordan (60%) (Al-Majali et al. 2008). PPR incidence rates in Turkey varied from 0.87% to 82.6%, with sheep (29.2%) outmatched goats (20%) (Ozkul et al. 2002).

The risk factors including sex, age and season reflected reasonable effects; that males (48.2%) were more impacted and seropositive for PPR than females (45.3%). (Table 2). There were several possible explanations for this result; According to Bangladeshi results, females are more likely than males to have higher antibody titers. Male goats are somewhat more vulnerable to PPR than females (Abdalla et al. 2012); but others showed that; while female goats and sheep are kept alive for breeding and milking, male animals are slaughtered at an early age (Khan et al. 2008).

Abubakar et al. (2015) reported that there was a positive correlation between PPR seropositivity and age and the animal's coming to pasture in their research on PPR, and they found that PPR seropositivity was higher in sheep aged 2 years and older. According to research from Turkey, India, Kenya, Pakistan, and Ethiopia, young animals are more susceptible to PPRV because they have lower antibody titers that serve as a protective barrier (Kozat and Sepehrizadeh 2017). The research reported that young animals provided higher positive percentages of PPR than older animals (Rahman et al., 2004). Animals aged 3 months to 2 years are the most seriously impacted in endemic regions. The severity of the illness is determined by the age, species, and immunity of the host (Kozat and Sepehrizadeh 2017). In addition, 200 serum samples were collected from animals aged 1 to 6 months for this investigation. According to the findings. The seropositivity ratios of age groups are shown in the table and figure. The disease's seropositivity rate rises between the ages of 2 and 6 months (Table 3). It can be interpreted that the increase in the presence of the disease is due to the lack of attention to adequate prophylactic applications.

Morbidity region that recorded the highest seropositivity rate of PPRV was Rashad region (66%) compared with other studied regions as Shown, Laylan, and Daquq as 45.8%, 47.2% and 28.6% respectively; PPR is most contagious when it manifests in a vulnerable population for the first time. In endemic regions, outbreaks can often happen, especially when additional animals are mixed in with the herd or introduced there (Table 4). We also explain the high rate of infection in the Rashad region compared to the rest of the areas due to the security strikes there in previous years, which led to the failure of veterinary teams to reach it to conduct vaccination campaigns organized by the government. The same is the case in the Shwan region, which is ranked second in terms of the high incidence of infection, and the reason for this is that it is a border area and it is difficult for the authorities in the province to control the movement of animals in it.

In this study, the incidence of clinical findings such as fever, mouth lesions, diarrhea and lacrimation was 90.4%, 76.5%, 82.9% and 44.7%, respectively (Table 5). Many researchers report that PPR will be evaluated as acute, subacute or subclinical according to clinical findings such as fever, mouth lesions, diarrhea and lacrimation (Munir 2014; Kozat and Sepehrizadeh 2017). Depression, mucous membrane congestion, oculo-nasal discharge, dyspnea, and a lot of aquatic diarrhoea are symptoms of infected animals dying within 4-5 days (Munir et al. 2013). Moreover; infants and lambs frequently experience acute sickness soon after their passive immunity becomes depleted (Munir 2014). Subacute or subclinical kinds appear to be particularly common in specific regions. In such circumstances, the sickness has intermittent effects and lasts 10 to 15 days. Later on, papules or pustules resembling infected ecthyma may develop. Because they encourage respiratory conditions that cannot be identified as PPR, invisible kinds are more hazardous. Typically, the only means to detect them is by serological surveys (Lefèvre et al. 1991).

Between February and April 2022, samples were collected from several locations in Kirkuk and its regions for this investigation. The Percentages of positivity were February 23.3%, March 44.9%, and April 56.5%. Iraq in general, and Kirkuk governorate in particular, suffered during the past two years from a great drought and a lack of rain, which led to a lack of green pastures, a lack of moisture and a large number of dust storms, starting from the March and April months, and this is the explanation for the high incidence of the disease in these months (Table 6).

A result, we conclude from this study that PPR is endemic in Kirkuk Governorate in Iraq and that the animals do not enjoy great protection against the disease because the strain used in vaccination is a non-local strain and the risk factors of age, gender, and geographical area played a role in the spread of the disease. It was necessary to establish a global plan to combat and eradicate small ruminant plagues by using several monitoring and preventative methods whether by following disinfection systems, immunization, quarantine, and applying health awareness among young farmers in all world regions.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENT

This study is summarized from the master thesis of Sarwat Khorshed RAHEEM.

AUTHOR CONTRIBUTIONS

Idea / Concept: SKR, SK
Supervision / Consultancy: SK
Data Collection and / or Processing: SKR
Analysis and / or Interpretation: SKR, SK
Writing the Article: SKR, SK
Critical Review: SKR, SK

REFERENCES

Abdalla AS, Majok AA, El Malik KH, Ali AS (2012). Sero-prevalence of peste des petits ruminants virus (PPRV) in small ruminants in Blue Nile, Gadaref and North Kordofan States of Sudan. *JPHE*, 4 (7), 59-64.

Abubakar M, Irfan M, Manzoor S (2015) Peste des petits ruminants in Pakistan; past, present and future perspectives. *J Anim Sci Techno,* 57, 32.

Abubakar M, Jamal SM, Arshed MJ, Hussain M, Ali Q (2009). Peste des petits ruminants virus (PPRV) infection; its association with species,

seasonal variations and geography. *Trop Anim Health Prod,* 41 (7), 1197-1202.

AL-Afaleq A, Abu Elzein E, AL-Naeem A, Amin M (2004). Serosurveillance for PPR and rinderpest antibodies in naturally exposed Saudi sheep and goats. *Vet Arhiv,* 74, 459-465.

Al-Majali AM, Hussain NO, Amarin NM, Majok AA (2008). Seroprevalence of, and risk factors for, peste des petits ruminants in sheep and goats in Northern Jordan. *Prev Vet Med,* 85, 1-8.

Altan E, Parida S, Mahapatra M, Turan N, Yilmaz H (2019). Molecular characterization of Peste des petits ruminants viruses in the Marmara Region of Turkey. *Transbound Emerg Dis,* 865-872.

Alwan GF, Alsaad KM (2022). Peste Des Petits Ruminants (PPR) of small ruminants in Iraq. *J Agric Vet Sci,* 20-23.

Baazizi R, Mahapatra M, Clarke BD et al. (2017). Peste des petits ruminants (PPR) A neglected tropical disease in Maghreb region of North Africa and its threat to Europe. *Plo S one,* 12 (4), 1-16.

Babashekh MO, Rashid PMA, Marouf AS, Raheem ZH, Amin KM (2014). Genetic characterization of peste des petitis ruminants' virus (PPRV) from Sulaimani/ Iraq by phylogenetic analysis and sequencing of nucleoprotein and fusion protein gene. *JZS,* 16 (3), 1-8.

Balamurugan V, Sen A, Venkatesan G et al. (2012). Peste des petits ruminants virus detected in tissues from an Asiatic lion (Panthera leo persica) belongs to Asian lineage IV. *J Vet Sci*, 13, 203-206.

Banyard AC, Parida S, Batten C et al. (2010). Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. *J Gen Virol,* 91 (12), 2885-2897.

Bazarghani TT, Charkhkar S, Doroudi J, Hassan EB (2007). Peste des Petits Ruminants (PPR) with Special Reference to PPR in Iran. *J Vet Med,* 53 (1), 17-18

FAO, OIE (2016). Peste des petits ruminants global eradication program.

Hoffmann B, Wiesner H, Maltzan J et al. (2011). Fatalities in Wild Goats in Kurdistan Associated with Peste Des Petits Ruminants Virus. *Transbound Emerg Dis,* 59 (2), 173-176.

Khan HA, Siddique M, Sajjad R, Abubakar M, Ashraf M (2008). The detection of antibody against peste des petits ruminants virus in sheep, goats, cattle and buffaloes. *Trop Anim Health Prod,* 40, 521-527.

Kozat S, Sepehrizadeh E (2017). Peste Des Petit Ruminants. *JIVS,* 1(2), 47-56.

Lefèvre PC, Daillo A, Schenkel S, Hussein S, Staak G (1991). Serological evidence of peste des petits ruminants in Jordan. *Vet Rec,* 110-128.

Munir M, Zohari S, Berg M (2013). Pathophysiology and Clinical Assessment of Peste des Petits Ruminants. In: Molecular Biology and Pathogenesis of Peste des Petits Ruminants Virus. Part of the Springer Briefs in Animal Sciences Book Series, 33-48.

Munir M (2014). Role of wild small ruminants in the epidemiology of peste des petits ruminants. *Transbound Emerg Dis,* 61 (5), 411-24.

Omani R, Gitao G, Gachohi J et al. (2019). Peste Des Petits Ruminants (PPR) in Dromedary Camels and Small Ruminants in Mandera and Wajir Counties of Kenya. *Adv Virol,* 1- 6.

Ozkul A, Akca Y, Alkan F et al. (2002). Prevalence, distribution,and host range of peste des petits ruminants virus. *Emerg Infect Dis,* 8 (7), 709-712.

Parida S, Muniraju M, Mahapatra M et al. (2015). Peste des petits ruminants. *Vet Microbiol,* 181, 190-106.

Rahman AU, Ashfaque M, Rahman SU, Akhtar M (2004). Pestie des petits ruminants antigen in mesenteric lymphnodes of goats slaughtered at D.I. Khan. *PakVet J,* 2, 159-160

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Yıldız S (2023).** Feed Supply and Animal Nutrition Habits of Van Province, Cattle Breeding Facilities. *Van Vet J*, 34 (2), 146-154. DOI: https://doi.org/10.36483/vanvetj.1284539

ISSN: 2149-3359 **Araştırma Makalesi** e-ISSN: 2149-8644

Van İli Büyükbaş Hayvancılık İşletmelerinin Yem Temini ve Hayvan Besleme Alışkanlıkları

Serhat YILDIZ*

Van Yüzüncü Yıl Üniversitesi, Gevaş Meslek Yüksekokulu, Veterinerlik, Laborant ve Veteriner Sağlığı Programı, 65700, Van, Türkiye

Geliş Tarihi: 17.04.2023 Kabul Tarihi: 14.07.2023

ÖZ

Bu çalışmada, Van İli büyükbaş hayvancılık işletmelerinin genel durumları ile yem temini ve hayvan besleme alışkanlıklarının belirlenmesi, işletmelerin temel sorunlarının saptanarak çözüm önerilerinde bulunulması amaçlanmıştır. Bu çalışmanın materyalini, Van Yüzüncü Yıl Üniversitesi DAP Çiftçi Eğitim Merkezi'nin bünyesinde yapılan eğitimlere, Van ilinden katılan 137 kursiyerlere uygulanan anketler oluşturmaktadır. Araştırma sonuçlarına göre, ankete katılanların %84.56'sının 15-50 yaş arasında olduğu, ortaokul ve altı eğitim alanların oranının ise %57.04 olmuştur. Kursiyerlerin %75.63'ünün 5-100 dekar, %18.49'unun 100-250 dekar, %5.88'inin ise, 250 dekar ve üzeri tarım arazisi işledikleri belirlenmiştir. Katılımcıların işletmelerinde ortalama olarak 40.89 baş her yaştan sığır ve 3.00 baş mandaya sahip oldukları; kaba ve kesif yem ihtiyaçlarını dışarıdan karşılayanların oranı sırasıyla %31.67 ve %50.00; meradan faydalananların oranı %78.23; yonca yetiştirenler %34.75 ve işletmesinde silaj kullananlar %35.25 olarak tespit edilmiştir. Kursiyerlerin %61.60'ının hayvanlar için hazırlanan rasyonları, kendilerine göre hazırladıkları, hayvanlara tuz ve vitamin-mineral ilavesi yapanların oranı %81.25 olarak bulunmuştur. Ankete katılan birçok yetiştiricinin, hayvanları günde iki defa yemledikleri (%58.87) ve ahır içinde sabit suluğu bulunan işletmelerin oranı ise %24.39 olarak tespit edilmiştir.

Anahtar Kelimeler: *Büyükbaş, Hayvan besleme, Hayvancılık işletmesi, Hayvan yemi.*

ABSTRACT

Feed Supply and Animal Nutrition Habits of Van Province, Cattle Breeding Facilities

In this study, it is aimed to determine the general situation of the cattle breeding enterprises in Van, feed supply and animal feeding habits, to determine the basic problems of the enterprises and to offer solutions. The material of this study consists of questionnaires made with 137 trainees from Van, who participated in the trainings opened within the body of Van Yüzüncü Yıl University DAP Farmer Training Center. According to the results of the research, 84.56% of the respondents were between the ages of 15-50, and the rate of those with secondary school or below education was 57.04%. It was determined that 75.63% of the trainees worked on 5-100 decares, 18.49% on 100-250 decares, and 5.88% on 250 decares and above. The participants had an average of 40.89 cattle of all ages and 3.00 buffaloes in their enterprises; the ratio of those who meet their roughage and concentrate feed needs from outside is 31.67% and 50.00%, respectively; the rate of those benefiting from the rangeland is 78.23%; clover growers 34.75% and those using silage in their farms were determined as 35.25%. It was found that 61.60% of the trainees prepared the animal rations according to themselves and 81.25% of the trainees added salt and vitamin-mineral combinations to the rations. It has been determined that many breeders participating in the survey feed the animals twice a day (58.87%) and the rate of enterprises with fixed drinkers in the barn is 24.39%.

Keywords: *Animal farm, Animal feed, Animal nutrition, Livestock.*

GİRİŞ

Tarihin ilk dönemlerinden beri önemli ekonomik faaliyetlerden birini oluşturan hayvancılık sektörü, günümüzde de insanlığa en faydalı alanların başında gelmektedir. Ülkelerin gelişmişlik durumuna bakılmaksızın, hayvancılık hayati bir öneme sahiptir. Bunun nedeni, ülke ekonomisine olan katkılarının yanında, insan beslenmesinde yeri doldurulamayan temel hayvansal besinlerin karşılanmasıdır. Hayvancılık et, süt, deri, kozmetik, ilaç gibi sektörlere hammadde sağlamanın yanı sıra, yem sanayi, et ve ürünleri sanayi, süt ve ürünleri sanayi, hayvansal ilaçlar, hayvancılık alet ve ekipmanları gibi alanlara istihdam oluşturmaktadır.

Sektör, ekonomide katma değer oluşturmasıyla kalkınmaya yardımcı olmakta ve milli geliri arttırmaktadır. Sürekli olarak artan dünya ve ülke nüfusu göz önüne alındığında, gıdaya özellikle hayvansal kaynaklı besinlere

✉ *Corresponding author: syildiz@yyu.edu.tr

olan ihtiyacın da katlanarak artacağı beklenmektedir. Ancak doğal kaynakların giderek azalması, küresel ısınmanın etkileri gibi nedenler, bu sektörde çağımızın en önemli sorunlarını oluşturmaktadır. Bu sorunlar göz önüne alınarak, kullanılan kaynakların daha verimli hale getirilmesi ve değerlendirilmesi, hayvansal üretimin arttırılması ve verimin arttırılması önem arz etmektedir (Ertaş ve Deniz 2018; İşler ve Ören 2021).

Hayvancılık sektörünün hem kültürel hem de stratejik olarak önemli bir rolü bulunmaktadır. Türkiye'deki üreticilerin hayvancılık için uygun iklim yapısına ve büyük bir potansiyele sahip olmasına rağmen, yıllardan bu yana alışılmış olan, kendi kendine yeterliliği kabullenen, kapalı bir üretim süreci yürütülerek hayvansal ürün ihtiyaçlarını karşılamışlardır. TÜİK 2022 yılı verilerine göre ülkemizde, 17 milyon 876 bin büyükbaş ve 58 milyon 448 bin küçükbaş olmak üzere toplamda 76 milyon 324 bin baş hayvan bulunmaktadır. Bu veriler ele alındığında Türkiye'nin bu sektördeki öneminin ne derece büyük olduğu görülmektedir. Benzer şekilde Van ili de hayvancılık sektöründe Türkiye içinde ilk sıralarda olup, etkili bir konumda yer almaktadır. (Yıldız ve Aygün 2021a/2021b; TÜİK 2022a).

Van ili Türkiye'nin doğusunda bulunması, arazi şekilleri, iklim şartları, bitki kompozisyonu, geniş mera alanlarının bulunması, tarımla uğraşan işletmelerinin özellikleri nedeniyle tarımsal faaliyetleri ön plana çıkmaktadır. Van ilinin belirgin özelliği yüksek dağlar, geniş plato ve ovalık alanlardan oluşmasıdır. İlin bu şekilde bir yapıya sahip olması tarımsal alanların sınırlı, çayır ve mera alanlarının da fazla olmasına, dolayısıyla hayvancılık faaliyetlerinin yoğunlaşmasına neden olmuştur. 2022 istatistiklerine göre her yaşta toplam 133.137 büyük baş ve 3.413.510 küçükbaş hayvan bulunan Van ili, Türkiye'de hayvancılık faaliyetlerinin yoğun olarak yapılan illeri arasında yer almaktadır. Bu sonuçlarla önceki yıllara göre, küçükbaş hayvan sayılarında artış, büyükbaş hayvan sayılarında bir azalmanın olduğunu göstermektedir (Ertaş ve Deniz 2018; Yıldız ve ark. 2019b; TÜİK 2022b).Ülkemizde, özelde de Van ilindeki hayvancılık sektörünün birçok sorunu bulunmaktadır. Bunlardan bazıları, işletmelerin genellikle küçük aile işletmeleri şeklinde olması, geleneksel yetiştiriciliğin daha çok tercih edilmesi, yetersiz bir mesleki ve kooperatif örgütlenmesinin olması, enerji ve özellikle yem başta olmak üzere girdi maliyetlerinin yüksek olması sayılabilir (İşler ve Ören 2021). Van ilinde yaşayanların çoğunluğu kırsal kesimde bulunmaktadır. Bu durum ili, tarım ve hayvancılıkta, önemli bir konuma getirmiştir. Belirli miktarda bulunan arazi kaynakları ile sınırlı üretim kaynaklarının daha iyi değerlendirilmesiyle, kırsalda yaşayanların, yaşam düzeylerinin daha iyi hale getirilmesi ve hayat standartlarının yükseltilmesi sağlanabilecektir (Arslan 2018; Yıldız ve ark. 2021). Van YYÜ DAP Çiftçi Eğitim Merkezi'nde gerçekleştirilen bu çalışmada, Van İli tarım ve hayvancılık faaliyetleriyle uğraşan yetiştiricilerin durumları, güncel hayvansal üretim kapasitelerinin belirlenmesi, gerekli olan yemlerin temini ve hayvan besleme alışkanlıklarının tespit edilmesi, sektörde üretimin azalma nedenlerinin belirlenerek temel sorunların tespiti, sektörün gelişmesi ve desteklenmesi için çözüm önerileri sunulması amaçlanmıştır.

MATERYAL VE METOT

Bu araştırma faaliyetinin etik kurul denetimine tabi olmadığı Van Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Etik Kurulunca belirtilmiştir.Tarih: 23/02/2023; Karar No: 2023/05-10.

Araştırmada materyal olarak, tümü Van ilinde ikamet eden büyükbaş hayvan yetiştiriciliği yapan ve Van Yüzüncü Yıl Üniversitesi DAP Çiftçi Eğitim Merkezi'nde gerçekleştirilen eğitim faaliyetlerine katılan, 137 kursiyerle bire bir yapılan anketler kullanılmıştır. Düzenlenen eğitim faaliyetlerine, Van ili İpekyolu, Tuşba, Edremit gibi merkez ilçeler ile Erciş ve Gevaş ilçelerinden katılımlar gerçekleşmiştir. Eğitimler Covid-19 Pandemi dönemine denk geldiğinden katılımlar beklenenden düşük olmuştur. Eğitimlere katılan kursiyerlerin tamamı kurslara kendi istekleri doğrultusunda katılmışlardır. Kurslarda katılımcılar, beş günlük süt sığırcılığı eğitimleri almışlardır. Ankete katılanların sayısının belirlenmesinde, bu çalışma materyalleri, örnek büyüklüğünün tespitinde kullanılan yöntemlere uymadığı için, eğitim alan tüm işletme sahiplerine anketler uygulanmıştır. Kurs faaliyetleri ve anketler 2019 yılı Kasım ayı ile 2022 yılı Ocak ayları arasında yapılmıştır.

İstatistiksel Analiz

Çalışma verisine ait tanımlayıcı istatistikleri incelemek için SAS (2014) istatistik paket programında 'means' ve 'freq' prosedürleri kullanılmıştır. Kategorik değişkenler arasındaki bağımsızlık testi için ki-kare ($\chi 2$) test istatistiğinden faydalanılmış ve SAS (2014) istatistik paket programında 'freq' prosedürlerinden faydalanılmıştır.

BULGULAR

Ankete Katılan Çiftçilerin Bazı Özellikleri

Araştırmada, Van ilinde büyükbaş hayvancılık faaliyetiyle uğraşan ve DAP Çiftçi Eğitim Merkezi'nde kurslara ve anketlere katılanların %84.56'sının 15-50 yaş arasında olduğu belirlenmiştir (Tablo 1).

Tablo 1: Kurslara ve anketlere katılan yetiştiricilerin bazı nitelikleri.

Table 1: Some qualifications of breeders participating in courses and surveys.

Yerleşim Yeri	N	%	YAŞ	N	%
İpekyolu	53	38.68	15- 20 yaşları arası	5	3.68
Tuşba	25	18.25	21-30 yaşları arası	43	31.62
Edremit	15	10.95	31-40 yaşları arası	38	27.94
Erciş	19	13.87	41-50 yaşları arası	29	21.32
Gevaş	25	18.25	51-60 yaşları arası	13	9.56
			61 yaş ve üzeri	8	5.88
Öğrenim Durumu			**Hayvancılık Faaliyetleri için Eğitim Alıp Almadığı**		
Okur-yazar değil	4	2.96	Aldım	28	20.74
Okur-yazar	4	2.96	Almadım	107	79.26
İlkokul mezunu	35	25.93			
Ortaokul mezunu	34	25.19			
Lise mezunu	37	27.41			
Üniversite	21	15.55			

*Bu sorular için birden fazla parametre tercih edilmiştir.

Tablo 2: Hayvancılık işletmelerinin genel durumu**.**

Table 2: General situation of livestock enterprises.

Tarım Arazisi Miktarları (Dekar)	**N**	**%**	**Hayvancılıkta Kullanılan Tarımsal Alet Ve Makinalar**	**N**	**%**
5-20 dekar	30	25.21	Traktör	47	40.17
20-50 dekar	17	14.29	Yem karma makinası	4	3.43
50- 100 dekar	43	36.13	Yem kırma makinası	4	3.43
100-250 dekar	22	18.49	Süt sağım makinası	3	2.56
250-500 dekar	1	0.84	Balya makinası	6	5.13
500-1 000 dekar	2	1.68	Diğer	40	34.19
1 000 dekar ve üzeri	4	3.36	İki veya daha fazla parametre seçimi*	13	11.09
Tarımsal Örgüt Üyelikleri			**Aylık Gelirler (TL)**		
Hiçbirine üye değil	93	67.88	2 000 den az	63	55.25
Tarımsal kalkınma kooperatifi	12	8.76	2 001-3 000	23	20.18
Sulama kooperatifi	2	1.46	3 001-4 000	22	19.30
Tarım kredi kooperatifi	9	6.57	4 001-5 000	1	0.88
DSYB'ne	8	5.84	5 001 ve üzeri	5	4.39
DMYB'ne	-	0.00	**Tarımsal Destek Durumu**		
İki parametre seçimi*	10	8.03	Evet	57	46.72
Üç parametre seçimi*	2	1.46	Hayır	65	53.28

Hayvan Türleri	**N**	**En Az**	**En Çok**	**Ortalama**	**Medyan**	**Varyans**	**Standart Sapma**	**Standart Hata**
İnek	92	1	57	8.14	6.00	79.22	8.90	0.93
Manda	2	1	5	3.00	3.00	8.00	2.23	2.00
Düve	20	1	112	11.95	4.00	616.68	24.83	5.55
Dana	24	1	50	9.50	4.00	162.43	12.75	2.60
Buzağı	33	1	80	8.30	5.00	206.22	14.36	2.50
Boğa	10	1	12	3.00	2.00	12.22	3.50	6.15

*Bu sorular için birden fazla parametre tercih edilmiştir.

İşletmelerin Genel Durumu

Van Yüzüncü Yıl Üniversitesi DAP Çiftçi Eğitim Merkezi'nde eğitimlere katılan, Van ili büyükbaş hayvan yetiştiricilerin %75.63'ünün 5-100 dekar, %18.49'unun 100-250 dekar, %5.88'inin ise, 250 dekar ve üzeri tarım arazisine sahip oldukları belirlenmiştir (Tablo 2).

İşletmede Yem Temini

Van ilinde büyükbaş hayvancılık faaliyetiyle uğraşan ve DAP Çiftçi Eğitim Merkezi'nde ankete katılan kursiyerlerinin yetiştirdikleri hayvanları için gerekli olan kaba yemlerin çoğunu kendilerinin yetiştirdiği (%42.50), kesif yemlerin çoğunu dışarıdan satın aldıkları (%50), arazilerinde yem bitkisi olarak en çok yonca yetiştirdikleri (%34.75), büyük çoğunluğu hayvanlarını meraya çıkardıkları (%78.23), hayvanlarına tuz ve mineral takviyesi yaptığı (%81.25) ve çoğunluğu hayvanlarını beslerken silaj yemlerinden faydalanmadıkları (%64.71) belirlenmiştir (Tablo 3).

İşletmede Hayvan Besleme Alışkanlıkları

Çalışmada, ankete katılanların birçoğu, hayvanların günde iki defa yemlendiği (%58.87), yemleme işleminin çuval kullanılarak (%68.91) yapıldığı, yoğun yem ve kaba yemler için ayrı yemliklerin olmadığı (%61.57) tespit edilmiştir. Katılımcıların %49.59'u hayvanlarını sulamak için suyu önlerine kedilerinin taşıdığı, %24.39'unun ise ahır içinde sabit sulukta hayvanların sulandığı bildirilmiştir (Tablo 4).

Tablo 3: İşletmelerde yem temini.
Table 3: Feed supply in enterprises.

Kaba Yem Temini	**N**	**%**	**Yetiştiriciliği Yapılan Yem Bitkisi Çeşidi**	**N**	**%**
Kendi yetiştiren	51	42.50	Yonca	41	34.75
Dışarıdan satın alan	38	31.67	Korunga	15	12.71
Hem kendi yetiştiren hem satın alan	31	25.83	Fiğ	3	2.54
Kesif Yem Temini			Silajlık mısır	3	2.54
Kendi yetiştiren	18	14.75	Diğer	11	9.32
Dışarıdan satın alan	61	50.00	İki parametre seçimi*	36	28.82
Hem kendi yetiştiren hem satın alan	43	35.25	Üç parametre seçimi*	6	5.07
Tuz ve Vitamin-Mineral Kullanımı			Dört veya daha fazla şık seçimi*	3	2.54
Evet	91	81.25	**Hayvanların Meraya Çıkma Durumu**		
Hayır	21	18.75	Evet	97	78.23
Silaj Yemi Kullanımı			Hayır	27	21.77
Evet	42	35.29			
Hayır	77	64.71			

*Bu sorular için birden fazla parametre tercih edilmiştir.

Tablo 4: Hayvan besleme alışkanlıkları.
Table 4: Animal feeding habits.

Kaba Yem Kullanımı	**N**	**%**	**Kesif Yem Kullanımı**	**N**	**%**
Buğday-arpa samanı	40	32.00	Buzağı yemi	2	1.69
Yonca	17	13.60	Süt yemi	14	11.86
Korunga	4	3.20	Besi yemi	26	22.03
Çayır otu	6	4.80	Arpa kırması	29	24.58
Silaj	2	1.60	Kepek	10	8.47
Şeker pancarı posası	-	0.00	Pamuk tohumu küspesi	-	0.00
İki parametre seçimi*	18	14.40	İki parametre seçimi*	16	13.56
Üç veya daha fazla şık seçimi*	38	30.40	Üç veya daha fazla şık seçimi*	21	17.81
Hayvanları Sulanma Şekli			**Rasyon Hazırlanırken İzlenilen Yol**		
Derede	12	9.76	Rasyon hazırlamayı bilen birinden yardım alıyorum	32	25.60
Köy ortak çeşmesinde	6	4.88	Komşu ya da arkadaşlar nasıl hazırlıyorsa öyle hazırlıyorum	10	8.00
Önlerine ben taşıyorum	61	49.59	Kendim göz kararı dengeli hazırlıyorum	77	61.60
Ahır içinde sabit sulukta	30	24.39	Rastgele ve düzensizce hazırlıyorum	3	2.40
Otomatik sulukta	7	5.69	İki parametre seçenler*	3	2.40
İki parametre seçenler*	7	5.69	**Günlük Yemleme Sayısı**		
Yemleme Şekli			Günde bir defa	4	3.23
Yem karma makinasıyla	2	1.68	İki defa	73	58.87
El arabasıyla	18	15.13	Üç defa	44	35.48
Çuvalla	82	68.91	Üçten fazla	3	2.42
Kovayla	14	11.76	**Yemliklerin Durumu**		
İki parametre seçenler*	3	2.52	Ayrı bir kaba ve kesif yemliği var	46	38.43
			Ayrı bir kaba ve kesif yemliği yok	75	61.57

*Bu sorular için birden fazla parametre tercih edilmiştir.

İşletmelerin Hayvan Besleme Alışkanlıkları ile Bazı Parametreler Arasındaki İlişki

Van ili büyükbaş hayvancılık işletmelerinde faaliyet gösteren ve DAP Çiftçi Eğitim Merkezi'nde eğitimlere anketlere ve katılanların hayvan besleme alışkanlıkları ile yaş, işletmenin bulunduğu yer, öğrenim düzeyi, hayvancılıkla ilgili eğitim alınıp alınmadığı, işlenen arazisi büyüklüğü ve tarım ve hayvancılık organizasyonlarına üyelik durumu parametreleri arasında Khi kare testi yapılmıştır (Tablo 5).

İşletmelerde Yemlerin Temin Edilmesi ile Bazı Değişkenler Arasındaki İlişkiler

Bu araştırmada, yemlerin temini ile ankete katılanların yaş, işletmenin bulunduğu yer, öğrenim düzeyi, hayvancılıkla ilgili eğitim alınıp alınmadığı, tarımsal arazisi büyüklüğü ve herhangi bir organizasyona üyelik durumu parametreleri arasında Khi kare testi yapılmıştır (Tablo 6).

Tablo 5: Hayvan Besleme Alışkanlıkları ile Önemli Bazı Değişkenler Arası İlişki.

Table 5: Relationship Between Animal Feeding Habits and Some Important Variables.

	Yaş		İşletmenin yeri		Öğrenim Düzeyi		Hayvancılıkla ilgili eğitim alınma şekli		Tarımsal arazilerin miktarları		Üye olunan tarım ve hayvancılık organizasyonları	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Hayvanların beslemesinde kullanılan kaba yemler	555.2258	0.253	128.8508	0.196	157.7962	0.144	31.7995	0.329	142.5831	0.923	428.8694	<.001
Hayvanların beslemesinde kullanılan kesif yemler	128.1208	0.190	91.6705	0.490	71.2788	0.998	24.2006	0.024	141.6428	0.161	324.4250	0.002
Hayvanların günlük yemleme Sayısı	7.6509	0.937	9.6813	0.644	11.6572	0.705	4.6728	0.197	36.4290	0.891	18.0812	0.984
Hayvanların yemlenme şekli	24.3203	0.757	19.1873	0.742	89.1192	<.001	10.5018	0.105	52.3916	0. 038	64.6721	0.523
Rasyonlar hazırlanırken izlenilen Yol	14.3353	0.813	21.2347	0.170	22.5300	0.313	5.4675	0.242	31.2575	0.147	45.6522	0.403
Hayvanların sulanma şekli	33.7908	0.890	35.1446	0.509	62.2240	0.045	8.4820	0.486	51.9575	0.554	161.5345	<.001
Hayvan yemliklerinin durumu	19.4026	0.496	23.9030	0.092	17.8338	0.598	6.0331	0.197	10.6221	0.992	25.5188	0.988

Tablo 6: Yemlerin Temin Edilmesi ile Önemli Değişkenler Arasındaki İlişkiler.

Table 6: Relationships Between Feed Supply and Important Variables.

	Yaş		İşletmenin yeri		Öğrenim Düzeyi		Hayvancılıkla ilgili eğitim alınma şekli		Tarımsal arazilerin miktarları		Üye olunan tarım ve hayvancılık organizasyonları	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Hayvanların beslemesinde kullanılan kaba yemler	15.3859	0.119	16.1015	0.041	10.3450	0.411	1.9043	0.386	7.7888	0.801	21.7915	0.472
Hayvanların beslemesinde kullanılan kesif yemler	17.9053	0.057	12.1011	0.147	9.6577	0.471	1.2246	0.542	4.7440	0.928	38.4257	0.016
Hayvanların meraya çıkarılma durumu	11.2930	0.046	7.5282	0.111	5.9286	0.313	4.1825	0.041	5.4862	0.483	6.5915	0.831
Hayvanlara silaj yemi verme durumu	1.9290	0.859	5.2174	0.266	6.5973	0.252	5.0139	0.025	2.4419	0.875	19.6139	0.051
Tuz ve vitamin-mineral verme durumu	4.0771	0.538	9.3927	0.052	5.3914	0.370	0.0330	0.856	2.3471	0.885	4.6314	0.948
İşletmede yem bitkileri yetiştirilmesi	64.4386	0.880	75.2997	0.158	64.5735	0.895	19.8126	0.229	90.1366	0.649	359.8905	<.001

TARTIŞMA VE SONUÇ

Araştırma konusu alan, Van ili büyükbaş hayvan yetiştiricilerinin %84.56'sının 15-50 yaş aralığında olduğu belirlenmiştir. Böyle yüksek bir oranda gençlerden oluşan bir kitlenin büyükbaş hayvancılık eğitimi almasının, ülke hayvancılığın geleceği açısından önemli bir durum olarak karşımıza çıkmaktadır (Tablo 1). Yapılan bazı çalışmalarda, yaş aralıkları birbirine yakın olmak kaydıyla, anketlere katılanların genç yaşta olanların oranları arasında varyasyonlar görülmüştür. Buna göre Şevik (2017) katılımcıların %95'inin, Karaturhan ve ark. (2014) %75'inin, Terin ve Ateş (2010) %62.7 ile Bakır ve Kibar (2019)'ın yaptığı çalışmada %71.9'unun genç yaşta olanların oranları olduğu belirlemiştir.

Anketlere katılanların eğitim düzeyleri incelendiğinde, %2.96'sının okuma-yazma bilmediği, %2.96'sının okuma-yazma bildiği, %25.93'ünün ilkokul mezunu, %25.19'unun ortaokul mezunu, %27.41'inin lise ve dengi okul bitirdikleri, %15.55'inin yükseköğretim mezunu olduğu belirlenmiştir. Çalışmada ankete katılanların birçoğunun (%79.26) yapılan hayvansal faaliyetler için herhangi bir yerden eğitim almadıkları bildirilmiştir (Tablo 1). Yapılan bir çalışmada, görüşülen işletme sahiplerinden %8.0'inin okuma yazma bilmediği, ilkokul mezunu olanların oranı %38.4 ve yükseköğretim mezunu olanların oranı ise %2.4 olduğu tespit edilmiştir (Şeker ve ark. 2012). Yapılan başka bir çalışmada (Yıldız ve ark. 2019a), ankete dahil edilen kursiyerlerden okuma yazma bilmeyenler %9.77, okuma yazma bilenler %8.20, ilk okul mezunları %39.06, orta okul mezunları %21.88, lise ve dengi okul mezunları %14.84 ve yükseköğretim mezunlarının oranı ise %5.86 olarak belirlenmiştir. Yapılan bir çalışmada (Bakır ve Kibar 2019), işletme sahiplerinin eğitim seviyesi incelenmiş ve %9.1'nin okur-yazar olmadığı, %51.5'inin ilkokul, %24.6'sının ortaokul, %13.5'inin lise ve %1.5'inin yükseköğretim mezunu olduğu bildirilmektedir. Hayvancılık faaliyetiyle uğraşan üreticilerin eğitim seviyelerinin düşük olduğu görülmektedir. Eğitim seviyelerinin artmasına paralel olarak ise, üreticilerin tarımsal etkinliklerinin ve modern tarım yöntemleri kullananların sayısının arttığı ifade edilmektedir.

Van ili büyükbaş hayvan yetiştiricisi olan ve eğitimlere katılanların yerleşim yerleri incelendiğinde; İpekyolu %38.68, Tuşba %18.25, Edremit %10.95, Erciş %13.87 ve Gevaş ilçesi %18.25 şeklinde gerçekleştiği belirlenmiştir (Tablo 1). Bakır ve Kibar (2020)'ın Muş İli besi işletmelerine ait bazı parametrelerin incelendiği çalışmalarında, ankete katılanların yerleşim yerleri yaklaşık olarak, Merkez ilçeden %16.48, Bulanık'tan %24.73, Hasköy'den %3.85, Korkut'tan %19.23, Malazgirt'ten %20.33 ve Varto ilçesinden %15.33 şeklinde olduğu bildirilmiştir. Yapılan bir çalışmada (Yıldız ve Deniz 2021), araştırmaya katılanların yerleşim yeri dağılımları Merkez ilçeden %55.94, Bulanık'tan %27.23, Hasköy'den %2.97, Korkut'tan %2.97, Malazgirt'ten %3.96 ve Varto ilçesinden %6.93 şeklinde olduğu belirlenmiştir.

İşletmelerde kaliteli ve ucuz yem bitkisi üretilebilmesi ve verimli bir hayvancılık yapılabilmesine olanak sağlayan kriterlerden biri de verimli tarım arazilerine sahip olmaktır (Yıldız ve Deniz 2021). Ankete katılan yetiştiricilerin %75.63'ünün 5-100 dekar, %18.49'unun 100-250 dekar, %5.88'inin ise, 250 dekar ve üzeri tarım arazisinden yararlandıkları belirlenmiştir (Tablo 2). Bakan ve Aydın (2016) tarafından yapılan bir çalışmada, Ağrı ilindeki sığırcılık işletmelerinin sosyo-ekonomik özellikleri araştırılmış ve incelenen işletmelerin ortalama olarak 111.4 dekar arazi büyüklüğüne sahip oldukları tespit edilmiştir. Diyarbakır ilinde manda yetiştiriciliği ile uğraşan yetiştiricilerin mevcut durumları ve sorunlarının araştırıldığı bir çalışmada (Turan 2019), 112 yetiştiricinin arazi ortalamasının 174.9 dekar olduğu; Güzel ve Aybek (2017)'in yaptığı çalışmada ise, işletme sahiplerinin arazi büyüklüğü ortalamasının 156 dekar olduğu belirlenmiştir.

Gelir düzeyinin, insan yaşam kalitesinin arttırılmasındaki en önemli faktörlerden biri olduğu bildirilmektedir (Yıldız ve ark. 2019b). Bu çalışmada ankete katılan yetiştiriciler incelenmiş ve birçoğunda aylık gelirin 3 000 TL'ndan daha düşük (%75.43) olduğu tespit edilmiştir (Tablo 2). Türkiye'de, 2018 yılında tarımsal işletmelerde işgücü ücretleri yapısına ait istatistiklerde, sürekli olarak tarımla uğraşan işçilerini ortalama aylık ücretleri 2 117 TL (TUIK 2018) olduğu bildirilmiştir. Yapılan bir çalışmada (Yıldız ve ark 2019b), işletme sahipleri içinde aylık geliri 3 000 TL ve altı olanların oranının %70.30 olduğu bildirilmiştir. Benzer bir çalışmada, aylık geliri 3 000 TL ve altı olan işletmelerin oranının ise, %86.11 olduğu bildirilmiştir (Yıldız ve Deniz 2021).

Zirai alet ve makineler gün geçtikçe gelişmiş ve farklılaşmış, tarım ve hayvancılıkla uğraşan yetiştiricilerin önemli bir yardımcısı olmuşlardır. İşlerin vaktinde yapılabilmesi, bitkisel ve hayvansal üretimin yapılmasını kolay hale getirmesi, bunların önemini arttıran faktörlerdendir (Kabaş 2021). Yapılan bu araştırmada, ankete katılan işletme sahiplerinin bazı zirai alet ve makinelerinin olduğu, ancak traktörün en çok sahip olunan zirai makine olduğu (%40.17) tespit edilmiştir. Bunun yanında, yem kırma makinasına (%3.43), yem karıştırma makinasına (%3.43), balya makinasına (%5.13) ya da süt sağım makinası (%2.56) olan işletmelerin oranı oldukça düşük olarak belirlenmiştir (Tablo 2). Yapılan çalışmada, Ağrı ilindeki sığırcılık işletmelerinin sosyo-ekonomik özellikleri incelenmiş ve işletmelerin %80.2'sinde en fazla sahip olunan zirai makinanın traktör olduğu tespit edilmiştir (Bakan ve Aydın 2016). Benzer çalışmalarda, işletmelerin sahip olduğu traktör oranını, Yıldız ve ark. (2019b) %68.15, Güzel ve Aybek (2017) ise %76 olarak belirlemişlerdir.

Tarımsal ürün miktarının artması, ürünlerin daha kaliteli hale getirilmesi ve yetiştiricilerin yaşam kalitelerinin yükseltilmesi üreticilerin etkili bir biçimde örgütlenmesiyle sağlanabilmektedir (Karaturhan ve ark. 2014; Taşan 2019). Yapılan bu araştırmada çalışmaya katılanların birçoğunun herhangi bir tarımsal örgüte üye olmadıkları (%67.88) belirlenmiştir (Tablo 2). Bakır ve Kibar (2020) tarafından yapılan bir araştırmada, Muş İli besicilik işletmesi sahiplerinden bir birliğe üye olanların oranı %39.3 ve bir kooperatife üye olanların oranı ise %15.9 olduğu bildirilmiştir. Yapılan bir çalışmada (Bakan ve Aydın 2016), Ağrı ilinde işletme sahiplerinden bir birliğe ya da kooperatife üye olmayanları oranı %96.2, yapılan başka bir çalışmada ise, işletme sahiplerinin %72.8'inin bir tarımsal örgüt üyeliğinin olduğu tespit edilmiştir (Turan 2019).

Tarımsal ürünler stratejik olduğundan dolayı, dünyada birçok ülke tarafından tarım alanındaki sektörler desteklenmektedir. Tarımsal destekler, tarım sektöründe elde edilen gelirleri ve verimliliği arttıran, kalitenin iyileşmesi ve üretimde istikrarın sağlanması gibi birçok önemli rolleri bulunmaktadır (Akın ve ark. 2018). Yapılan bu çalışmada, işletmelerin %46.72'inin tarımsal desteklerden faydalandıkları, %53.28'inin faydalanmadıkları belirlenmiştir (Tablo 2). Bakan ve Aydın (2016) tarafından Ağrı ilinde yapılan bir araştırmada, ele alınan işletmelerden çoğunun tarımsal bir

destekten faydalandığı, başka bir çalışmada (Aksoy ve ark. 2014), birli üyeliği bulunan işletme sahiplerinin %46.6'sının tarımsal bir destekten faydalandığı, Yıldız ve ark. (2019b) ise %41.82 olarak tespit etmişlerdir.

Bu araştırma kapsamında incelenen, Van ili büyükbaş sığırcılık işletmelerin sahip oldukları hayvan sayıları incelenmiş ve işletme başına ortalama her yaştan 40.89 baş sığıra, 3.00 baş mandaya sahip oldukları tespit edilmiştir (Tablo 2). Benzer bir çalışmada Yıldız ve Deniz (2021), Muş ili için bu oranları baş sığır için 37.8 ve manda için 9.33 baş şeklinde; Bakır ve Kibar (2019), yine Muş ili süt sığırcılığı işletmelerinde bu oranı ortalama 56.6 olarak; Bakan ve Aydın (2016) ise, Ağrı ili için bu oranı 19.9 baş şeklinde tespit etmişlerdir.

Verimli hayvancılığın önkoşullarından biri de, kaliteli yemlerin kullanılması ve hayvanların ihtiyacı olan çevre şartlarının iyileştirilmesidir. Yemleme hayvancılıkta önemli olmasına rağmen hayvanların yeterince beslendiği söylenemez. İşletmede yemlerle ilgili masraflar toplam işletme giderlerinin %60-70'ini oluşturabilmektedir. Bundan dolayı yem ve yemleme ile ilgili yapılması gereken planlamalarla yeni, ucuz ve kaliteli yem kaynakları araştırılmalıdır. Böylece bu konuda yapılacak çalışmalar hayvancılığın geleceği açısından önem arz etmektedir (Kutlu ve ark. 2003; Özek 2022). Bu araştırmada, çalışmaya katılanlardan, hayvanların ihtiyacı olan kaba yemleri, kendi yetiştirenlerin oranı %42.50, işletme dışından alanların oranı %31.67 ve %25.83'ünün ise, hem kendisi tarafından yetiştirildiği hem de işletme dışından alındığı belirlenmiştir (Tablo 3). Yetiştiricilerin tarım-hayvancılık potansiyellerinin değerlendirildiği bir araştırmada (Yıldız ve ark. 2019b), %46.55'inin işletmelerin ihtiyaç duydukları kaba yemleri, kendilerinin ürettiği, %21.55'inin işletme dışından temin ettiği, hem kendileri üreten, hem de dışarıdan temin edenlerin oranı ise, %18.97 olduğu tespit edilmiştir. Yapılan bir çalışmada (Karakuş ve Akkol 2013), işletmelerin %12.26'ının ihtiyaç duydukları kaba yemlerin kendileri tarafından üretildiği, %17.22'inin işletme dışından temin ettiği, %70.52'inin ise, hem kendileri tarafından üretildiği, hem de dışarıdan temin edildiği bildirilmektedir.

Çalışmaya katılan işletme sahiplerinin, hayvanlarına daha çok buğday-arpa samanı (%32.00) verdikleri daha az oranda yonca (%13.60) ve silaj yemleri (%1.60) verdikleri belirlenmiştir (Tablo 3). Budağ ve Keçeci (2013) tarafından Van ilinde gerçekleştirilen bir araştırmada, sığır besisi yapan işletme sahiplerinin %87'si buğday ve arpa samanını, %66'sı yoncayı, %35'i korungayı, %45'i çayır kuru otunu, ve %54'ü de mercimek samanını tercih etmişlerdir. Bakır ve Demirel (2001)'in yaptığı bir çalışmada, ankete katılan işletmelerde kullanılan kaba yem kaynaklarının %84.1'inin samandan, %72.2'sinin kuru ottan, %15.3'ünün kesten ve %12.8'inin ise, yaş şeker pancar posasından oluşturdukları bildirilmiştir.

Bu çalışmada ankete katılan yetiştiricilerin, kesif yem temini konusunda, %14.75'inin kendilerinin yetiştirdiği, %50.00'sinin dışarıdan temin ettiği ve %35.25'inin ise hem kendilerinin yetiştirdiği hem de dışarıdan satın aldıkları belirlenmiştir (Tablo 3). Yapılan bir araştırmada (Yıldız ve ark 2019b), ankete katılan yetiştiricilerin kesif yem temini konusunda, kullanılan kesif yemlerin kendileri tarafından üretenlerin oranı %23.11, işletme dışından satın alanların oranı %43.56, yetiştiricilerin %25.33'ünün ise hem kendilerinin ürettiği, hem de dışarıdan aldığı tespit edilmiştir. Yapılan bir çalışmada (Karakuş ve Akkol 2013), ankete katılanlar içinde kesif yemleri işletmede üretenlerin oranı %5.65, dışarıdan satın alanlar %15.86, hem kendilerinin ürettiği, hem de dışarıdan alanların oranını %78.49 olarak belirlemişlerdir. Yıldız ve Deniz (2021)'in yaptığı bir çalışmada, işletme sahiplerinin hayvanlarına kesif yemleri, iki ya da daha fazla yem hammaddesini karıştırarak verenlerin yanında (%50.55), sadece arpa kırması(%27.27), kepek (%15.66) ya da süt yemi (%3.54) verdikleri belirlenmiştir. Bakır ve Demirel (2001) tarafından yapılan bir çalışmada, işletme sahipleri içinde kesif yem kaynağı olarak kepek kullananların oranı %71.5, süt yemi %69.3, arpa kırığı %5.6 ve besi yemi kullananların oranı %2.2 olarak belirlenmiştir

Yem bitkileri tarımına yer vermeyen işletmelerinin uzun vadede rantabl olmaları mümkün değildir. Bu nedenle, özellikle gelişmiş ülkelerde, tarımsal üretimin mihenk taşı görevi yem bitkileri oluşturmaktadır. Bu durum, yem bitkilerinin işlenen tarım toprağını koruma, verimli hale getirme ve hayvanların ihtiyaç duyduğu ucuz ve kaliteli yem ihtiyacını karşılaması gibi öneminden kaynaklanmaktadır (Yıldız ve Deniz 2021). Bu nedenle, her işletmede yem bitkileri üretiminin yapılması gerekir. Bu çalışmada, ankete katılan işletmeler içinde yem bitkisi yetiştiriciliği olarak %34.75'inin sadece yonca, %2.54'inin sadece silajlık mısır, %12.71'inin korunga ve %2.54'inin ise fiğ yetiştirdiği belirlenmiştir. %36.43'ünün de iki veya daha fazla yem bitkisini birlikte yetiştirdikleri tespit edilmiştir (Tablo 3). Yapılan bir araştırmada (Şahin ve Yılmaz 2008a), çalışmaya katılan işletmelerin ortalama 29.38 da alanda yem bitkisi, 20.67 da alanda yonca ve 8.71 da alanda ise korunga yetiştirildiği; benzer bir araştırmada ise (Şahin ve Yılmaz 2008b), ortalama 26.87 da alanda yem bitkisi ekiminin yapıldığı, 20.14 da alanda yonca, 5.34 da alanda korunga ve 1.39 da alanda ise silajlık mısır yetiştirildiği tespit edilmiştir. Demir ve ark. (2013) tarafından Kars ili süt sığırcılığı işletmeleri ile ilgili yapılan bir araştırmada, yem bitkisi üreten işletmelerin oranının %88.7 olduğu ve korunga ve fiğin en fazla yetiştiriciliği yapılan yem bitkisi olduğu belirlenmiştir.

Bu araştırmada ankete katılan işletme sahiplerinden %81.25'inin hayvanlarının ihtiyacı olan tuz ve vitamin-mineral takviyesini yaptıkları, %18.75'inin ise yapmadıkları tespit edilmiştir (Tablo 3). Yapılan bir araştırmada (Yıldız ve ark. 2019b), çalışmaya dahil edilen işletmelerin %86.94'ü vitamin mineral ve tuz ilavesi yaparken, %13.06'sının ise yapmadığı, yapılan başka bir araştırmada (Karakuş ve Akkol 2013) yetiştiricilerin %57.95'inin vitamin mineral ve tuz ilavesini yaptığı, %42.05'inin ise yapmadığı belirlenmiştir.

Bu çalışmada, hayvanlarını merada otlatan işletme sahiplerinin oranı %78.23 olarak belirlenmiştir (Tablo 3). Yapılan bir çalışmada (Yıldız ve ark. 2019b), ele alınan işletmelerde %90.87 oranında yetiştiricinin hayvanlarını meraya çıkarttığı tespit edilmiştir. Şahin ve Yılmaz (2008a)'ın yaptığı çalışmada %76.22 olan bu oran; Yıldız ve Deniz (2021)'in çalışmasında %95.45; Demir ve ark. (2013)'nın çalışmasında, %87.6 olarak belirlenmiştir.

Kârlı bir hayvancılığın önemli faktörlerinden biri olan silaj yemleri, bu çalışmaya katılan yetiştiricilerin ancak %35.29'u tarafından hayvanlarının beslenmesinde kullanmışlardır (Tablo 3). Yapılan bir araştırmada (Aksoy ve ark. 2014), yetiştiricilerin %11.3'ünün birliğe üye olduğu ve silaj yemi kullandıkları, %2.0'sinin ise birliğe üye olmayan ve silaj yemi kullanmayanlardan oluştuğu belirlenmiştir. Demir ve ark. (2013) tarafından yapılan bir çalışmada, işletme sahiplerinin %88.3'ü hayvanları beslerken silaj yemleri kullanmadıkları belirlenmiştir. Yapılan bir araştırmada (Yıldız ve ark. 2019b), çalışmaya dahil edilen yetiştiricilerin %18.40'ının silaj yemlerinde

yararlanırken; Yıldız ve Deniz (2021)'in yaptığı çalışmada silaj kullanım oranı %50.53 olarak belirlenmiştir.

Büyükbaş hayvancılık işletmelerinde kendi imkanlarıyla elde edilen ya da satın alınan kaba ve kesif yemlerle hayvanlar beslemektedir. Bu yemlerle karışımlar hazırlanırken, yemlerin içeriklerinin ve hayvanların ihtiyaçlarına dikkat edilmediği görülmektedir. İşletme sahiplerinin mevcut imkanlarını kullanarak ve hayvanların ihtiyaçları dikkate alınarak, dengeli rasyonlar oluşturulmalı, bunun için de işletmecilerin ilgili kuruluşlarca eğitilerek teknik bilgilerle donatılmaları gerekmektedir (Bakır ve Tugay 2008). Yapılan bu araştırmada, yetiştiricilerin %58.87'sinin hayvanlarını günde iki defa yemledikleri, %68.91'inin yemleme işlemini çuval kullanarak yaptığı ve %61.57'sinin farklı yemler için farklı yemliklerin bulunmadığı tespit edilmiştir (Tablo 4).

Güzel ve Aybek (2017) tarafından yapılan bir araştırmada, işletme sahipleri sahip oldukları hayvanların günde iki defa yemlendiğini bildirmişlerdir. Bakır (2002) tarafından yapılan bir çalışmada, yemliklerin ahırların tamamında duvara bitişik olarak yerleştirdikleri, bunların betonarme, ahşap veya saç malzemeden yapıldığı, yem yolunun ise olmadığı, yemleme işleminin hayvanlar arasına girilerek yapıldığı bildirilmiştir. Yapılan bir araştırmada (Turan 2019), çalışmaya dahil edilenlerin %73.5'inin hayvan barınaklarında beton yemlikler kullandıkları, metal yemlik kullananlar %12.9, plastik yemlik kullananlar %8.8 ve tahta yemlik kullananların oranı ise, %4.8 olduğu belirlenmiştir.

Bu çalışmaya katılan işletme sahiplerinin %61.60'ı hayvanlarına verilen rasyonların kendi göz kararlarına göre; %25.60'ı rasyonların hazırlanması konusunda deneyimli birilerinden yararlandıkları belirlenmiştir (Tablo 4). Bakır ve Demirel (2001) tarafından yapılan bir araştırmada, verdikleri kaba ve kesif yemlerin işletmede var olduğu için kullandıkları, rasyonlarda silaj yemlerinin yer almadığı, hayvanların yaşama ve verim durumları göz önüne alınmadan yemlendiği tespit edilmiştir. Demir ve ark. (2013) tarafından yapılan bir çalışmada ise, yetiştiricilerin çoğunluğunun hayvanları kendi bilgi ve tecrübeleri doğrultusunda besledikleri bildirilmiştir.

Bu çalışmada katılımcıların %49.59'u hayvanlara suyu önlerine kedilerinin taşıyarak suladıkları, %24.39'unun ise hayvanların ahır içinde bulunan sabit suluklarda sulandığı tespit edilmiştir (Tablo 4). Yapılan bir araştırmada (Turan 2019), yetiştiricilerin yaz döneminde mandalarını çeşmede (%10.2), çeşme+nehirde (%34.7) ve çeşme+nehir+kuyu suyu (%32.7) ile suladıkları bildirilmektedir. Aydın ve ark. (2016) tarafından yapılan bir araştırmada ise, işletmelerde hayvanların, %34.3'ünün önlerine taşınmasıyla, %34.3'ünün yemlikler içine su doldurularak, %22.4'ünün otomatik suluklar yardımıyla, %4.6'sının çeşme kullanılarak ve % 4.3'ünün ise yalaklardan suladığı tespit edilmiştir.

Van ili büyükbaş hayvancılık işletmelerinden kurslara ve ankete katılanların bazı özellikleri arasında Khi kare testi uygulanmıştır. Bu değişkenler arasında, hayvan besleme alışkanlıklarıyla yetiştiricilerin yaşı, işletmenin bulunduğu yer (ikametgahı), öğrenim durumları, hayvancılıkla ilgili eğitim alınıp alınmadığı durumları, sahip olunan tarım arazisi miktarları ve herhangi bir tarımsal organizasyona üyelik durumları bulunmaktadır (Tablo 5).

Buna göre, ankete katılanların öğrenim durumları ile hayvanlara yem verme ve sulama şekli arasında; hayvancılık eğitimi alınması ile hayvan beslemede kullanılan kesif yemler değişkenleri arasında; işlenen tarım arazisi miktarları ile hayvanların yemlenme şekli değişkenleri arasında; tarımsal organizasyonlara üyelik durumu ile hayvanların beslenmesinde kullanılan kaba yemler, hayvanların beslenmesinde kullanılan kesif yemler ve hayvanların sulanma şekli parametreleri aralarında anlamlı ilişkiler ($p<0.05$) tespit edilmiştir. Çalışmada ele alınan parametreler içinde bireylere ait yaşlar ve işletmenin bulunduğu yer ile hayvanların beslenme durumları arasında anlamlı bir ilişki olmadığı tespit edilmiştir.

Yine bu araştırmada, yemlerin temin edilme durumu ile yetiştiricilere ait yaşlar, işletmelerin yerleri, öğrenim durumları, hayvancılıkla ilgili herhangi bir eğitim alınıp alınmadığı durumları, sahip olunan tarımsal arazi miktarları ve herhangi bir tarım ve hayvancılık organizasyonuna üyelik parametreleri arasında yine Khi kare testi yapılmıştır (Tablo 6). Tablo incelenmiş ve araştırmada ele alınan yetiştiricilerin öğrenim durumları ve işlenen tarım arazileri ile yemlerin temin edilme durumları arasında anlamlı bir ilişki olmadığı belirlenmiştir. Ankete katılanların yaş durumları ile hayvanların meraya çıkarılma durumu; işletmenin yeri ile kaba yemlerin temin edilmesi; hayvancılıkla ilgili eğitim alınıp alınmadığı parametreleriyle, hayvanların meraya çıkarılma parametreleri ve hayvanların silaj yemleriyle besleme durumu parametreleri arasında; tarımsal organizasyonlara üyelik ile kesif yem temin edilme durumu ve işletmede yem bitkileri yetiştirme parametreleri arasında anlamlı bir ilişki olduğu ($p<0.05$) belirlenmiştir.

Sonuç olarak; Türkiye'nin doğusunda bulunan Van ilinde, arazi şekilleri, iklim şartları, bitki kompozisyonu, geniş mera alanlarının bulunması, tarımla ve hayvancılıkla uğraşan işletmelerinin özellikleri nedeniyle, hayvancılığın ön plana çıktığı görülmektedir. Van ilinde hayvancılık faaliyetleriyle uğraşan üreticilerin, daha da bilinçli hale getirilmesi ile bu insanların büyükbaş hayvancılık kapasitelerinin arttırılması mümkündür. Ayrıca bu araştırmada, ankete katılanların çoğunluğunun genç yaşta olduğu ve bu durumun bölgede hayvancılık potansiyelinin gelişmesi açısından umut vadettiği; yetiştiricilerin eğitim seviyelerinin çok düşük olmamasına rağmen, hayvancılıkla ilgili herhangi bir eğitim alanların oranlarının oldukça düşük olduğu, bu nedenle Van ili ve çevresinde uygun hayvan besleme alışkanlıklarının olmadığı görülmüştür.

Söz konusu işletmelerde yeterli hayvan varlığının olmadığı, işletme ve hayvan sayılarının her yıl giderek azaldığı tespit edilmiştir. Bölgedeki yetiştiricilerin küçük arazilere sahip olması nedeniyle istenilen düzeyde tarımsal faaliyet yapamamaktadır. Bundan dolayı işletmeler için gerekli olan kaba ve konsantre yemlerin temin edilmesini zorlaştırmıştır. Küçük işletmeler nedeniyle, bölgede tarım ve hayvansal teknolojiden yeterince yararlanılamamaktadır. Hayvanların büyük bir kısmının meraya çıkarılıyor olması, hayvancılığın ekonomikliği açısından olumlu görülürken, buğday-arpa samanları gibi düşük kaliteli yemlerin fazla kullanılması, silaj gibi kaliteli kaba yemlerinin kullanım yetersizliği olumlu görülmemektedir.

ÇIKAR ÇATIŞMASI

Yazarlar bu çalışma için herhangi bir çıkar çatışması olmadığını beyan ederler.

TEŞEKKÜR VE BİLGİLENDİRME

Çalışma kapsamında sayın Prof. Dr. Suphi DENİZ'e ve sayın Doç. Dr. Suna AKKOL'a katkılarından dolayı teşekkür ederim.

YAZAR KATKILARI

Fikir/Kavram: SY
Denetleme/Danışmanlık: SY
Veri Toplama ve/veya İşleme: SY
Analiz ve/veya Yorum: SY
Makalenin Yazımı: SY
Eleştirel İnceleme: SY

KAYNAKLAR

Akın S, Kara A, Tutkun, M (2018). Diyarbakır Damızlık Sığır Yetiştiricileri ve Damızlık Koyun Keçi Yetiştiricileri Birliği özelinde hayvancılık destekleri hakkında üretici görüşlerinin belirlenmesi. *DUFED*, 7 (1), 21-26.

Aksoy A, Güler İO, Terin, M (2014). Erzurum İli Damızlık Sığır Yetiştiricileri Birliğine üye olan ve olmayan üreticilerin belirli özellikler açısından karşılaştırılması. *JAFAG,* 31 (3), 82-90.

Arslan Ö (2018). Muş ili özelinde doğal tarım ve hayvancılığa dayalı sürdürülebilir bir ekonomik gelişim. *Anemon*, 6 (1), 75–90.

Aydın R, Güler O, Yanar M ve ark. (2016). Erzurum İli Hınıs İlçesi sığırcılık işletmelerinin barınak özellikleri üzerine bir araştırma. *KSÜ Doğa Bil Derg*, 19 (1), 98-42.

Bakan Ö, Aydın R (2016). Ağrı İli süt sığırcılığı işletmelerinin sosyo-ekonomik özellikleri. *Atatürk Üniversitesi Ziraat Fak Derg*, 47 (2), 113-122.

Bakır G, Demirel M (2001). Van İli ve ilçelerindeki sığırcılık işletmelerinde kullanılan yem çeşitleri ve hayvan besleme alışkanlıkları. *YYÜZF Tar Bil Derg*, 11 (1), 29-37.

Bakır G, Kibar M (2019). Muş İlinde bulunan süt sığırcılığı işletmelerinin bazı yapısal özelliklerinin Crosstab analiziyle belirlenmesi. *KSÜ Tarım ve Doğa Derg*, 22 (4), 609-619.

Bakır G, Kibar M (2020). Muş İli besi sığırcılığı işletmelerinin bazı yapısal özelliklerinin belirlenmesi. *KSÜ Tarım ve Doğa Derg,* 23 (6), 1687-1697.

Bakır G (2002). Van İlindeki özel süt sığırcılığı işletmelerinin yapısal durumu. *J Agric Sci,* 12 (2), 1-10.

Budağ C, Keçeci Ş (2013). Van'da büyükbaş hayvan besilerinde kullanılan yemler ve besi şekillerine ilişkin bir anket çalışması. *YYÜ Fen Bil Enst Derg*, 18 (1-2), 48-61.

Demir P, Elmalı DA, Işık S, Tazegül R, Ayvazoğlu C (2013). Kars İli süt sığırcılık işletmelerinde yem kullanımı ve hayvan besleme alışkanlıklarının ekonomik önemi. *Atatürk Üniversitesi Vet Bil Derg,* 8 (3), 229-236.

Ertaş N, Deniz O (2018). 1991 sonrasında Van'da küçükbaş hayvancılığın gelişim seyri ve sorunları. TÜCAUM 30. Yıl Uluslararası Coğrafya Sempozyumu, 3-6 Ekim 2018, Ankara

Güzel M, Aybek A (2017). Kahramanmaraş İli süt sığırcılığı işletmelerinin mekanizasyon yapısı. *KSÜ Doğa Bil Derg,* 20 (2), 148-159.

İşler H, Ören HGÜ (2021). Dünya'da, Bölgelerde ve Türkiye'de hayvancılık sektörü. *Sosyal ve Beşeri Bil Araşt Derg*, 22 (48), 72-95.

Kabaş Ö (2021). Tarımsal mekanizasyonun dünyada ve Türkiye'deki yeri. Erişim tarihi: 21.06.2021. Erişim adresi: https: //arastirma.tarimorman.gov.tr/batem/Belgeler/Kutuphane/Teknik%20Bilgiler/tarimsal%20mekanizasyon.pdf

Karakuş F, Akkol S, (2013). Van İli küçükbaş hayvancılık işletmelerinin mevcut durumu ve verimliliği etkileyen sorunların tespiti üzerine bir araştırma. *YYÜ Fen Bil Enst Derg,* 18 (1-2), 09-16.

Karaturhan B, Şevik T, Yıldız Ö (2014). Yetiştirici birliklerinin tarımsal kalkınmaya etkileri üzerine bir araştırma: Edirne damızlık sığır yetiştiricileri birliği örnek olayı. *Ege Üniv Ziraat Fak. Derg,* 51 (2), 175-184.

Kutlu H, Gül A, Görgülü M (2003). Türkiye hayvancılığının sorunları ve çözüm yolları. I. damızlık hayvan-kaliteli yem. *Yem Mag Derg.* 34, 40-46.

Özek K (2022). TR22 Güney Marmara Bölgesinde büyükbaş ve küçükbaş hayvancılığın durumu, kaba yem üretimi, yeterliliği ve hayvan beslemedeki önemi. *Iğdır Üniversitesi FBED,* 12 (2), 1187-1200.

SAS (2014). SAS/STAT. Statistical analysis system for Windows. Released version 9.4. SAS Institute Incorporation, Carry, NC, USA

Şahin K, Yılmaz İH (2008a). Van İli'nde yem bitkileri tarımı, mera kullanımı ve sosyo ekonomik yapı üzerine bir araştırma. *AÜZF Tar Bil Derg*, 14 (4), 414-419.

Şahin K, Yılmaz İH (2008b). Van ili Gürpınar ilçesinde yem bitkileri üretimi ve sorunları üzerine bir araştırma. *AÜZF Tar Bil Derg,* 14 (1), 16-21.

Şeker İ, Tasalı H, Güler H (2012). Muş ilinde sığır yetiştiriciliği yapılan işletmelerin yapısal özellikleri. *FÜ Sağ Bil Vet Derg,* 26 (1), 9-16.

Şevik T (2017). Edirne İli Lalapaşa İlçesi süt sığırcılığı eğitiminin tarımsal yayım açısından değerlendirilmesi. Yüksek Lisans Tezi. Namık Kemal Üni. Fen Bilimleri Enstitüsü, Tekirdağ

Taşan M (2019). Türkiye'de tarımda üretici örgütlenmesi. *Uluslararası Anadolu Zir Müh Bil Derg,* (Özel Sayı 1), 77-85.

Terin M, Ateş HÇ (2010). Çiftçilerin örgütlenme düzeyi ve örgütlerden beklentileri üzerine bir araştırma: Van İli örneği. *Ege Üniv Zir Fak Derg,* 47 (3), 265-274.

Tugay A, Bakır G (2008). Giresun yöresindeki sığırcılık işletmelerinde kullanılan yem çeşitleri ve hayvan besleme alışkanlıkları. *Atatürk Üniv Zir Fak Derg,* 39 (2), 231-239.

Turan M (2019). Diyarbakır manda yetiştiriciliğinin mevcut durumu, sorun ve çözüm önerilerinin belirlenmesi. Yüksek Lisans Tezi. Dicle Üniversitesi Fen Bilimleri Enstitüsü. Diyarbakır.

TÜİK (2018). Tarımsal işletme işgücü ücret yapısı, Erişim tarihi: 10.04.2021. Erişim adresi: https://tuikweb.tuik.gov.tr/PreHaberBultenleri.do;jsessionid=RBs9fYSLTtyFvHcQCG2Jq49pMb66wHD2yNjJrwcJh2v22JZ85qm!765828690?id=30820.

TÜİK (2022a). Hayvansal üretim istatistikleri. https://data.tuik.gov.tr/ Erişim tarihi: 06.03.2023.

TÜİK (2022b). Tarım göstergeleri. Erişim tarihi: 06.03.2023. Erişim adresi: https://biruni.tuik.gov.tr/ilgosterge/?locale=tr.

Yıldız S, Akkol S, Deniz S (2019a). Van Yüzüncü Yıl Üniversitesi DAP Çiftçi Eğitim Merkezi'nde eğitim alan kursiyerlerin tarım-hayvancılık okuryazarlığı kapasitelerinin değerlendirilmesi. *YYÜ Fen Bil Enst Deri*, 24 (2), 133-141.

Yıldız S, Akkol S, Deniz S (2019b). Van Yüzüncü Yıl Üniversitesi DAP Çiftçi Eğitim Merkezi'nde eğitim alan kursiyerlerin tarım-hayvancılık potansiyellerinin değerlendirilmesi. *Van Vet J*, 30 (3), 151-157.

Yıldız S, Deniz S (2021). Muş İli Damızlık Sığır/Manda Yetiştiricileri Birliklerine üye işletmelerin yem temini ve hayvan besleme alışkanlıkları. *Iğdır Üniversitesi FBED,* 11 (4), 3280-3291.

Yıldız A, Aygün T (2021a). Van ili Merkez ilçede küçükbaş hayvancılık faaliyetleri ve genel sorunlar: I. İşletmelerin yapısal özellikleri. *JASP,* 4 (1), 23-36.

Yıldız A, Aygün T (2021b). Van ili Merkez ilçede küçükbaş hayvancılık faaliyetleri ve genel sorunlar: II. İşletmelerde yetiştirme işleri. *JASP,* 4 (1), 37-53.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Aydın SS, Denek N (2023).** The Effect of Lyophilized and Frozen Natural Lactic Acid Bacteria on Alfalfa Silage Quality Preparedin Different Waysand Stored For Different Periods of Time. *Van Vet J*, 34 (2), 155-162. DOI: https://doi.org/10.36483/vanvetj.1285065

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

The Effect of Lyophilized and Frozen Natural Lactic Acid Bacteria on Alfalfa Silage Quality Prepared in Different Ways and Stored for Different Periods of Time

Sadık Serkan AYDIN[1]* Nihat DENEK[1]

[1] *Harran University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Disease, 63210, Şanlıurfa, Türkiye*

Received: 18.04.2023 Accepted: 02.06.2023

ABSTRACT Within the scope of this study, it was aimed to determine the effect of the groups with the highest LAB numbers determined as a result of storage for one and three months on alfalfa silage quality by freezing fermented lactic acid bacteria (LAB) liquids prepared with different levels of sucrose addition (5-10%) and incubation (2 and 5 days) for different periods of time in deep freezer and by drying via lyophilization process according to the results obtained from the previous study.In the study, groups consisted of control, 2D5%STsL (lyophilized with TRIS (Ts) after 2 days incubation with 5% sucrose addition), 2D10%SDsL (lyophilized with DMSO (Ds) after 2 days incubation with 10% sucrose addition), 5D10%SDsL (DMSO (Ds) additive lyophilized after 5 days of incubation with 10% sucrose addition), and 5D5%STsD (TRIS (Ts) additive deep freezer after 5 days of incubation with 5% sucrose addition).In the study, LAB count, CO_2, lactic acid (LA) content, acetic acid (AA) content, pH, NH_3-N/TN, and butyric acid (BA) values were statistically significant between the groups at the end of the one-month storage period. Crude protein (CP), pH, LA, and BA values were found to be statistically significant between the groups at the end of the three-month storage period in the study.

Keywords: *Alfalfa, Probiotics, Silage.*

ÖZ

Farklı Şekillerde Hazırlanarak Değişik Sürelerde Depolanan Liyofilize Edilmiş ve Dondurulmuş Doğal Laktik Asit Bakteri Sıvılarının Yonca Silaji Kalitesi Üzerine Etkisi

Bu çalışma kapsamında, önceki çalışmadan elde edilen sonuçlarına göre farklı seviyelerde sükroz ilavesi (%5-10) ve farklı sürelerde inkübasyonla (2 ve 5 gün) hazırlanmış fermente edilmiş laktik asit bakteri (LAB) sıvılarının derin dondurucuda dondurularak ve liyofilizasyon işlemi ile kurutularak bir ve üç ay süre ile depolanması sonucunda belirlenen en yüksek LAB sayılarına sahip grupların yonca silajı kalitesi üzerine etkisi belirlenmesi amaçlanmıştır. Çalışmada gruplar kontrol, 2D5%STsL (%5 sükroz ilavesi ile 2 gün inkübasyon sonrasında TRİS (Ts) katkılı liyofilize), 2D10%SDsL (%10 sükroz ilavesi ile 2 gün inkübasyon sonrasında DMSO (Ds) katkılı liyofilize), 5D10%SDsL (%10 sükroz ilavesi ile 5 gün inkübasyon sonrasında DMSO katkılı liyofilize) ve 5D5%STsD'den (%5 sükroz ilavesi ile 5 gün inkübasyon sonrasında TRİS katkılı derin dondurucu) oluşuyordu.Çalışmada bir aylık depolama süresi sonunda LAB sayısı, CO_2, laktik asit (LA) içeriği, asetik asit (AA) içeriği, pH, NH_3-N/TN ve bütirik asit (BA) değerleri gruplar arasında istatistiksel olarak anlamlı bulunmuştur. Çalışmada üç aylık depolama süresi sonunda ise gruplar arasında ham protein (CP), pH, LA ve BA değerleri istatistiksel olarak anlamlı bulunmuştur.

Anahtar Kelimeler: *Probiyotik, Silaj, Yonca.*

INTRODUCTION

In order to improve silage quality, live bacterial cultures called microbialinoculants have recently been utilized. For this purpose, commercially produced microbialinoculants preparations contain mostly LAB.

Lactic acid bacteria positively improve fermentation by minimizing the growth of aerobic bacteria, yeasts and mold that will compete for the substrate in the environment as a result of the formation of anaerobic conditions in the silo. Lactic acid bacteria inactivate plant protease enzymes by lowering the pH in the silo and reduce the degradation of silage plant proteins, as well as preventing the development of undesirable microorganisms in aerobic silage fermentation (Muck 1996). The desired anaerobic conditions in the silo can be achieved by ensuring that the ensiled material has the

✉ *Corresponding author: sadik.aydin@harran.edu.tr

appropriate dry matter (DM) content, is broken down to the appropriate size, the silo is filled quickly, compacted sufficiently, and closed quickly and airtight. With the formation of anaerobic conditions in the silo, LAB present in the natural microflora of the ensiled product ferment water-soluble carbohydrates (WSC) into various organic acids, mostly lactic acid. These acids, which are the fermentation product of lactic acid bacteria, increase the hydrogen ion level in the silo to a level that inhibits the growth of microorganisms whose activities in the silo are undesirable. As a result, the increase in lactic acid production in the silo and the consequent decrease in pH value inhibits the growth of all remaining microorganisms in the silo (McDonald et al. 2002).

This study, it was aimed to investigate the effect of different storage methods, incubation times, and preservative-added natural lactic acid bacterial liquid inoculant on the quality of alfalfa silage.

MATERIAL AND METHODS

Ethics committee approval was not required as the study did not include (live or dead animals or their tissues, slaughterhouse materials, procedures with waste fetuses).

Formation of Test groups

In the study, the LAB liquids were frozen and lyophilized and stored for one and three months and the groups with the highest LAB numbers at the end of each storage period were added to the silages prepared from alfalfa plants. In this context, at the end of one month storage period, in addition to the control group, 2D5%STsL, 2D10%SDsL, 5D10%SDsL and 5D5%STsD groups with the highest LAB numbers were prepared as treatment group silages. At the end of three months storage period, 2D5%SDsL, 5D5%STsL, 5D5%STsL,5D10%STsL and 5D10%STsDD groups with the highest LAB numbers were prepared as treatment group silages in addition to the control group.

Activation of Fermented LAB Liquids Lyophilized and Frozen in Deep freezer

Within the scope of the study, lyophilized, dried and deep-frozen LAB fluids were stored for one and three months. The lyophilized groups were left to thaw for 3 hours at room temperature by adding 1 ml of distilled water and the deep-frozen groups were left to thaw for 3 hours at room temperature without adding distilled water, and then microbiological analyses were performed. At the end of each storage period (one and three months), four groups of LAB liquids with the highest LAB numbers were added to the silages prepared from alfalfa plants. In this context, as a result of the microbiological analyzes performed at the end of one month storage, while 2D5%STsL, 2D10%SDsL, 5D10%SDsL and 5D5%STsD groups with the highest LAB numbers constituted the treatment groups, the silage groups were formed so that the alfalfa silage without additives constituted the control group. At the end of the microbiological analysis performed after three months of storage, 2D5%SDsL, 5D5%STsL, 5D10%STsL and 5D10%10STsD groups with the highest LAB numbers constituted the treatment groups, while unamended alfalfa silage constituted the control group.

Preparation of Alfalfa silage

In the silages prepared at the end of one month storage period, the alfalfa plant (*Medicagosativa L*) used as silage material was harvested at the period of second harvest and 20% flowering. In the silages prepared at the end of the three-month storage period, the alfalfa plant, which was used as silage material, was harvested in the period of fifth harvest and when the flowering was 20%. The alfalfa plant, which was used as silage material in the study, was broken into 5-7 cm pieces by mean. The silages prepared at the end of each storage period contained LAB liquids obtained at the end of one month storage period (2D5%STsL, 2D10%SDsL, 5D10%SDsL and 5D5%STsD) according to the results of the control (without additives) and the first test; LAB liquids obtained from frozen and/or lyophilized LAB liquids with the highest LAB count at three months storage period (2D5%SDsL, 5D5%STsL, 5D10%STsL and 5D%10STsD) were prepared by spraying the fresh silage material at a dose of 10^5cfu/ml. At this stage, in order to apply the LAB liquids added to the silage material homogeneously, LAB liquids were added into 10 ml of pure water for each kilogram of fresh silage material and sprayed with hand sprays independently of each other. In the control (no additive) group, the amount of pure water added to the treatment group was sprayed in order to homogenize the DM effect in the treatment groups. The silages were compressed in 1.5 liter glass jars with 4 replicates for control (without additive) and each treatment group and silaged in an airtight manner. Thus, 20 jars of silage were prepared for each storage period (one month and three months). The prepared silages were stored for 60 days at room temperature in a dark environment by covering the jars.

Determination of Silage Composition

Control (without additive) and LAB liquid-added alfalfa silages were opened at the end of the sixty-day ensiling period and the effects of fermented LAB liquid additives on alfalfa silage quality were determined. The silages prepared within the scope of the test (at the end of one and three months storage period) were opened at the end of the fermentation period, and after the 3-5 cm part of the top of the jars was discarded, 100 ml of pure water was added to 25 g of silage sample taken homogeneously and disintegrated with the help of a mixer for 2 minutes and the pH value of the silage liquid obtained was quickly measured with a pH meter (Hanna-HI-9813) (Polan et al. 1998).

Ammonia nitrogen ratio (NH_3-N/TN, %) values in total nitrogen (TN) content of the silages obtained were determined according to the method reported by AOAC (1990). Lactic acid and volatile fatty acids (butyric (BA), acetic (AA) and propionic acid (PA)) concentrations were determined by high pressure liquid chromatography (HPLC) according to the method reported by Suzuki and Lund (Suzuki and Lund 1980). Aerobic stability values of the silages obtained within the scope of the study were determined according to the method reported by Ashbell et al. (1991). In the preparation of the silages, the WSC content of alfalfa plant used as silage material was determined according to the method reported by Dubois et al. (1996) and the buffering capacity (BC) was determined according to the method reported by Playne and McDonald (1966). The part of the silages used in the crude nutrient analysis of the silages evaluated within the scope of the second test was dried at room temperature and ground in a laboratory mill (Şimşek Laborteknik) to pass through a 1 mm of sieve and made ready for analysis. The DM, crude ash (CA) and crude protein (CP) contents of the obtained silages and alfalfa plant used as silage material were determined by the method reported by AOAC (2005). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined according to the method reported by Van Soest et al. (2018).

Statistical Analysis

The effects of LAB liquid addition on the quality of alfalfa silage were evaluated by analysis of variance. The difference between the averages obtained in the test was determined by Duncan multiple comparison test ($p<0.01$) and SAS (1989) package program was used for this purpose.

RESULTS

Alfalfa silages added with lactic acid bacteria liquids stored for one month

In this study, the values of DM, CA, CP, ADF and NDF were determined as 20.30%, 9.95%, 23.70%, 37.96% and 39.14%, respectively; buffering capacity was 620 meq/kg DM; and WSC content was determined as 71.10 g/kg DM, according to DM basis of alfalfa plant used in the preparation of alfalfa silages obtained by adding LAB liquids with the highest LAB values at the end of one month storage period. The effects of LAB liquids with the highest LAB values on the crude nutrient values of alfalfa silage at the end of one month storage period are presented in Table 1.

There was no statistical difference ($p>0.01$) between the LAB liquids with the highest LAB values at the end of one month storage period on the crude nutrient values of alfalfa silage. The effects of LAB liquids with the highest LAB values on the fermentation quality of alfalfa silage at the end of one month storage period are presented in Table 2. When the pH, NH_3-N/TN, CO_2, LA, AA and BA values of the silages obtained were analyzed, the differences between the groups were found to be statistically significant ($p<0.01$).

In addition, propionic acid was not detected in any of the alfalfa silages prepared by adding the LAB liquids with the highest LAB values to the alfalfa plant at the end of one month storage period.

Table 1: The effect of LAB liquids with the highest LAB values on crude nutrient values of alfalfa silage at the end of one month storage period.

Groups	DM	CA	CP	ADF	NDF
Control	17.72	11.57	23.34	30.06	31.57
2D5%STsL	18.33	10.90	24.02	29.99	32.56
2D10%SDsL	18.24	11.05	24.31	29.45	31.65
5D10%SDsL	17.67	11.44	23.36	30.99	32.04
5D5%STsD	18.19	11.25	23.82	32.86	30.83
SEM	0.091	0.085	0.145	0.412	0.310
p	p>0.01	p>0.01	p>0.01	p>0.01	p>0.01

DM: Dry matter %, **CA:** Crude ash (DM%), **CP:** Crude protein (DM%), **ADF:** Acid Detergent Fiber (DM%), **NDF:** Neutral Detergent Fiber (DM%), **2D5%STsL:** lyophilized with TRIS (Ts) after 2 days incubation with 5% sucrose addition, **2D10%SDsL:** lyophilized with DMSO (Ds) after 2 days incubation with 10% sucrose addition, **5D10%SDsL:** DMSO (Ds) additive lyophilized after 5 days of incubation with 10% sucrose addition, **5D5%STsD:** (TRIS (Ts) additive deep freezer after 5 days of incubation with 5% sucrose addition.

Table 2: The effect of LAB liquids with the highest LAB values on the fermentation quality of alfalfa silage at the end of one month storage period.

Groups	pH	NH_3-N/TN	CO_2	LA	AA	PA	BA
Control	5.01^{ab}	50.71^{b}	9.07^{a}	28.41^{b}	13.29^{c}	0.00	5.31^{b}
2D5%STsL	4.75^{b}	40.57^{c}	6.03^{c}	45.89^{a}	18.49^{bc}	0.00	3.10^{c}
2D10%SDsL	4.76^{b}	42.93^{c}	5.42^{c}	35.44^{b}	19.50^{ab}	0.00	3.65^{c}
5D10%SDsL	5.33^{a}	55.07^{a}	7.17^{b}	26.96^{b}	24.25^{ab}	0.00	7.42^{a}
5D5%STsD	5.06^{ab}	53.76^{ab}	6.92^{b}	25.64^{b}	25.31^{a}	0.00	7.51^{a}
SEM	0.066	1.385	0.296	1.946	1.133	-	0.465
p	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01

a-c: Values with different letters in the same column were found to be different ($p<0.01$), **NH_3-N/TN:** Ammonia nitrogen, **CO_2:** Carbondioxide, **LA:** Lactic acid g/kg DM, **AA:** Asetic acid g/kg DM, **PA:** Propionic acid g/kg DM, **BA:** Butyric acid g/kg DM, **2D5%STsL:** lyophilized with TRIS (Ts) after 2 days incubation with 5% sucrose addition, **2D10%SDsL:** lyophilized with DMSO (Ds) after 2 days incubation with 10% sucrose addition, **5D10%SDsL:** DMSO (Ds) additive lyophilized after 5 days of incubation with 10% sucrose addition, **5D5%STsD:** (TRIS (Ts) additive deep freezer after 5 days of incubation with 5% sucrose addition.

Alfalfa silages added with Lactic Acid Bacterial Liquids Stored for Three months

In this study, the values of DM, CA, CP, ADF and NDF were determined as 23.80%, 10.81%, 19.73%, 37.44% and 43.11%, respectively; buffering capacity was determined as 500 meq/kg DM; and WSC content was determined as 76.20 g/kg DM, according to the DM basis of the alfalfa plant used in the preparation of alfalfa silages obtained by adding LAB liquids with the highest LAB values at the end of three months storage period.

The effects of LAB liquids with the highest LAB values on the crude nutrient values of alfalfa silage at the end of three months storage period are presented in Table 3.When Table 3 was examined, it was found that while the differences between the groups in terms of DM, CA, ADF and NDF values of the silages obtained were not statistically significant (p>0.01), the differences between the groups in terms of CP values were statistically significant (p<0.01).

The effect of lactic acid bacteria liquids with the highest LAB values on the fermentation quality of alfalfa silage at the end of three months storage period is presented in Table 4.

When Table 4 is examined, while the differences between the groups in terms of CO_2 and AA values of the silages obtained were not statistically significant (p>0.01), the differences between the groups in terms of pH, NH_3-N/TN, LA and BA values were found to be statistically significant (p<0.01).

Table 3: The effect of LAB liquids with the highest LAB values on crude nutrient values of alfalfa silage at the end of three months storage period.

Groups	DM	CA	CP	ADF	NDF
Control	23.78	11.56	20.07[a]	28.92	35.07
2D5%STsL	23.49	11.50	19.45[b]	29.79	34.83
2D10%SDsL	23.95	11.17	19.22[b]	31.55	35.57
5D10%SDsL	23.75	11.38	19.53[b]	30.59	34.81
5D5%STsD	23.98	11.41	19.23[b]	30.61	35.87
SEM	0.158	0.052	0.087	0.337	0.321
p	p>0.01	p>0.01	p<0.01	p>0.01	p>0.01

[a-c]: Values with different letters in the same column were found to be different (p<0.01), **DM:** Dry matter %, **CA:** Crude ash (DM%), **CP:** Crude protein (DM%), **ADF:** Acid Detergent Fiber (DM%), **NDF:** Neutral Detergent Fiber (DM%), **2D5%STsL:** lyophilized with TRIS (Ts) after 2 days incubation with 5% sucrose addition, **2D10%SDsL:** lyophilized with DMSO (Ds) after 2 days incubation with 10% sucrose addition, **5D10%SDsL:** DMSO (Ds) additive lyophilized after 5 days of incubation with 10% sucrose addition, **5D5%STsD:** (TRIS (Ts) additive deep freezer after 5 days of incubation with 5% sucrose addition.

Table 4: The effect of LAB liquids with the highest LAB values on the fermentation quality of alfalfa silage at the end of three months storage period.

Groups	pH	NH_3-N/TN	CO_2	LA	AA	PA	BA
Control	5.15[a]	29.66[a]	5.12	14.19[b]	14.25	0.00	4.31[a]
2D5%STsL	4.91[c]	25.40[b]	4.55	26.09[a]	18.50	0.00	2.92[b]
2D10%SDsL	4.92[c]	27.08[ab]	4.84	25.41[a]	16.99	0.00	2.98[b]
5D10%SDsL	4.90[c]	26.82[ab]	4.54	23.68[a]	15.02	0.00	2.95[b]
5D5%STsD	5.03[b]	29.12[a]	4.56	12.67[b]	17.57	0.00	4.12[a]
SEM	0.024	0.440	0.121	1.375	0.665	-	0.415
p	p<0.01	p<0.01	p>0.01	p<0.01	p>0.01	-	p<0.01

[a-c]: Values with different letters in the same column were found to be different (p<0.01), **NH_3-N/TN:** Ammonia nitrogen, **CO_2:** Carbondioxide, **LA:** Lactic acid g/kg DM, **AA:** Asetic acid g/kg DM, **PA:** Propionic acid g/kg DM, **BA:** Butyric acid g/kg DM, **2D5%STsL:** lyophilized with TRIS (Ts) after 2 days incubation with 5% sucrose addition, **2D10%SDsL:** lyophilized with DMSO (Ds) after 2 days incubation with 10% sucrose addition, **5D10%SDsL:** DMSO (Ds) additive lyophilized after 5 days of incubation with 10% sucrose addition, **5D5%STsD:** (TRIS (Ts) additive deep freezer after 5 days of incubation with 5% sucrose addition.

DISCUSSION AND CONCLUSION

At the end of one month storage period, the DM values (18.33%, 18.24% and 18.19%) of the 2D5%STsL, 2D10%SDsL and 5D5%STsD additive groups obtained by adding the LAB liquids with the highest LAB values were not statistically different from the control group, but numerical increases were determined in the treatment groups (Table 1). It is accepted that silages with less than 10-12% DM loss during the ensiling process of the silage material are considered to be fermented in the desired direction, and silages with more than 20% DM loss are considered to have undesired fermentation (Kung 2008). In this study, compared to the control group silage, it was observed that the DM loss of alfalfa plant used as silage material (20.30% DM) was less than 12% in alfalfa silages obtained by adding LAB liquids with the highest LAB values at the end of one month storage period (2D5%STsL, 2D10%SDsL and 5D5%STsD). At the end of the three-month storage period, the DM values (Table 3) of the additive groups prepared by adding LAB liquids with the highest LAB values (2D5%SDsL, 5D5%STsL, 5D10%STsL and 5D10%STsD) were determined as 23.49%, 23.95%, 23.75% and 23.98%, respectively, and there was no statistical difference with the value obtained from the control group (23.78%). At the end of the three-month storage period of this study, it was observed that there was generally no DM loss of the alfalfa plant (23.80% DM) used as silage material in the control and all additive silages prepared at the end of the three-month storage period. In this study, among the alfalfa silages obtained by adding LAB liquids with the highest LAB values at the end of one month storage period, the numerical increase in DM values (18.33% and 18.24%) in the 2D5%STsL and 2D10%SDsL additive groups and the low pH and butyric acid values and high lactic acid values in these groups suggest that the activity of homofermentative LAB was higher in these groups. At the end of one month storage period, the CP value of alfalfa plant used in silage preparation was determined as 23.70% DM. Compared to the control silage, there was no statistical difference in CP values (Table 1) at the end of one month storage period, but numerical increases were determined. At the end of the three-month storage period, the CP value of alfalfa plant used in silage preparation was determined as 19.73% DM. There was no statistical difference between the CP values of the additive groups prepared at the end of the three-month storage period and the control group (Table 3). In this study, the high CP value (23.70% DM) of alfalfa plant used as silage material at the end of one month storage period may be due to the fact that the plant was harvested and ensiled at the beginning of the second harvest and flowering when it was still green. At the end of one- and three-months storage period, it was observed that LAB liquids had no effect on ADF and NDF values (Table 1 and Table 3) of alfalfa silages obtained by adding LAB liquids with the highest LAB values. The results obtained in this study in terms of ADF and NDF parameters were consistent with the report that LAB has little or no effect on the cellulose value of silages due to its lack of degradative effect on cell wall elements (Muck 1996).

The pH values of silages are affected by many factors such as the LAB species used as inoculant source, the buffering capacity of the plant, the WSC content, the structure of mycobialflora present in the plant and the process applied in the preparation of the silage. When the pH values of alfalfa silages prepared with the addition of LAB liquids with the highest LAB values at the end of one month storage period were analyzed, the values obtained from the 2D5%STsL and 2D10%SDsL additive silages (4.75 and 4.76) were numerically lower than the value obtained from the control group silage (5.01), while the values obtained from the 5D10%SDsL and 5D5%STsD additive groups (5.33 and 5.06) were similar to the control group (Table 2). When the pH values of alfalfa silages prepared with the addition of LAB liquids with the highest LAB values at the end of the three-month storage period were analyzed; the values obtained from the additive silages were lower ($p<0.01$) than the value obtained from the control group silage (Table 4).

The pH values (4.75 and 4.76) obtained from the 2D5%STsL and 2D10%SDsL additive groups of the silages prepared at the end of one month storage period were found to be close to Kung and Shaver (2001)'s report that the pH value should be in the range of 4.3-4.7 for quality legume silages. Among the alfalfa silages prepared with the addition of LAB liquids with the highest LAB values at the end of one and three months of storage period, the pH values in the groups with 2D5%STsL and 2D10%SDsL at the end of one month storage period and 2D5%SDsL, 5D5%STsL, 5D10%STsL and 5D10%STsD at the end of three months of storage period were found to be lower than the control and other additive groups, probably because the lactic acid content in these groups was higher than the other groups. The increase in silage pH values with the increase in AA and decrease in LA values can be explained by the fact that acetic acid is a weaker acid than lactic acid (Keleş 2009). When the lactic acid, acetic acid, pH and NH_3-N/TN values of alfalfa silages prepared with the addition of LAB liquids with the highest LAB values at the end of one month storage period of this study were evaluated in general, it was concluded that homofermentative LAB species were more effective in the silo in the 2D5%STsL and 2D10%SDsL groups, and heterofermentative LAB species were more effective in the silo in the 5G%10SDsL and 5D5%STsD groups (Table 2). When lactic acid, acetic acid, pH and NH_3-N/TN values of alfalfa silages prepared with the addition of LAB liquids with the highest LAB values at the end of the three-month storage period of this study were evaluated in general; it is thought that homofermentative LAB species are more effective in the silo in the groups with 2D5%SDsL, 5D5%STsL and 5D10%STsL, and heterofermentative LAB species are more effective in the silo in the group added with 5D10%STsD (Table 4). In this study, the pH values (5.01 and 5.15) of the control silages prepared at the end of one and three months storage period were lower than other studies (Bai et al. 2020; Hu et al. 2020; Li et al. 2020; Yang et al. 2020; Huo et al. 2021; Sun et al. 2021; Wang et al. 2023) and the expected result; this may be due to the number of epiphytic microorganisms carried by the alfalfa plant used in the preparation of silages, its species, vegetation period, withering process and chopping size (Spoelstra and Hindle 1989). In this study, the alfalfa plant used in the preparation of silages was shredded in 3-5 cm length and the plant enzymes released due to this process activated the bacteria that were previously on the plant but not active, especially increasing the LAB population (Lin et al. 1992) and the silage was well compressed.

When the NH_3-N/TN values of alfalfa silages prepared with the addition of LAB liquids with the highest LAB values at the end of one month storage period were examined (Table 2), the values obtained from 2D5%STsL and 2D10%SDsL additive silages (40.57% and 42.93%) were lower ($p<0.01$) than the value obtained from the control group silage (50.71%). At the end of one month storage

period, the NH_3-N/TN value obtained from the alfalfa silages prepared with the addition of LAB liquids with the highest LAB values from the 5G%10SDsL group (55.07%) was higher ($p<0.01$) than the value obtained from the control group silage (50.71%). When the NH_3-N/TN values of the alfalfa silages prepared with the addition of LAB liquids with the highest LAB values at the end of the three-month storage period were examined (Table 4), the value obtained from 2D5%SDsL-added silage (25.40%) was lower ($P<0.01$) than the value obtained from the control group silage (29.66%). These results were found to be consistent with our study in many studies conducted with the addition of fermented LAB liquid to alfalfa plants, which decreased the NH_3-N values of the silages obtained due to the addition of the additive (Bai et al. 2020; Huo et al. 2021; Sun et al. 2021; Li et al. 2022; Na et al. 2022). It has been reported that the addition of homofermentative LAB inoculant generally decreases the silage NH_3-N value, while the inoculation of *L. buchneri*, which is heterofermentative LAB, decreases the number of yeasts and molds in silages and increases NH_3-N production (Kung and Ranjit 2001; Nsereko et al. 2008). During the proteolysis event occurring in the silo, protease enzymes in the plant break down the proteins in the structure of the plant into peptides and amides, especially amino acids and ammonia. As a result, increases in silage NH_3-N values are formed (Yang et al. 2020). The fact that whether the silo is well compressed or not, the ratio of lactic acid production in the silo, and the DM content of the silage plant are closely related to the silage NH_3-N value. Proteolysis decreases due to the increase in lactic acid in the silo (Davies et al. 1998). In this study, the higher lactic acid values (45.89 and 35.44 g/kg DM) in the 2D5%STsL and 2D10%SDsL additive groups (45.89 and 35.44 g/kg DM) compared to the control group (28.41 g/kg DM) among the alfalfa silages prepared by adding LAB liquids with the highest LAB values at the end of one month storage period can be considered as a reason for the low NH_3-N/TN values in the 2D5%STsL and 2D10%SDsL additive groups. Carpintero et al. (1979) reported that silage can be considered as quality silage if the silage NH_3-N/TN value is 11% or less. In this study, when the NH_3-N/TN values (Tables 2 and 4) of the silages prepared at the end of one- and three-months storage period were evaluated in general, the values obtained were found to be much higher than the values reported by Carpintero et al. (1979) and it was concluded that proteolysis occurred intensively in alfalfa silages prepared in this study.

The number of LAB contaminating the plant before harvesting can vary from $1x10^1$cfu/g to $1.0x10^7$cfu/g and there may be differences in the number and types of LAB contaminating the plants to be silaged. It has been reported that the withering process, hot environmental conditions and the period and number of harvestings have an effect on the number of epiphytic LAB in fresh alfalfa material (Lindgren et al. 1985; Lin et al. 1992). In this study, the plant material used in the silages prepared at the end of the one-month storage period was the second form and was prepared from alfalfa harvested in the period when the air and environmental temperature had not yet risen (May). The plant material used in the silages prepared at the end of the three-month storage period was the fifth form and was prepared from alfalfa harvested during the period when the air and environmental temperature was at its highest (July), and the silage plant was withered before silage preparation in both periods. It is reported that in order for LAB inoculants added to the silage to be effective, the dose value used should be higher than the number of natural LAB in the structure of the plant to be silaged (Pahlow and Honig 1986). The reason why the pH and NH_3-N values of the silages prepared with the addition of LAB liquids stored for one and three months in this study did not reach the expected and desired results may be thought to be due to the fact that the number of epiphytic LAB on the alfalfa plant used as silage material was more than 10^5cfu/g and the application dose of LAB liquids used in this study was insufficient.

When the lactic acid values (Table 2) of the silages prepared by adding the LAB liquids with the highest LAB values at the end of one month storage period were examined, the LA value (45.89 g/kg DM) obtained from the 2D5%STsL additive group was higher than the value obtained from the control group silage (28.41 g/kg DM) ($p<0.01$). When the lactic acid values (Table 4) of the silages prepared at the end of three months storage period were examined, the values obtained from 2D5%SDsL, 5D5%STsL and 5D10%STsL additive groups (26.09, 25.41 and 23.68 g/kg DM) were higher than the values obtained from control and 5D10%STsD additive silages (14.19 and 12.67 g/kg DM) ($P<0.01$). In this study, lactic acid values of 2D5%STsL and 2D10%SDsL group silages prepared at the end of one month storage period were found to be compatible with the values obtained from some previous studies (Hu et al. 2020; Sun et al. 2021; Huo et al. 2022). It is thought that the increase in lactic acid value in silages prepared by adding fermented LAB liquids may be due to the decrease in silage pH value due to the fermentation of LAB in the silo (Weinberg et al. 1988). In a quality silage, the lactic acid ratio should be 65-70% of total silage acids (Kung and Shaver 2001). At the end of one month storage period of this study, while the lactic acid ratios of the control, 5D10%SDsL and 5D5%STsD additive groups in total silage acids (60%, 46% and 44%) were below the specified ratio, the lactic acid ratios of the 2D5%STsL and 2D10%SDsL additive groups in total silage acids (62% and 66%) were found to be close. At the end of the three-month storage period of this study, lactic acid ratios (50%, 58%, 60%, 61% and 42%) in total silage acids of the control, 2D5%SDsL, 5D5%STsL, 5D10%STsL and 5D10%STsD additive groups were below the ratios reported by Kung and Shaver (Kung and Shaver 2001).

When the acetic acid values (Table 2) of alfalfa silages prepared with the addition of LAB liquids with the highest LAB values at the end of one month storage period were examined; the AA values obtained from the 2D10%SDsL, 5D10%SDsL and 5D5%STsD additive groups (19.50, 24.25 and 25.31 g/kg DM) were higher than the value obtained from the control group silage (13.29 g/kg DM) ($p<0.01$). Compared to the control group, the increases in acetic acid values of the silages due to the addition of fermented LAB liquid were found to be compatible with the results obtained from some studies on this subject (Bai et al. 2020; Yang et al. 2020; Drouin et al. 2022). When the acetic acid values of the silages prepared at the end of the three-month storage period (Table 4) were examined, the AA values obtained from the additive groups were found to be similar to the value obtained from the control group, although they increased numerically.

When CO_2 values related to aerobic stability parameter of alfalfa silages prepared by adding LAB liquids with the highest LAB values at the end of one month storage period were examined, CO_2 values obtained from all additive groups were lower than the value obtained from the control group silage (9.07 g/kg DM) ($p<0.01$), and although there was no statistical difference in the silages

prepared at the end of three months storage period, a numerical decrease was determined (Table 2, Table 4). There is a direct relationship between silage acetic acid value and aerobic stability values of silage. Acetic acid has an inhibitory effect against microorganisms that cause silage spoilage after the silage is opened and prevents the growth and activity of yeasts (Taylor et al. 2002; Danner et al. 2003). It was observed that the aerobic stability value of silage increased due to the increase in the amount of acetic acid produced by heterofermentative LAB and the aerobic degradation time of silage were prolonged during the feeding process (Kung and Ranjit 2001). When acetic acid and CO_2 values related to aerobic stability parameters of alfalfa silages prepared with the addition of LAB liquids with the highest LAB values at the end of the three-month storage period were examined; acetic acid values obtained from all additive groups increased numerically although there was no statistical difference from the values obtained from the control group silages, while CO_2 values decreased numerically although there was no statistical difference from the value obtained from the control group silage (5.12 g/kg DM) (Table 4). The effect of acetic acid on reducing CO_2 production, i.e. increasing aerobic stability values, was observed significantly in the silages prepared at the end of three months storage period. Heterofermentative LAB increases the production of acetic acid due to the increase in the amount of WSC in the silo. For this reason, lactic:acetic acid ratio in silages varies according to the content of fermentable WSC. In the present study, the DM value of alfalfa plant used for the silages prepared at the end of one month storage period (20.30%) was lower than that of alfalfa plant used for the silages prepared at the end of three months storage period (23.80%). The reason why the CO_2 production values of the control and additive silages prepared at the end of one month of storage were higher than the CO_2 production values of the silages prepared at the end of three months of storage may be due to the fact that the DM value of the alfalfa plant used as silage material was low in the silages prepared at the end of one month storage period.

When the butyric acid values of the silages prepared at the end of one month storage period (Table 2) were examined, the values obtained from 2D5%STsL and 2D10%SDsL additive groups (3.10 and 3.65 g/kg DM) were lower than the value obtained from the control group (5.31 g/kg DM), while the values obtained from 5D10%SDsL and 5D5%STsD additive groups (7.42 and 7.51 g/kg DM) were higher ($p<0.01$). When the butyric acid values of the silages prepared at the end of three months storage period were examined (Table 4), it was determined as 4.31 and 4.12 g/kg DM in the control and 5D10%STsD group silages; compared to the control group, butyric acid values in 2D5%SDsL, 5D5%STsL and 5D10%STsL groups were 2.92, 2.98 and 2.95 g/kg DM lower, respectively ($p<0.01$). The lower butyric acid values obtained from 2D5%STsL and 2D10%SDsL at the end of one month storage period and 2D5%SDsL, 5D5%STsL and 5D10%STsL additive groups at the end of three months storage period compared to the control group can be explained by the low pH values and high lactic acid values in these groups. The fact that butyric acid increased the silage pH value is due to the fact that butyric acid is a weaker acid compared to lactic acid. Clostridial fermentation is reported to occur in silages prepared from silage materials with dry matter content lower than 30-35% (Kendall 1978). In this study, the high CP values (23.70% and 19.73% DM), but low DM (20.30% and 23.80%) and WSC contents (71.10 and 76.20 g/kg DM) of alfalfa plants used as silage material at the end of one- and three-months storage periods are thought to be due to the inadequacy of lactic acid production, which is necessary to inhibit the growth of Clostrodial bacteria (Aydın 2014). In the silages prepared at the end of one and three months of storage period in this study, the low values of the DM and WSC of alfalfa plant used as silage material suggest that saccharolytic Clostridia may have converted the WSC in the plant structure and the organic acids formed in the silage into butyric acid (Mcdonald 1981).

In a quality legume silage, it is preferred to have a DM value of 30-40%, pH value of 4.3-4.7, lactic acid value of 70-80 g/kg DM, acetic acid value of 20-30 g/kg DM, propionic and butyric acid values of 5 g/kg DM, and NH_3-N/TN value of about 10-15% (Kung and Shaver 2001). In this study, DM and lactic acid values of the silages obtained by adding LAB liquids to the silages prepared at the end of one- and three-months storage periods were lower than the specified values and pH values were high. In this study, while the acetic acid values obtained from all of the additive groups obtained by adding LAB liquids to the silages prepared at the end of one month storage period and butyric acid values obtained from 2D5%STsL and 2D10%SDsL groups were found to be compatible with the report of Guo et al. (2020), the acetic and butyric acid values obtained from all of the silages prepared at the end of three months storage period were found to be low.

In the study, the effects on alfalfa silage quality were researched by adding the fermented LAB liquids with the highest LAB values from the frozen and lyophilized LAB liquids for one and three months to the silages prepared from alfalfa plants in four groups for each storage period. At the end of one month storage period, it was observed that CO_2 values decreased, and aerobic stability values increased in alfalfa silages prepared by adding LAB liquids with the highest LAB values. The highest lactic acid content was determined in the group dried by lyophilization process and TRIS (Ts) addition at the end of two days incubation period with 5% sucrose addition (2D5%STsL); the highest acetic acid content was determined in the group dried by lyophilization process and TRIS (Ts) addition at the end of five days incubation period with 5% sucrose addition (5D5%STsD). At the end of the three-month storage period, it was observed that pH values decreased, and lactic acid values increased in alfalfa silages prepared by adding LAB liquids with the highest LAB values. It was observed that the silage fermentation quality was partially improved when the additives obtained by lyophilizing the LAB liquids obtained by incubation at different levels of sucrose and incubation for different periods of time and storage in deep freezer (1 and 3 months) were added to the silages prepared from alfalfa plants. In this study, fermented LAB liquids were generally added to the silages at the dose (10^5cfu/g in fresh silage material) reported for the addition of LAB inoculants. However, considering that alfalfa plants, which were the silage material in this study, had low WSC values and high buffering capacity, the reason why the expected and desired results were not obtained in terms of pH and NH_3-N/TN parameters in this study may be due to the insufficient dose used in the study. It is thought that the additive dose should be higher than 10^5cfu/g to fresh silage material in future studies with legumes.

In this study, considering the high viability rates obtained from the lyophilization and drying of LAB liquids with TRIS (Ts) and DMSO (Ds) cryoprotectants, it is seen that the obtained LAB liquids have a high potential to be used as silage additives and to be commercialized. However, it

was concluded that the lyophilized LAB liquid should be researched in comparison with commercial LAB inoculants in large silos and in silages to be prepared with different silage materials in order to use the application in practice.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENT

This study is summarized from the doctoral thesis of Sadık Serkan AYDIN.

AUTHOR CONTRIBUTIONS

Idea / Concept: SSA, ND
Supervision / Consultancy: SSA, ND
Data Collection and / or Processing: SSA, ND
Analysis and / or Interpretation: SSA, ND
Writing the Article: SSA, ND
Critical Review: SSA, ND

REFERENCES

Ammann P, Pfisterer M, Fehr T, Rickli H (2004). Raised cardiac troponins. *Br Med J,* 328, 1028-1029.

Anas AA, Wiersinga WJ, de Vos AF, van der Poll T (2010). Recent insights into the pathogenesis of bacterial sepsis. *Neth J Med,* 68, 147-152.

AOAC (1990). Official Method of Analysis. XV. Edition. Association of Official Analytical Chemistry, Washington.

AOAC (2005). Official Methods of Analysis. XVIII Edition. Association of Official Analytical Chemiststry, Arlington.

Ashbell G, Weinberg ZG, Azrieli A, Hen Y, Horev, B (1991). A simple system to study the aerobic determination of silages. *Can Agric Eng*, 34, 171-175.

Aydın SS (2014). Farklı Sukroz Seviyeleri ve İnkubasyon Sürelerinde Hazırlanan Fermente Edilmiş Doğal Laktik Asit Sıvısının, Laktik Asit Bakterileriile Yonca Silajı Kalitesine Etkisi. Yüksek Lisans tezi, Harran Üniversitesi, Sağlık Bilimleri Enstitüsü, Şanlıurfa, Türkiye.

Bai J, Xu D, Xie D at al. (2020). Effects of antibacterial peptide-producing Bacillus subtilis and Lactobacillus buchneri on fermentation, aerobic stability, and microbial community of alfalfa silage. *Bioresour Technol*, 315, e123881.

Carpintero CM, Henderson AR, McDonald P (1979). The effect of some pre-treatments on proteolysis during the ensiling of herbage. *Grass Forage Sci*, 34 (4), 311-315.

Danner H, Holzer M, Mayrhuber E, Braun R (2003). Acetic acid increases stability of silage under aerobic conditions. *Appl Environ Microbiol*, 69 (1), 562-567.

Davies DR, Merry RJ, Williams AP et al. (1998). Proteolysis during ensilage of forages varying in soluble sugar content. *J Dairy Sci*, 81 (2), 444-453.

Drouin P, Tremblay J, da Silva ÉB, Apper E (2022). Changes to the microbiome of alfalfa during the growing season and after ensiling with Lentilacto bacillus buchneri and Lentilacto bacillus hilgardii inoculant. *J of Appl Microbiol*, 133 (4), 2331-2347.

DuBois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956). Colorimetric method for determination of sugars and related substances. *Anal Chem*, 28 (3), 350-356.

Guo L, Yao D, Li Det al. (2020). Effects of lactic acid bacteria isolated from rumen fluid and feces of dairy cows on fermentation quality, microbial community, and in vitro digestibility of alfalfa silage. *Front Microbiol*, 10 (2998), 1-11.

Hu Z, Niu H, Tong Q et al. (2020). The microbiota dynamics of alfalfa silage during ensiling and after air exposure, and the metabolomics after air exposure are affected by Lactobacillus casei and cellulase addition. *Front Microbiol*, 11 (519121), 1-17.

Huo W, Wang X, Wei Z et al. (2021). Effect of lactic acid bacteria on the ensiling characteristics and in vitro ruminal fermentation parameters of alfalfa silage. *Ital J Anim Sci*, 20 (1), 623-631.

Huo W, Zhang Y, Zhang L et al. (2022). Effect of lactobacilli inoculation on protein and carbohydrate fractions, ensiling characteristics and bacterial community of alfalfa silage. *Front Microbiol*, 13 (1070175), 1-9.

Keleş G (2009). Homofermantatif ve Heterofermantatif Laktik Asit Bakterilerinin Mısır Silajının Kimyasal Kompozisyonu ile Konya Merinosu Toklularda Performansa Etkileri. Doktora Tezi, Selçuk Üniversitesi, Fen Bilimleri Enstitüsü, Konya, Türkiye.

Kendall NVG (1978). Anormal silages and silage related disease problems. Literature Review on Fermantation of Silage-A Review. In: Grants-In-Aid Committee. National Feed Ingredients Association. West Des Moines, lowa.

Kung JrL, Ranjit NK (2001). The effect of Lactobacillus buchneri and other additives on the fermentation and aerobic stability of barley silage. *J Dairy Sci*, 84 (5), 1149-1155.

Kung L, Shaver R (2001). Interpretation and use of silage fermentation analysis reports. *Focus on Forage*, 3 (13), 1-5.

Li R, Jiang D, Zheng M et al. (2020). Microbial community dynamics during alfalfa silage with or without clostridial fermentation. *Sci Rep*, 10 (1), 1-14.

Li Z, Li F, Xie D et al. (2022). Effects of Bacteriocin-Producing Lactiplantibacillus Plantarum on Fermentation, Dynamics of Bacterial Community, and Their Functional Shifts of Alfalfa Silage with Different Dry Matters. *Ferment*, 8 (12), 690.

Lin C, Bolsen KK, Brent BE, Fung DYC (1992). Epiphytic lactic acid bacteria succession during the pre-ensiling and ensiling periods of alfalfa and maize. *J Appl Bacteriol*, 73 (5), 375-387.

Lindgren S, Pettersson K, Kaspersson A, Jonsson A, Lingvall P (1985). Microbial dynamics during aerobic deterioration of silages. *J Sci Food Agric*, 36 (9), 765-774.

Mcdonald P (1981). The Biochemistry of Silage. Edition, John Wiley & Sons. New York, United States.

McDonald P, Edwards RA, Greenhalgh JFD et al. (2002). Animal Nutrition. VI. Edition. Longman Scientific & Technical, Essex, Longman Scientific & Technical, Essex.

Muck RE (1996). Inoculation of silage and its effects on silage quality. In: Informational Conference with Dairy and Forage Industries. United States.

Na N, Qili M, Wu N et al. (2022). Bacterial community and fermentation quality of ensiling alfalfa with commercial lactic acid bacterial additives. *Front Microbiol*, 789 (836899), 1-11.

Nsereko VL, Smiley BK, Rutherford WM et al. (2008). Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber. *Anim Feed Sci Technol*, 145 (1-4), 122-135.

Pahlow G, Honig H (1986). Wirkungsweise Und Einsatzgrenzen Von Silage Impfkulturen Aus Milchsäurebakterien. *Das Wirtschaftseigene Futter*, 32 (1), 20-35.

Playne MJ, McDonald P (1966). The buffering constituents of herbage and of silage. *J Sci Food Agric*, 17 (6), 264-268.

Polan CE, Stieve DE, Garrett JL (1998). Protein preservation and ruminal degradation of ensiled forage treated with heat, formic acid, ammonia, or microbial inoculant. *J Dairy Sci*, 81 (3), 765-776.

Spoelstra SF, Hindle VA (1989). Influence of wilting on chemical and microbial parameters of grass relevant to ensiling. *Neth J Agri Sci*, 37 (4), 355-364.

Sun L, Jiang Y, Ling Q et al. (2021). Effects of adding pre-fermented fluid prepared from red clover or Lucerne on fermentation quality and in vitro digestibility of red clover and Lucerne silages. *Agriculture*, 11 (5), 454.

Suzuki M, Lund C W (1980). Improved gas-liquid chromatography for simultaneous determination of volatile fatty acids and lactic acid in silage. *J Agric Food Chem*, 28 (5), 1040-1041.

Taylor CC, Ranjit N J, Mills J A, Neylon J M, Kung Jr L (2002). The effect of treating whole-plant barley with Lactobacillus buchneri 40788 on silage fermentation, aerobic stability, and nutritive value for dairy cows. *J Dairy Sci*, 85 (7), 1793-1800.

Van Soest PV, Robertson J B, Lewis BA (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci*, 74 (10), 3583-3597.

Wang Y, Ke W, Lu Q, Zhang G (2023). Effects of Bacillus coagulans and Lactobacillus plantarum on the Fermentation Characteristics, Microbial Community, and Functional Shifts during Alfalfa Silage Fermentation. *Anim*, 13 (5), 932.

Weinberg ZG, Ashbell G, Azrieli A (1988). The effect of applying lactic bacteria at ensilage on the chemical and microbiological composition of vetch, wheat and alfalfa silages. *J Appl Bacteriol*, 64 (1), 1-7.

Yang F, Wang Y, Zhao, S, Wang Y (2020). Lactobacillus plantarum inoculants delay spoilage of high moisture alfalfa silages by regulating bacterial community composition. *Front Microbiol*, 11 (1989), 1-13.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Çak B, Keskin S, Yılmaz O, Demirel AF (2023).** Correspondence Analysis to Visualize the Relationships between Alpha-S1 Casein and Beta-Lactoglobulin Gene Polymorphisms in Norduz Sheep. *Van Vet J*, 34 (2), 163-167. DOI: https://doi.org/10.36483/vanvetj.1287808

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Correspondence Analysis to Visualize the Relationships between Alpha-S1 Casein and Beta-Lactoglobulin Gene Polymorphisms in Norduz Sheep

Bahattin ÇAK[1,*] Sıddık KESKİN[2] Orhan YILMAZ[1] Ahmet Fatih DEMİREL[1]

[1] *Van YuzuncuYil University, Faculty of Veterinary Medicine, Department of Zootechnics, 65080, Van, Türkiye*
[2] *Van YuzuncuYil University, Faculty of Medicine, Department of Biostatistics, 65080, Van, Türkiye*

Received: 26.04.2023 Accepted: 25.07.2023

ABSTRACT Correspondence analysis (CA) is one of the multivariate statistical analysis techniques which examines the relationships between different nominal variable categories in two-way or contingency tables. The aim of this study is to investigate the relationships between Alpha-S1 casein and Beta-lactoglobulin gene polymorphisms in Norduz sheep by the CA method. In this study, genotype frequencies of CSN1S1 and BLG genes in Norduz sheep (n=102) were used as categorical variables. As a statistical method, descriptive statistics of characteristics were presented as count and percent and the chi-square (χ^2) test and CA in this study were performed to explore the relationships among the genotype frequencies of Beta-lactoglobulin and Alpha-S1 casein genes. The results of this study indicated that CA can be contributed to a graphical display for categories of nominal variables. Animal breeders can utilize CA as an analytical technique and graphical representation for categorical data. According to the results of this study, the first and second dimensions jointly accounted for 52.25% of the total inertia and "AA" allele of CSN1S1 has the greatest significance in the first dimension, while "BB" allele of BLG has the greatest significant in the second dimension. Therefore, it would be useful to investigate the effects of βLactoglobulin and αS1-Casein genotypes on various yield traits in larger population of Norduz sheep.

Keywords: *Alpha (S1)-casein, Beta-Lactoglobulin, Correspondence analysis, Norduz sheep.*

ÖZ

Norduz Koyunlarında Alfa-S1 Kazein ve Beta-Laktoglobulin Gen Polimorfizmleri Arasindaki İlişki için Uyum Analizi

Uyum analizi (CA), çok değişkenli istatistik analiz yöntemlerinden biridir ve iki yönlü veya olasılık tablolarındaki nominal değişken kategorileri arasındaki ilişkileri araştırır. Bu çalışmanın amacı Norduz Koyunlarında Alpha-S1 Kazein ve Beta-Laktoglobulin gen polimorfizmleri arasındaki ilişkilerin Uyum Analizi yöntemi ile araştırılmasıdır. Bu çalışmada, Norduz koyunlarında (n=102) CSN1S1 ve BLG genlerine ait genotiplerin frekansları kategorik değişkenler olarak kullanılmıştır. İstatistik yöntem olarak, özelliklerin tanımlayıcı istatistikleri sayı ve yüzde olarak belirtilmiş ve Beta-laktoglobulin ve Alfa-S1 kazein genlerinin genotip frekansları arasındaki ilişkileri araştırmak için ki-kare (χ^2) testi ve Uyum analizi yapılmıştır. Bu çalışmanın sonuçları, Uyum analizinin, nominal değişken kategorileri için grafiksel bir gösterime katkıda bulunabileceğini göstermiştir. Hayvan ıslahçıları, CA'yı kategorik veriler için analitik bir teknik ve grafiksel gösterim olarak kullanabilir. Bu çalışmanın sonuçlarına göre; birinci ve ikinci boyutlar birlikte, toplam varyasyonun (inertia) %52.25'ini açıklamış ve CSN1S1'in "AA" alleli birinci boyutta önemlilik gösterirken, BLG'nin "BB" alleli ise ikinci boyutta önemlilik göstermiştir. Böylece β-Lactoglobulin ve αS1-Casein genotiplerinin çeşitli verim özellikleri üzerindeki etkilerinin daha büyük Norduz koyun popülasyonunda araştırılması faydalı olabilir.

Anahtar Kelimeler: *Alfa(S1)-kazein, Beta-Laktoglobulin, Uyum analizi, Norduz koyunu.*

INTRODUCTION

Sheep breeding is one of the very important sector of livestock production in Turkey. The production system of sheep in Turkey is mainly extensive, intensive and semi-extensive, and more focused on the utilization of grasslands and pasture areas. In addition, there is seen a seasonal movement of sheep.

According to TUIK data for 2021, the number of sheep in Turkey is 45 177 690 heads, while according to TURKVET

✉ *Corresponding author: bahattincak@yyu.edu.tr

data for 2022, the total number of sheep in Van is 2 800 327 heads.

Gürpınar is one of the largest districts of Turkey with a surface area of 4.063 km² within the boundaries of Van province (Ezelhan et al. 2021). Norduz sheep are bred in the Van province Gürpınar District Norduz Region. Norduz sheep are bred on low and high pastures in the Norduz region, which is rich in natural resources and has rough and sloping land, vegetation and water. Norduz sheep are fat-tailed, they have high endurance, survivability and adaptability in the region where they are raised (TAGEM 2009).

For many people, milk is an essential source of nutrients. Compared to horse or donkey milk, ruminant milk typically has less lactose and more casein, fat, vitamins, and minerals. Between ruminants and nonruminants, as well as between several breeding variations of the same species and between individual animals, milk content varies significantly. Additionally, milk has bioactive components that have been shown to have positive effects on health (Kalyankar et al. 2016).

In terms of human nutrition, milk is crucial. Today's dairy sector is focused on producing an expanding variety of milk products, and technological aspects of milk are receiving an increasing amount of attention (Frajman and Dovc 2004). In animal breeding, the genetic polymorphisms of milk proteins are quite interesting (Barillet et al. 2005; Çelik and Özdemir 2006). The selection of dairy cattle is focused primarily on the improvement of yield and composition of milk (Dybus et al. 2002). Classical selection methods require a long time, intensive labor, and high cost, and provide slow genetic progress. However, the developments in the field of molecular genetics in the last 20-30 years have made it possible to benefit from genetic markers that show a high correlation with the yield trait emphasized, as well as enable the identification of high-yielding breeders at young ages and regardless of gender (Kabasakal et al. 2015).

In recent years, studies investigating the relationships between molecular markers and polymorphisms in these markers and different yield traits have gained importance in improving the population in terms of important yield traits in animal husbandry (Čítek et al. 2006). Known as the main protein of milk, caseins are the part that does not precipitate after the reaction of milk with acid (Yardibi 2008). They are produced by mammalian epithelial cells (Gaiaschi et al. 2001).

Milk protein includes components that have several different and featured protein combinations. Especially complex casein is known as the main fraction of milk proteins. Casein is easily separated by precipitation with acid. The rest of the proteins are whey protein or serum proteins. Whey protein dissolving in semi saturated ammonium solution is named alpha-lactalbumin and if it is not dissolving in semimature ammonium then it is named beta-lactoglobuline (β-lg). Caseins, beta-lactoglobulin and alpha-lactalbumin are synthesized in the mammary epithelial cells. Contrary to this, immunoglobulin and serum albumin are absorbed from the blood. (Demirci 1995).

Correspondence analysis (CA) is one of the multivariate statistical analysis methods and explores the relationships among the categories of nominal variables in the two-way or contingency tables. In addition to nominal data, binary or ordinal data can also be analyzed by CA without any distributional assumptions. This method also visualizes the relationships among the categories in two dimensional spaces. Thus, in this study, Alpha-S1 Casein and Beta-lactoglobulin gene polymorphisms were investigated by PCR-RFLP method and the data were analyzed with CA for visualization of the relationships among the genotypes in two dimensional spaces in Norduz sheep.

MATERIAL AND METHODS

Van Yuzuncu Yil University Animal Experiments Local Ethics Committee granted authorization for this work under permit number 2020/05-04.

Data Set

Categorical data used in this research were obtained from the project numbered TSA-2020-8930. The data set used in this study was composed of alpha-S1 casein and Beta-lactoglobulin genotype frequencies in Norduz sheep (n=102).

Statistical Analysis

Descriptive statistics for the studied characteristics were presented as count and percent. Chi-square test and CA were performed to explore the relationships among the genotype frequencies of Beta-lactoglobulin and Alpha-S1 casein genes.

CA is one of the exploratory statistical methods which analyzes simple two-way or contingency tables containing categorical, ordinal, or binary data. The method aims to present the relationships between the categories of variables in two two-dimensional spaces.

The basic algorithm of CA can be summarized as follows (Greenacre and Blasius 2006): Let two variables with I rows and J columns be in a contingency table. n is the sample size and N is the grand total. $\mathbf{P}$ is the correspondence matrix with elements $p_{ij} = n_{ij}/ n$.

Corresponding to each element p_{ij} of $\mathbf{P}$ matrix is a row sum ($p_{i.} = n_{i.} / n$) and column sum $p_{.j} = n_{.j} / n$) denoted by r_i and c_j respectively. r_i and c_j are marginal relative frequencies and called as row and column masses, respectively. Masses play dual roles in CA and serve to center and to normalize the correspondence matrix (Greenacre and Blasius 2006).

The expected relative frequencies of the p_{ij} are $r_i c_j$, provided that the variables in the row and column are independent.

Centering involves calculating differences (p_{ij}- $r_i c_j$) between observed and expected relative frequencies, and normalization involves dividing these differences by the square roots of $r_i c_j$, leading to a matrix of standardized residuals; $s_{ij} = (p_{ij} - r_i c_j) / (r_i c_j)^{1/2}$

In matrix notation, this is written as;

$\mathbf{S} = \mathbf{D}_r^{-1/2}(\mathbf{P} - \mathbf{r}\mathbf{c}^T)\,\mathbf{D}_c^{-1/2}$ (Greenacre and Blasius 2006).

"where $\mathbf{r}$ and $\mathbf{c}$ are vectors of row and column masses, and $\mathbf{D}_r$ and $\mathbf{D}_c$ are diagonal matrices with these masses on the respective diagonals. The sum of squared elements of the matrix of standardized residuals is $\sum_i \sum_j s_{ij}^2$= trace ($\mathbf{S}\mathbf{S}^T$) and called the *total inertia."* Total inertia accounts for total variance in the cross- table.

The relations or association structure of the S matrix can be revealed by Singular Value Decomposition (SVD) as follows (Greenacre and Blasius 2006):

$\mathbf{S} = \mathbf{U}\mathbf{\Sigma}\mathbf{V}^T$

Where $\mathbf{\Sigma}$ is the diagonal matrix with singular values in descending order:

$\sigma_1 \geq \sigma_2 \geq \ldots \geq \sigma_S > 0$ (where S is the rank of matrix $\mathbf{S}$)

The columns of **U** and **V** are called left singular vectors, and right singular vectors, respectively. This is orthonormal [$\mathbf{U}^T\mathbf{U} = \mathbf{V}^T\mathbf{V} = \mathbf{I}$]. "The connection between the SVD and the eigenvalue decomposition can be seen in the following"(Greenacre and Blasius 2006):

$$\mathbf{S}^T\mathbf{S} = \mathbf{V}\boldsymbol{\Sigma}\mathbf{U}^T\mathbf{U}\boldsymbol{\Sigma}\mathbf{V}^T = \mathbf{V}\boldsymbol{\Sigma}^2\mathbf{V}^T = \mathbf{V}\boldsymbol{\Lambda}\mathbf{V}^T$$

$$\mathbf{S}\mathbf{S}^T = \mathbf{U}\boldsymbol{\Sigma}\mathbf{V}^T\mathbf{V}\boldsymbol{\Sigma}\mathbf{U}^T = \mathbf{U}\boldsymbol{\Sigma}^2\mathbf{U}^T = \mathbf{U}\boldsymbol{\Lambda}\mathbf{U}^T$$

The SVD provides all the results we need to make CA visualization. "The principal and standard coordinates can be calculated for the row and column categories" (Greenacre and Blasius 2006):

Principal coordinates of rows: $\mathbf{F} = \mathbf{D}_r^{-1/2}\mathbf{U}\boldsymbol{\Sigma}$

Standard coordinates of rows: $\mathbf{A} = \mathbf{D}_r^{-1/2}\mathbf{U}$

Principal coordinates of columns: $\mathbf{G} = \mathbf{D}_c^{-1/2}\mathbf{V}\boldsymbol{\Sigma}$

Standard coordinates of columns: $\mathbf{B} = \mathbf{D}_c^{-1/2}\mathbf{V}$

The proportion of inertia explained would be $[(\sigma_1^2+\sigma_2^2/\sum_s \sigma_s^2]$ or $[(\lambda_1 + \lambda_1) /\sum_s \lambda_s]$ (Greenacre and Blasius 2006)

For visual representation in CA, maximum number of dimensions is [(I, J) -1]. However, for a simple interpretation, in general, only the first two dimensions are used for graphical visualization of the association among the categories (Beh 2004). Thus, in the study, the configuration of the genotypes is shown in two-dimensional space. All statistical calculations were performed using the SPSS (ver. 20) statistical software, with a 5% significant level for statistical significance.

RESULTS

Allele and Genotype Frequencies of the αS1-Casein and β-Lactoglobulin Gene

A and C allele frequencies of the αS1-Casein gene in Norduz sheep were identified 0.01 and 0.99, respectively. Also, The AA, AC and CC genotype frequencies of the αS1-Casein gene were identified as 0.0, 2.9 and 97.1%, respectively.

A and B allele frequencies of the β-Lactoglobulin gene in Norduz sheep were identified 0.52 and 0.48, respectively. Also, the genotype frequencies of the β-Lactoglobulin gene were identified 17.6% (AA), 69.6% (AB) and 12.7% (BB), respectively. Distribution of alleles for CSN1S1 and BLG genes is shown in Figure 1

The Contingency of Genotype Frequencies of the αS1-Casein and β-Lactoglobulin Gene

The contingency table of the frequencies is shown in Table 1. As shown in Table 1, among the 99 AC categories of CSN1S1, AB of BLG is the most common genotype (69.7%) followed by AA (17.2%), and BB (13.1%). According to the chi-square test, there was no statistically significant relationship between CSN1S1 and BLG variables (p= 0.661). However, this test cannot provide a visual representation of the relationships among the categories of CSN1S1 and BLG. Thus, CA was carried out based on "the analysis of the contingency table" for graphically representing these relative frequencies.

Correspondence Analysis (CA)

The results of the CA analysis are summarized in Table 2. As shown in Table 2, the first and second dimensions jointly accounted for 52.25% of the total inertia. According to CA results, the configuration of the categories is presented in Figure 2. "In Figure 2, the origin on the map corresponds to the centroid of each variable." In the study, "AC" is the most common genotype and located closest to the origin.

In Figure 2, the horizontal axis stands in for dimension 1 and the vertical axis for dimension 2. In Figure 2, we can see that "AA" of CSN1S1 is the component that is most distant from the origin and, hence, has the greatest significance along dimension 1. Similar to dimension 1, dimension 2 shows that "BB" of BLG is the most significant.

Table 1: Contingency tables for genotype frequencies of Beta-lactoglobulin and Alpha-S1 casein genes.

CSN1S1		BLG			Total
		AA	AB	BB	
AA	Count	1	2	0	3
	% within CSN1S1	33.3%	66.7%	0.0%	100.0%
	% within BLG	5.6%	2.8%	0.0%	2.9%
	% of Total	1.0%	2.0%	0.0%	2.9%
AC	Count	17	69	13	99
	% within CSN1S1	17.2%	69.7%	13.1%	100.0%
	% within BLG	94.4%	97.2%	100.0%	97.1%
	% of Total	16.7%	67.6%	12.7%	97.1%
Total	Count	18	71	13	102
	% within CSN1S1	17.6%	69.6%	12.7%	100.0%
	% within BLG	100.0%	100.0%	100.0%	100.0%
	% of Total	17.6%	69.6%	12.7%	100.0%
Chi-Square = 0.829; p = 0.661					

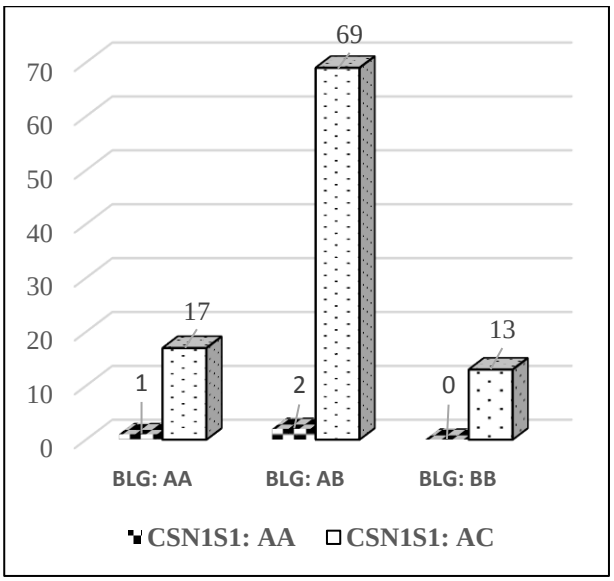


Figure 1: Distribution of alleles for CSN1S1 and BLG genes.

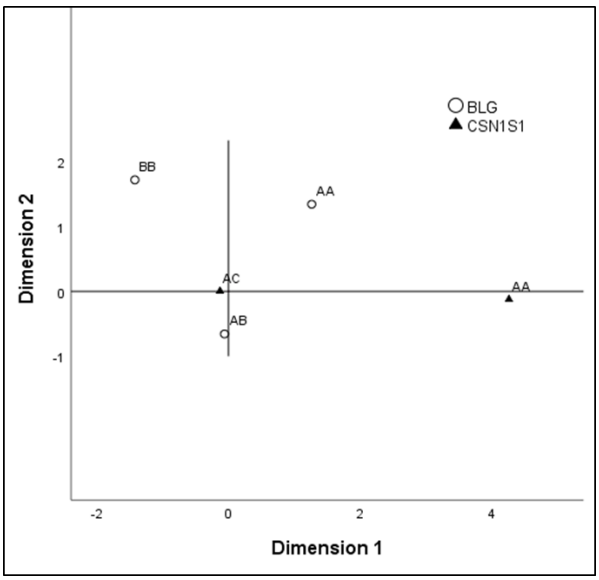


Figure 2: Configuration in two dimensional spaces for genotype frequencies of Beta-lactoglobulin and Alpha-S1 casein genes.

Table 2: Summarized results for CA.

Model Summary				
Dimension	**Cronbach's Alpha**	**Variance Accounted for**		
		Total (Eigenvalue)	**Inertia**	**% of Variance**
1	0.165	1.090	0.545	54.507
2	0.001	1.000	0.500	49.995
Total		2.090	1.045	
Mean	0.086	1.045	0.523	52.251
		Dimension 1	**Dimension 1**	**Mean**
CSN1S1		0.549	0.000	0.275
BLG		0.541	0.999	0.770
Active Total		1.090	1.000	1.045
% of Variance		54.507	49.995	52.251

DISCUSSION AND CONCLUSION

These findings demonstrated that between the "AA" of CSN1S1 and the other genotypes exhibit the most significant difference or biggest divergence from independence. The second most important difference is between "BB" of and the other genotypes. The other genotypes being more closely related to the origin imply that the deviations from the predicted proportions are relatively minimal.

Although distances between categories of CSN1S1 and BLG are not mathematically defined, their degree of ''clustering'' or closeness of points on the map with regard to their angle from the origin and points in the same quadrant can be used as guidelines to interpret relationships between row and column variables.

The clusters allow us to visualize how the BLG and CSN1S1 categories are related. According to the first dimension, it can be stated that AA" of CSN1S1 and BLG cluster on the right side of the map. Thus, it can be stated that "AA" of CSN1S1 is highly and negatively associated with "BB" of BLG. Similarly, when the considering second dimension, "AA" and "BB" of BLG locate the upper side of the map while "AB" locates the lower side. Thus, it can be also noted that "AB" of BLG is negatively associated with AA" and "BB" according to the second dimension.

Hirschfeld introduced the concept of CA in the statistical literature for the first time in 1935 (Hirschfield 1935) however recently has the method begun to rise in favor. Since the analysis is done at the level of the individual answer categories rather than the variable level, this method retains the categorical character of the variables. CA's main objective is to graphically display the most significant links between the variable response categories (Benzécri 1992). The chi-square distance between the response categories is the association metric applied in CA (Clausen 1998). Larger observed proportions do not predominate the distance computation relative to smaller proportions according to the measure's mathematical structure (Nagpaul 1999). Thus, compared to other multivariate methods based on the correlation coefficient (Hill 1974), for which no such standardization is carried out, CA offers a more accurate measure of association. As mentioned by Sourial et al. (2010) CA is a flexible method in terms of underlying distributional assumptions, therefore this method can be used for the data set which consisted of categorical, binary, or ordinal variables.

As a result of this study, it was determined that the A allele of the β-Lactoglobulin gene and the AB genotype were more common, the C allele was absent, the C allele and the CC genotype of the αS1-Casein gene were more common, and the AA genotype was absent in Norduz sheep. Thus, according to the results of this study, it would be useful to investigate the effects of β-Lactoglobulin and αS1-Casein genotypes on various yield traits in larger a population of Norduz sheep. In addition, it can be stated that CA can be contributed to graphical display for categories of nominal variables. Thus, Animal Breeders can utilize CA as an analytical technique and graphical representation for categorical data.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENT

The authors would like to thank to Scientific Research Projects Coordination Unit of Van Yuzuncu Yil University (the project number TSA-2020-8930) for financial supporting.

AUTHOR CONTRIBUTIONS

Idea / Concept: BÇ, SK
Supervision / Consultancy: BÇ, SK, OY, AFD
Data Collection and / or Processing: BÇ, OY, AFD
Analysis and / or Interpretation: BÇ, SK
Writing the Article: BÇ, SK, OY, AFD
Critical Review: BÇ, SK, OY, AFD

REFERENCES

Barillet F, Arranz JJ, Carta A (2005). Mapping quantitative trait loci for milk production and genetic polymorphisms of milk proteins in dairy sheep. *Genet Sel Evol*, 37 (1), 109-123.

Beh EJ (2004). Simple Correspondence Analysis: A Bibliographic Review. *Int Stat Rev*, 72 (2), 257-284.

Benzécri JP (1992). Correspondence Analysis Handbook. Marcel Dekker, New York.

Çelik Ş, Özdemir S (2006). β-Lactoglobulin Variants in Awassi and Morkaraman Sheep and their Association with the Composition and Rennet Clotting Time of the Milk. *Turkish J Vet Anim Sci*, 30 (6), 539-544.

Čítek J, Panicke L, Řehout V, Procházková H (2006). Study of genetic distances between cattle breeds of central Europe. *Czech J Anim Sci*, 51 (10), 429-436.

Clausen SE (1998). Applied Correspondence Analysis: An Introduction. Sage Publications, Thousand Oaks.

Demirci M (1995). Süt Teknolojisine Giriş. Trakya Üniversitesi Ziraat Fakültesi Yayınları, Tekirdağ.

Dybus A (2002). Associations between Leu/Val polymorphism of growth hormone gene and milk production traits in Black-and-White cattle. *Arch Tierz Dummerstorf,* 45, 421-428.

Ezelhan Ş, Keleş A, and İşler S (2021). Macrofungal biodiversity of Gürpınar (Van) district. *Ant J Bot*, 5 (1), 23-28.

Frajman P, Dovc P (2004). Milk production in the post-genomic era. *Acta Agric Slov*, 84 (2), 109-119.

Gaiaschi A, Beretta B, Poiesi C et al. (2001). Proteolysis of β-casein as a marker of grana padano cheese ripening. *J Dairy Sci*, (84), 60-65.

Greenacre M, Blasius J (2006). Multiple Correspondence Analysis and Related Methods. CRC Press, Boca Raton.

Hill MO (1974). Correspondence analysis: A neglected multivariate method. *Appl Stat*, 23(3), 340-354.

Hirschfield HO (1935). A connection between correlation and contingency. *Proc Camb Phil Soc*, 31, 520-524.

Kabasakal A, Dündar E, Ün C, Seyrek K (2015). Analysis of kappa-casein (κ-casein) gene of associated with milk yield on Turkish Grey cattle breed. *Van Vet J*, 26 (2), 87-91.

Kalyankar SD, Khedkar CD, Patil AM, Deosarkar SS (2016). Milk: Sources and composition. Benjamin C, Paul MF, Fidel T (eds). The Encyclopedia of Food and Health. (pp. 741-747). Elsevier, Amsterdam.

Nagpaul PS (1999). Guide to advanced data analysis using IDAMS software. New Delhi: United Nations Educational, Scientific and Cultural Organization.

Sourial N, Wolfson C, Zhu B et al. (2010). Correspondence analysis is a useful tool to uncover the relationships among categorical variables. *J Clinepidemiol*, 63 (6), 638-646.

TAGEM(2009). Türkiye çiftlik hayvanları genetik kaynakları kataloğu. Tarım ve Orman Bakanlığı, Tarımsal Araştırmalar Genel Müdürlüğü, Ankara.

Yardibi H (2008). Ruminantlarda süt proteinleri ve polimorfizmi. *İstanbul Üniv Vet Fak Derg*, 34 (3), 29-35.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Takcı A, Ekıcı M, Kıvrak MB (2023).** The Effect of Vitamin and Mineral Supplementation in Different Forms on Placenta and Birth Weight and Reproductive Performance in Kangal Sheep. *Van Vet J*, 34(2), 168-173. DOI: https://doi.org/10.36483/vanvetj.1297898

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

The Effect of Vitamin and Mineral Supplementation in Different Forms on Placenta and Birth Weight and Reproductive Performance in Kangal Sheep

Abdurrahman TAKCI[1,*] Mehmet EKİCİ[2] Mehmet Buğra KIVRAK[1]

[1] *Sivas Cumhuriyet University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, 58000, Sivas, Türkiye*
[2] *Sivas Cumhuriyet University, Faculty of Veterinary Medicine, Department of Physiology, 58000, Sivas, Türkiye*

Received: 16.05.2023 Accepted: 13.07.2023

ABSTRACT The objective of this study was to evaluate the out-of-season reproductive performance and lamb birth weight of Kangal ewes orally administered a bolus of vitamin and mineral premix or mineral premix given as injection with at 40-45 days postpartum during the anestrous period. In total, 78 primiparous Kangal ewes with were randomly allocated to three experimental groups. Estrus induction protocol was performed and a ram was introduced to the ewes at the 80th days postpartum. Ewes in the group 1 a dose of 2 mL of injectable mineral solution at 40-45 days before oestrous synchronisation, was administered at once (n = 25). As for group 2, at 40-45 days before oestrous synchronisation, a mineral bolus was given orally once (n = 27). To the ewes in the group 3 a dose of 2 mL of physiological saline was given once to the animals (n = 26) simultaneously with group 1 and group 2. Reproductive parameters such as estrus and pregnancy rates; single, twin, triplet, and multiple pregnancy rates; litter size; embryonic mortality; fecundity; and dystocia rates were evaluated. In addition, the placentas were weighed to evaluate the effect of mineral supplements on placentation. There were no significant differences between the groups in terms of parameters above ($p>0.05$). However, the rate of dystocia was significantly lower in group 2 compared to Groups 1 and 3 ($p<0.05$). In conclusion, as a result, it was determined that slow-releasing boluses could not produce efficacy during the throughout pregnancy.

Keywords: *Mineral, Postpartum, Pregnancy, Reproduction, Sheep.*

ÖZ

Kangal Koyunlarında Farklı Formlarda Verilen Vitamin ve Mineral Desteğinin Plasenta ve Doğum Ağırlığı ve Reprodüktif Performansa Etkisi

Bu çalışmanın amacı, doğumdan sonraki 40-45 günde anöstrus döneminde oral olarak verilen bolus vitamin ve mineral premiks veya enjeksiyonluk olarak uygulanan mineral premiksin Kangal koyunlarının sezon dışı üreme performanslarını ve kuzu doğum ağırlıklarını değerlendirmektir. Toplamda 78 adet primipar Kangal koyunu rastgele üç grubuna ayrıldı. Hayvanlar senkronize edildi ve postpartum 80. günde koç katımı yapıldı. Grup 1'deki koyunlara östrus senkronizasyonundan 40-45 gün önce 2 mL enjekte edilebilir mineral solüsyonu tek doz olarak uygulandı (n=25). Grup 2'ye ise, östrus senkronizasyonundan 40-45 gün önce oral olarak bir kez mineral bolus verildi (n=27). Grup 3'teki koyunlara grup 1 ve grup 2 ile eş zamanlı olarak hayvanlara (n = 26) bir kez 2 mL fizyolojik tuzlu su verildi. Östrus oranları gibi üreme parametreleri; gebelik oranları; tek, ikiz, üçüz ve çoğul gebelik oranları; bir batında yavru sayısı; embriyonik ölüm; doğurganlık; ve güç doğum oranları değerlendirildi. Ek olarak, mineral takviyelerinin plasentasyon üzerindeki etkisini değerlendirmek için plasentalar tartıldı. Yukarıdaki parametreler açısından gruplar arasında anlamlı fark yoktu ($p>0.05$). Ancak, güç doğum oranı Grup 2'de Grup 1 ve Grup 3'e göre anlamlı olarak daha düşüktü ($p<0.05$). Sonuç olarak, uzun salınımlı bolusların tüm gebelik boyunca etkinlik oluşturamadığı belirlendi.

Anahtar Kelimeler: *Gebelik, Koyun, Mineral, Postpartum, Üreme.*

INTRODUCTION

Sheep farming contributes significantly to the economic existence of many small low-input households that form part of their social culture, particularly in developing countries (Kosgey et al. 2006). Sheep are seasonal polyestrous animals that give birth once a year and undergo prolonged anoestrous periods. To improve the economic contribution of sheep, efforts should be made to boost their reproductive efficiency by utilising straightforward and affordable solutions (Asaduzzaman et al. 2021). Reproduction management is the most important factor that determines the sustainability of sheep farms (Sharkey et al. 2001). Although various

✉ *Corresponding author: abdurrahmantakci@cumhuriyet.edu.tr

methods have been used to successfully control reproduction in different regions of the world during several breeding seasons and in numerous breeds of sheep, a management strategy for the most productive reproduction has not yet been identified (Yu et al. 2018). Therefore, studies aimed at increasing reproductive success are needed (Abecia et al. 2012).

Minerals, such as phosphorus (P), calcium (Ca), magnesium (Mg), iodine (I), manganese (Mn), copper (Cu), selenium (Se), and zinc (Zn) are responsible for successful sheep reproduction. Vitamins and minerals are known to be insufficient in pastures where sheep are grazed, which negatively affects their reproductive performance (Garg et al. 2003; Robinson et al. 2006). Trace elements and vitamins may have significant effects on fertility and reproductive performance in sheep (Robinson et al. 2006). Several vitamins (such as A and E), which are essential components of biological processes, including fertility and embryonic development, can improve reproductive performance, reduce oxidative stress from mating and pregnancy, and maintain fertility in sheep (Kamiloğlu et al. 2017). Deficiencies in many vitamins and minerals, such as I, Cu, Mg, Mn, Se, and vitamins A and E cause calm oestrous, anovulation, abortion, and the birth of difficult-to-live lambs (Smith and Sherman 2009). Inadequate intake of trace minerals may impair reproductive function (Hostetler et al. 2003). Microminerals play an important role in the stability of secondary molecules and the intracellular system that protects the cells from free radicals. Since microminerals are components of hormones and changes in the plasma levels of these minerals can affect hormone synthesis and reproduction, microminerals may have an impact on endocrine activity (Kumar et al. 2011). The reproductive performance of sheep fed in pastures, such as oocyte development, oestrous, ovulation, implantation, and embryonic and foetal development processes, is generally insufficient without the reinforcement of premix, block, bolus, and injectable minerals (Vázquez-Armijo et al. 2011).

Vitamin and mineral mixtures are being studied using different synchronisation protocols aimed at increasing the reproductive performance of sheep in and out of season (Awawdeh et al. 2019; Kuru et al. 2020; Robinson et al. 2006). However, no studies have investigated the effectiveness of different mineral and vitamin mixes on reproductive data and lamb parameters in Kangal sheep during the postpartum period. The purpose of this study was to investigate the effect of vitamin-trace element mixtures in injectable and bolus forms on reproductive parameters (oestrous rate, pregnancy rate, number of pregnancies, fertility, and dystocia rate) in Kangal sheep subjected to postpartum oestrous synchronisation during the nonbreeding season.

MATERIAL AND METHODS

Location

The study was carried out in a sheep farm between February-July with the following coordinates: latitude: 39.83371433796894, longitude: 36.34688098838113, and altitude: 1290 m in Sivas Province, Türkiye. During the study, the weather conditions in the current location were as follows: in April, temperature, relative humidity, and rainfall were 12±3 °C, 62%, and 76 mm (annual average 11%), respectively, while in June these data were 20±2 °C, 66%, and 66.62 mm (annual average 9 %), respectively.

Animals and treatment schedule

This study was approved by the Sivas Cumhuriyet University Animal Experiments Ethics Committee (Approval No: 65202830-050.04.04-702 numbered and 10.12.2022 dated).

The material used in this study consisted of 78 healthy primiparous Kangal sheep (2-2.5-year-old). 10 Kangal rams between the ages of 4 and 6, which had proven their fertility were used. At the beginning of the study, the average live weight of Kangal sheep was 53±5 kg and the body condition score (BCS) was between 2.5 and 3.25, while the live weight of the Kangal rams was 102±5 kg and BCS was between 3.0 and 3.5. From the beginning to the end of the study, pasture flora was used as feed. Rams were fed 250 g of barley flakes daily in addition to the grazing pasture.

Kangal sheep breeders have abandoned milk production in recent years. The milk of the sheep constituting the study material was used only to feed the offspring. Estrous stimulation was performed after weaning. In conclusion, the milk yield was not monitored in this study. When the existing maternal ewes reached 40–45 days postpartum, each group was further divided into three groups containing 25, 27, and 26 ewes each. Exogenous mineral applications were performed as described below during the animal division of the groups.

From an injectable mineral mixture (Activate, Alke, Turkey), which included 2.5 mg copper equivalents of copper gluconate, 1.25 mg sodium selenite, 5 mg manganese equivalents of manganese gluconate and 5 mg zinc equivalents of zinc gluconate, a 2 mL dose was administered intramuscularly to maternal ewes in Group 1.

Kangal ewes in Group 2 were administered a bolus (TMR Nutrition Min Vit Sheep Bolus, Biochem Turkey) using a swallowing applicator. The bolus contains the following: vitamin A (5.000.000 IU/gr) 135 mg, vitamin D_3 (5.000.000 IU/gr) 1240 mg, (3b302) cobalt hydroxide carbonate 2.596 mg, vitamin E (50%) 4063 mg, magnesium citrate 12.023 mg, manganese (manganese sulfate) 15.575 mg, 3b202 iodine (calcium iodine) 15.575 mg, carbon oxide 24.339 mg, omega 3 67.720 mg, zinc (zinc oxide) 167.000 mg, dicalcium phosphate 179.000 mg, magnesium oxide 223.800 mg, and beeswax 284.000 mg.

The ewes in Group 3 were injected 2 mL of saline intramuscularly.

When the animals in all groups reached 65–70 days postpartum, they were separated and weaned from their lambs. Complete involution of the udder lasted for 15 days after weaning. An estrus induction protocol was performed to theall ewes in three groups. Induction protocol is displayed in figure 1. Pregnancy was confirmed by ultrasonography twice: 30 days (45th day) and 60 days (75th day) after ram introduction. The births were monitored and recorded. The lambs were weighed after weaning by their mothers for the first half hour. The lambs were monitored for 28 days to determine their viability. Births were considered dystocia if they were delivered in a longer time than required for any reason (foetal or maternal) and only with intervention (Jacobson et al. 2020). During the first 28 days after birth (in the neonatal stage), lochia and body temperature were controlled throughout the day to detect infectious diseases. Lochia, foul odour, serosanguinous discharge, and high fever were considered positive signs of infection.

In the postpartum period, the effects of exogenous mineral supplements on reproductive parameters such as oestrous rate, pregnancy rate, twin pregnancy rate, triplet pregnancy rate, multiple pregnancy rate, lambing rate, embryonic mortality rate, fecundity, and dystocia rate, as well as lamb live birth weight, placental weight, and lamb viability were assessed (Table 1 and Table 2).

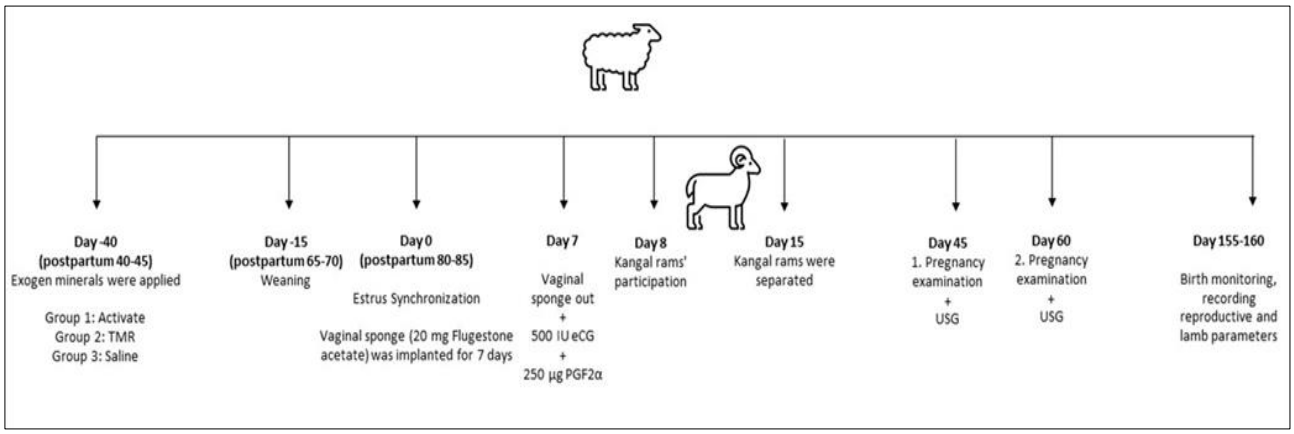


Figure 1: The synchronization protocol and schedule of minerals (Injectible and oral bolus) administered to the ewes (group 1 n=25, group 2 n=27, group 3 n=26).

Table 1: The effect of different treatments on the reproductive performance of Kangal sheep.

Reproductive parameters	Groups			p value
	Group 1 (n = 25)	Group 2 (n = 27)	Group 3 (n = 26)	
Estrous rate	21 (84 %)	23 (85.2 %)	18 (69.2 %)	> 0.05
Pregnancy rate	17 (68 %)	19 (70.4 %)	15 (61.5 %)	> 0.05
Twin pregnancy rate	8 (32 %)	11 (40.7 %)	7 (26.9 %)	> 0.05
Triplet pregnancy rate	2 (8 %)	2 (7.4 %)	1 (3.8 %)	> 0.05
Multiple pregnancy rate	10 (40 %)	13 (48.1 %)	8 (30.8 %)	> 0.05
Lambing rate	17/17 (1)	19/19 (1)	13/15 (0.87)	> 0.05
Embryonic mortality rate	0 (0 %)	0 (0 %)	2 (7.7 %)	> 0.05
Fecundity rate	29/17 (1.71)	34/19 (1.79)	22/15 (1.47)	> 0.05
Dystocia rate	$5/17^{b}$ (29.4 %)	$2/19^{a}$ (10.5 %)	$3/13^{b}$ (23.1 %)	0.018

p<0.05 is statistically significance according to one-way ANOVA and post hoc Duncan test. Data expressed as mean ± SD. Uncalculated: Statistical analysis could not be performed since there was one birth of 3.

Table 2: Effect of bolus and injectable mineral mixture on some lamb birth parameters in Kangal sheep.

Lamb parameters	Groups			p value
	Group 1 (n = 25)	Group 2 (n = 27)	Group 3 (n = 26)	
Lamb weight (single) (kg)	5.02 ± 1.24	4.99 ± 0.98	4.97 ± 0.29	> 0.05
Lamb weight (twin) (kg)	4.16 ± 0.59	4.49 ± 0.15	4.05 ± 0.20	> 0.05
Lamb weight (triplet) (kg)	3.13 ± 0.11	3.94 ± 0.37	uncalculated	uncalculated
Placenta weight (g)	429.14 ± 28.19	420.83 ± 16.10	418.80 ± 18.44	> 0.05

p<0.05 is statistically significance according to one-way ANOVA and post hoc Duncan test. Data expressed as mean±SD. Uncalculated: Statistical analysis could not be performed since there was one birth of 3.

Pregnancy examination

Pregnancy was examined twice: as first pregnancy examination via transrectal ultrasonographic method 1 month (45^{th} day) after participation of the ram, and second pregnancy examination via transabdominal ultrasonographic method 2 months later (75^{th} day). If an animal was pregnant at the first control but not at the second control, early embryonic death was considered. To determine pregnancies and offspring counts in the early period, a B-mode, linear-array ultrasonography device (Mindray DP50/Vet/US) containing a 5.0-7.5-MHz rectal probe was used in the supine position to determine embryonic and fetal losses, or transabdominally in the following days of pregnancy through the rectal route.

Statistical Analysis

Data were analysed using SPSS version 26 (IBM Corp., Armonk, NY, USA). Reproductive parameters (oestrus rate, pregnancy rate, twin pregnancy rate, triplet pregnancy rate, multiple pregnancy rate, number of pregnancies, embryonic mortality rate, fecundity, and dystocia rate) were analysed using the chi-square test. Lamb parameters (Lamb weight of single, twin, and triple, and placental weight) were analysed by one-way ANOVA and post hoc Duncan's test, and the results were expressed as mean±standard deviation (SD). Statistical significance was set at $p<0.05$.

RESULTS

No infection was found in any animal in any group based on measurements of lochia and body temperature taken during the first 5 days following delivery. The first 28 days after birth showed no lamb mortality in any of the three groups.

Table 1 shows the reproductive parameters. There were no significant differences in the estrous rate, pregnancy rate, twin pregnancy rate, triplet pregnancy rate, multiple pregnancy rate, lambing rate, embryonic mortality rate, or fecundity between the groups ($p>0.05$). The rate of dystocia in the sheep in Group 2 treated with TMR was significantly lower than that in Group 1 treated with Activate and the control group treated with saline ($p<0.05$). The parameters related to the lambs are listed in Table 2. There were no significant differences between the groups in terms of lamb and placental weights ($p>0.05$).

DISCUSSION AND CONCLUSION

To the best of our knowledge, no study has investigated the effects of mineral supplementation in different forms (injectable and bolus) on reproductive and lamb parameters before oestrus synchronisation (progesterone-containing sponge + eCG + PGF2α) in postpartum Kangal sheep during the non-breeding season. Previous studies (Karadas 2014; Kivrak et al.2022) have reported that there is a trace element deficiency in the rangelands of Central Anatolia where the present study was conducted. Trace element deficiency has been observed in previous studies on pastures of the Central Anatolia region (Alper and Taşova 2019; Karadas 2014; Kivrak et al.2022). Therefore, neither macro- nor trace-mineral analyses of the rangeland forage were performed in our study.

In the previous study in which oestrous synchronisation was performed using a progestogen sponge and eCG + PGF2α outside of breeding season on Kangal sheep, the oestrous rate as 71.19%, and the pregnancy rate was 31.03% (Gonzalez-Bulnes et al.2020). In our study, we found the oestrus rate to be 84% in the group administered Activate, 85.2% in the group administered TMR, and 69.2% in the control group administered physiological saline. In addition, the pregnancy rate in our study was 68% in the group administered Activate, 70.4% in the group administered TMR, and 61.5% in the group administered saline. Although there was no significant difference, the rate of twin, triplet, and total multiple pregnancies in Kangal ewes in the groups that received TMR and Activate was quantitatively higher than that in the control group. In the TMR group, these values were higher compared to the out-of-season pregnancy rates obtained with different estrous synchronisations, as reported in a previously published meta-analysis (Cizmeci et al. 2022). In a previous study, which also included findings supporting our study, it was reported that the percentage of oestrous and multiple pregnancy rates were quantitatively high, and the pregnancy rate was significantly higher in Pırlak sheep administered a mineral mixture of soft capsules (Toryum) (Kuru et al. 2020). However, in another study, the proportion of twins born from ewes administered the bolus was reported to be significantly higher than that in untreated ewes (Hemingway et al. 2001). Lactation-related changes in the concentrations of macro and trace elements in the blood of sheep during the postpartum period have been reported previously (Antunović et al. 2021). In this study, the blood Ca concentration was lowest in the early lactation period, higher in the late period, and highest in the middle period. As lactation progresses, there is a significant increase in the concentration of Mg, Co, and Cd in the blood and a significant decrease in the concentration of Na, Fe, Cu, Zn, Mo, and Se (Antunović et al. 2021). Trace element deficits have been found to have a deleterious impact on reproductive efficiency in sheep and goat (Vázquez-Armijo et al. 2011). In our study, we compensated for the trace element deficiency in the postpartum period and increased the oestrous and pregnancy rates quantitatively in the non-breeding season.

Trace element supplementation (Cu, Mn, Zn, Fe, Co, and Se) increases lambing rates under deficient conditions, but only Se provides good evidence that embryo development during implantation is impacted (Gürdoğan et al. 2006). In a previous study, ewes receiving a bolus had a higher lambing percentage than those administered Cu or copper oxide injections and the control group (Hemingway et al. 2001). In our study, this was 1 in the Activate group, 1 in the TMR group, and 0.87 in the control group. The quantity of mineral combinations, particularly Se, enhanced the lambing rate.

Foetal mortality, embryonic loss, and embryonic implantation are caused by low levels of Se, Zn, and Cu (Vázquez-Armijo et al. 2011). Previous research has shown the possibility of ruminal boluses with higher bioavailability improving reproductive efficiency, embryo number, embryo quality (Kuru et al. 2020; Mitchell et al. 2007), and multiple births (Hemingway et al. 2001). Research on Kangal sheep demonstrated no embryonic mortality in the control group when progesterone sponge + eCG + PGF2α were used in synchronisation (Cizmeci et al. 2022). In our study, embryonic mortality rates were 0 % in the Activate group, 0 % in the TMR group, and 7.7 % in the control group. Because of the content of the mineral mixture, embryonic mortality was not observed in the Kangal sheep treated in our study.

Most sheep breeds exhibit seasonal polyestrous behaviour; thus, exogenous hormone treatments are required to induce oestrous outside of the breeding season. Increased litter size and financial gain are key goals in this situation. Supplementation of ewes with Se and I before mating can increase fecundity by decreasing perinatal mortality and increasing lambing percentage (Grace and Knowles 2012).

Koyuncu and Yerlikaya (2007) reported that Se (1.31) and Se + Vit E (1.48) supplementation significantly increased the fecundity rate compared to the control group (1.15). Although vitamin E and Se supplementation in Awassi ewes did not cause a statistical difference in fecundity, it was reported to be higher than that in the control group (Awawdeh et al. 2019). In our study, the

fecundity rate was found to be 1.71 in the group receiving Activate, 1.79 in the group receiving TMR, and 1.47 in the control group, which is consistent with the findings of this study.

Dystocia and other reproductive problems have been linked to deficiencies in Ca, Mg, P, Cu, Se, Zn, and Mn, among other minerals (Molefe and Mwanza 2020). Ovarian function can be affected by a change in the Ca:P ratio by inhibiting the pituitary gland, resulting in a prolonged first oestrous and ovulation, delayed uterine involution, increased frequency of dystocia, placental retention, and uterine prolapse (Kumar 2003). In high-yielding dairy cows under heat stress, bolus mineral administration reduced the incidence of dystocia numerically but not statistically. However, the relationship between sheep dystocia and the prevalence of trace mineral deficiencies remains largely unknown. The dystocia rates in our study were as follows: Activate group (29.4%); TMR group (10.5%); and control group (23.1%). This finding suggests that addressing trace mineral imbalance that develops during the postpartum period may decrease the incidence of dystocia.

A previous study reported that the bolus had no effect on lamb birth weights compared to the non-treatment group (Garín et al. 2003). In another study, Zn, Se, and Co as slow-release ruminal bolus supplements in advanced pregnant Mehraban ewes were reported to increase lamb birth weights compared with the non-treatment group (Aliarabi et al. 2019). In contrast, pre-breeding vitamin E and Se injections do not affect the birth weight of lambs of Mehraban sheep (Farahavar et al. 2020). In our study, no statistically significant differences were found between the injectable and bolus mineral mixes in terms of lamb birth weights. This may be related to the duration of the mineral supplementation.

Nutritional imbalances, such as vitamin E and selenium deficiency, have been observed to decrease placental size and reduce foetal development and birth weight in surviving lambs (Freer and Dove 2002). According to a recent study, Se and vitamin E deficiencies may not be severe enough to prevent the placenta from growing normally or reduce the birth weight of lambs (Farahavar et al. 2020). A previous sheep study reported no effect of maternal Se diet on total placental weight (348.8±13.8 g) or cotyledon weight (96.1±4.9 g) (Vonnahme et al. 2010). In our study, there was no difference in the placental weights between the treated and non-treated groups, which is consistent with the results of these studies.

Our study has a few limitations. The injectable mineral combination was first administered as a single dosage. Second, the mineral combinations were tested only during the postpartum period. Oestrous synchronisation and testing at different stages of pregnancy should be conducted in future studies. Third, the number of animals in the study groups should be increased in future studies. Finally, blood will be collected from the animals in the group, and the hormonal and biochemical parameters will be monitored in future studies.

In this study, the effectiveness of injectable and bolus mineral mixes on reproductive and lamb-related parameters before oestrous synchronisation in postpartum Kangal sheep during the non-breeding season was evaluated. In this study, TMR administered as a bolus decreased the rate of dystocia and caused numerical improvements in other reproductive parameters, with Activate as an injectable mineral. Further studies with wider participation are required to determine the effectiveness of these mineral mixes in Kangal sheep.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: AT
Supervision / Consultancy: AT
Data Collection and / or Processing: AT, ME
Analysis and / or Interpretation: MBK
Writing the Article: ME
Critical Review: AT, MBK

REFERENCES

Abecia JA, Forcada F and González-Bulnes A (2012). Hormonal control of reproduction in small ruminants. *Anim Reprod Sci,* 130 (3-4), 173–179.

Aliarabi H, Fadayifar A, Alimohamady R, Dezfoulian AH (2019). The effect of maternal supplementation of zinc, selenium, and cobalt as slow-release ruminal bolus in late pregnancy on some blood metabolites and performance of ewes and their lambs. *Biol Trace Elem Res,* 187, 403–410.

Alper A and Taşova H (2019). İç Anadolu Bölgesi tarım topraklarının bazı verimlilik parametrelerinin belirlenerek haritalanması. *Mediterr Agric* Sci, 32, 1–6.

Antunović Z, Mioč B, Lončarić Z et al. (2021). Changes of macromineral and trace element concentration in the blood of ewes during lactation period. Czech J Anim Sci, 66 (4), 129–136.

Asaduzzaman M, Alam MG, Jha PK, Farida B (2021). On-farm Management, Breeding Practice and Constraints Between Two Sheep Breeds in Bangladesh. *J Anim Prod,* 62 (1), 15–24.

Ataman MB, Akoz M, Akman O (2006). Induction of synchronized oestrus in Akkaraman cross-bred ewes during breeding and anestrus seasons: the use of short-term and long-term progesterone treatments. *Rev Med Vet (Toulouse)*, 157 (5), 257–260.

Awawdeh MS, Eljarah AH, Ababneh MM (2019). Multiple injections of vitamin E and selenium improved the reproductive performance of estrus-synchronized Awassi ewes. *Trop Anim Health Prod*, 51, 1421–1426.

Cizmeci SU, Kivrak MB, Takci A, Dinc DA, Coskun B (2022). Evaluation of hormonal protocols for induction of synchronized estrus on reproductive indices in Kangal-Akkaraman ewes during the outbreeding season. *Small Rumin Res,* 216, 106787.

Farahavar A, Rostami Z, Alipour D, Ahmadi A (2020). The effect of pre-breeding vitamin E and selenium injection on reproductive performance, antioxidant status, and progesterone concentration in estrus-synchronized Mehraban ewes. *Trop Anim Health Prod*, 52 (4), 1779–1786.

Freer M, Dove H (2002). Sheep Nutrition. I. Edition. CAB International, Wallingford.

Garg MR, Bhanderi BM, Sherasia PL (2003). Macro and micro-mineral status of feeds and fodders in Kota district of Rajasthan. *Indian J Anim Nutr*, 20 (3), 252–261.

Garín D, Caja G and Bocquier F (2003). Effects of small ruminal boluses used for electronic identification of lambs on the growth and development of the reticulorumen. *J Anim Sci,* 81 (4), 879–884.

Gonzalez-Bulnes A, Menchaca A, Martin GB, Martinez-Ros P (2020). Seventy years of progestagen treatments for management of the sheep oestrous cycle: Where we are and where we should go. *Reprod Fertil Dev,* 32 (5), 441–452.

Grace ND and Knowles SO (2012). Trace element supplementation of livestock in New Zealand: meeting the challenges of free-range grazing systems. *Vet Med Int,* 2012, 1-8.

Gürdoğan F, Yildiz A, Balikci E (2006). Investigation of serum Cu, Zn, Fe and Se concentrations during pregnancy (60, 100 and 150 days) and after parturition (45 days) in single and twin pregnant sheep. *Turkish J Vet Anim Sci*, 30 (1), 61–64.

Hemingway RG, Parkins JJ, Ritchie NS (2001). Enhanced reproductive performance of ewes given a sustained-release multi-trace element/vitamin ruminal bolus. *Small Rumin Res*, 39 (1), 25–30.

Hostetler CE, Kincaid RL, Mirando MA (2003). The role of essential trace elements in embryonic and fetal development in *livestock. Vet J*, 166 (2), 125–139.

Kamiloğlu NN, Kacar C, Güven A, et al. (2017). Changes in lipid peroxidation, glutathione and fertility in tuj sheep after combined administration of vitamin A and E and passive immunization with testosterone antibodies. *Kafkas Univ Vet Fak Derg*, 23 (3), 459–465.

Karadas F (2014). Scientific data on selenium status in Turkey. *Agric Sci,* 5 (2), 87-93.

Kivrak MB, Takci A, Bölükbaş B, Yüksel M (2022). Aşım sezonunda senkronize edilen Kangal ırkı koyunlarda vitamin ve mineral desteğinin gebelik oranları üzerine etkisi. *Eurasian J Vet Sci*, 38 (2), 115–121.

Kosgey IS, Baker RL, Udo HMJ, Van Arendonk JAM (2006). Successes and failures of small ruminant breeding programmes in the tropics: a review. *Small Rumin Res,* 61 (1), 13–28.

Koyuncu M and Yerlikaya H (2007). Effect of selenium-vitamin E injections of ewes on reproduction and growth of their lambs. *S Afr J Anim Sci,* 37 (4), 233–236.

Kumar S 2003. Management of infertility due to mineral deficiency in dairy animals. Proc ICAR Summer Sch "Advance Diagnostic Tech Ther Approaches to Metab Defic Dis Dairy Anim Held IVRI, Izatnagar, UP (15th July to 4th Aug) 128–137.

Kumar S, Pandey AK, AbdulRazzaque WA, Dwivedi DK (2011). Importance of micro minerals in reproductive performance of livestock. *Vet World*, 4 (5), 230.

Kuru M, Kuru BB, Sogukpinar O et al. (2020). Oestrus synchronisation with progesterone-containing sponge and equine chorionic gonadotropin in Pirlak ewes during the non-breeding season: can Toryum improve fertility parameters? *J Vet Res,* 64 (4), 573–579.

Mitchell LM, Robinson JJ, Watt RG et al. (2007). Effects of cobalt/vitamin B12 status in ewes on ovum development and lamb viability at birth. *Reprod Fertil Dev*, 19 (4), 553–562.

Molefe K and Mwanza M (2020). Effects of mineral supplementation on reproductive performance of pregnant cross-breed Bonsmara cows: An experimental study. *Reprod Domest Anim*, 55 (3), 301–308.

Robinson JJ, Ashworth CJ, Rooke JA, Mitchell LM, McEvoy TG (2006). Nutrition and fertility in ruminant livestock. *Anim Feed Sci Technol,* 126 (3-4), 259–276.

Sharkey S, Callan RJ, Mortimer R, Kimberling C (2001). Reproductive techniques in sheep. *Vet Clin North Am Food Anim Pract,* 17 (2), 435–455.

Smith MC and Sherman DM (2009). Goat medicine. John Wiley & Sons.

Vázquez-Armijo JF, Rojo R, López D, et al. (2011). Trace elements in sheep and goats reproduction: a review. *Trop Subtrop Agroecosystems,* 14 (1), 1–13.

Vonnahme KA, Luther JS, Reynolds LP et al. (2010). Impacts of maternal selenium and nutritional level on growth, adiposity, and glucose tolerance in female offspring in sheep. *Domest. Anim. Endocrinol*, 39 (4), 240-248.

Yu XJ, Wang J, Bai YY (2018). Estrous synchronization in ewes: The use of progestogens and prostaglandins. *Acta Agric Scand Sect A—Animal Sci*, 68 (4), 219–230.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Koşal V, Keleş ÖF, Kömüroğlu AU, Başbuğan Y (2023).** Investigation of Reproductive Parameters in Male Geriatric (3 years old) Rats. *Van Vet J*, 34 (2), 174-179. DOI: https://doi.org/10.36483/vanvetj.1301038

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Investigation of Reproductive Parameters in Male Geriatric (3 years old) Rats

Volkan KOŞAL[1,*] Ömer Faruk KELEŞ[2] Ahmet Ufuk KÖMÜROĞLU[3] Yıldıray BAŞBUĞAN[4]

[1] *Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Artificial Insemination, 65080, Van, Türkiye*
[2] *Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Pathology, 65080, Van, Türkiye*
[3] *Van Yuzuncu Yil University, Health Service Vocational School of Higher Education, 65080, Van, Türkiye*
[4] *Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Internal Medicine, 65080, Van, Türkiye*

Received: 23.05.2023 Accepted: 03.07.2023

ABSTRACT Many health problems are seen due to aging. One of these is problems in the reproductive system. Reproductive system problems are caused by lower urinary system symptoms, prostate diseases, low fertility, testicular dysfunction. The aim of this study was to compare reproductive parameters of geriatric (3 years old) and young (3 months old) rats. A 3-year-old rat is equivalent to an average 90-95-year-old human. For this purpose, sperm analysis, testicular and prostate histopathology, testicular oxidative stress parameters were examined in geriatric (3 years old) and young (3 months old) rats. In the analysis results, it was determined that sperm motility ratio decreased ($p<0.001$), abnormal sperm ratio increased ($p<0.001$) and sperm density decreased ($p<0.001$) in the geriatric group. Statistically, oxidative stress parameters MDA, AOPP, T-SH levels increased ($p<0.05$) and CAT level decreased ($p<0.05$) in geriatric group. Histopathologically, degeneration, necrosis and irregular alignments were observed in the tubulus seminiferous contortus in the geriatric group. Hyperplasia and dilatation of the prostate gland were detected in the geriatric group. As a result of this study, it is thought that reproductive performance in geriatric male rats is very low, and the probability of reproduction is very difficult.

Keywords: *Geriatri, Prostate, Rat, Sperm, Testis.*

ÖZ

Geriatrik (3 Yaşlı) Erkek Ratlarda Üreme Parametrelerinin Araştırılması

Yaşlanmaya bağlı olarak pek çok sağlık sorunu görülmektedir. Bu sorunlardan biri de üreme sistemindeki problemlerdir. Üreme sistemi sorunlarına alt üriner sistem semptomları, prostat hastalıkları, düşük dölverimi, testis fonksiyon bozuklukları neden olur. Bu çalışmadaki amaç geriatrik (3 yaş) ve genç (3 aylık) sıçanların üreme parametrelerinin karşılaştırılması oldu. 3 yaşındaki bir rat ortalama 90-95 yaşındaki bir insana denk gelmektedir. Bu amaçla geriatrik (3 yaş) ve genç (3 aylık) ratlarda sperm analizi, testis ve prostat histopatolojisi, testiküler oksidatif stres parametreleri incelendi. Analiz sonuçlarında geriatric grupta sperm motilite oranının düştüğü ($p<0.001$), anormal sperm oranının arttığı ($p<0.001$), sperm yoğunluğunun azaldığı ($p<0.001$) belirlendi. İstatistiksel olarak geriatric grupta oksidatif stress parametrelerinin MDA, AOPP, T-SH seviyelerinin arttığı ($p<0.05$) ve CAT seviyesinin düştüğü ($p<0.05$) tespit edildi. Histopatolojik olarak geriatrik grupta tubulus seminiferous contortuslarda dejenerasyon, nekroz ve düzensiz dizilimler gözlendi. Geriatik grupta prostat bezinde hiperplazi ve dilatasyon tespit edildi. Bu çalışma verileri sonucunda geriatrik erkek ratlarda üreme performansının çok düşük olduğu ve üreme olasılığının çok zor olduğu düşünülmektedir.

Anahtar Kelimeler: *Geriatri, Prostat, Sıçan, Sperm, Testis.*

INTRODUCTION

Aging is a natural process observed in all living species. The age of 65 and over is accepted as geriatric throughout the world. Many health problems are encountered in this process. It can be classified as digestive, respiratory, circulatory, excretory, nervous, musculoskeletal, reproductive systems (Elsawy and Higgins 2011, Kammerlander et al. 2010; Thakur et al. 2013). Reproductive system problems are caused by lower urinary system symptoms, prostate diseases, low fertility and testicular dysfunction (Corona et al. 2010; Auerbach et al. 2012; Donna et al. 2015).

Many changes occur in the testis due to aging; reduction in volume, dysfunction in Sertoli cells, decrease in the number of germ cells, degeneration of Leydig cells, decrease in testosterone production etc. Depending on these changes, decrease in sperm count, increase in

✉ *Corresponding author: volkan_kosal@hotmail.com

abnormal sperm ratio, increase in dead-live sperm ratio, decrease in motility ratio, and increase in sperm DNA damage can be observed (Hu et al. 2013; Lindor 2014; Francisco et al. 2015; Mattigk et al. 2020).

Changes in androgen metabolism and regulation of apoptosis cause enlargement of the prostate gland. This problem, called benign prostatic hyperplasia, is thought to be caused by the more active form of testosterone, dihydrotestosterone (DHT). It is a common problem in older men (Rosette et al. 2001; McVary 2006; Kramer et al. 2006; Raymond et al. 2009).

Reactive oxygen species and free radicals increase with aging. Free radicals have a direct effect on cell growth and development, and from these direct effects on cell life; imbalance in redox homeostasis occurs, the antioxidant enzyme mechanism cannot function adequately, and radical formation increases. ROS attack DNA, protein, lipids and molecules in all structures. It causes cell membrane damage with DNA molecule oxidation, genetic messenger DNA damage, cell division arrest, uncontrolled growth, malignancy, lipid peroxidation (Sabuncuoğlu and Özgüneş 2011; Öğüt and Atay 2012; Tabakoğlu and Durgut 2013; Özcan et al. 2015).

The aim of this study is to compare the changes in reproductive parameters, testicular oxidative stress and testicular histopathology in geriatric and young rats.

MATERIAL AND METHODS

The study was undertaken under agreement no. 2021/12-06 of Van Yuzuncu Yil University Animal Experiments Local Ethics Committee, dated 23/12/2021.

Animals

Adult, pathogen-free, male Albino Wistar rats were obtained from Van Yuzuncu Yil University. Animals were fed ad libitum and kept with 12 hours of light and 12 hours of dark per day. The living areas had an average temperature of 26 °C and 60 % relative humidity.

Groups

We used 16 Albino Wistar rats.

Group 1 (n:8): 3-4 months old animals with an average weight of 150-200 gr (Figure 1 B). Group 2 (n:8): 36 months old animals with an average weight of 400-450 gr (Figure 1 A).

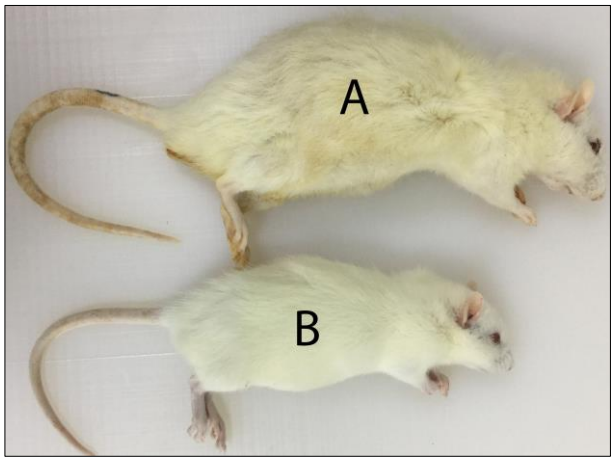


Figure 1: Macroscopic view of young and geriatric rat. A: Macroscopic view of geriatric group rats. B: Macroscopic view of young group rats.

Sperm Examination

Motility examination: The sperm sample was obtained by epididymis puncture immediately after sacrifice and was placed on a glass slide on the heating table set to 38 °C. The coverglass was closed at an angle of 45° and motility (in %) detected by microscopy at 40x magnification (Hafez and Hafez 2016).

Density analysis: After epididymal puncture, 0.1 ml of sperm sample was added to Eppendorf tubes with 0.5 ml Hayem solution (Norateks, Germany). Sperm count per ml was calculated on a Thoma cell counting chamber (Hafez and Hafez 2016).

Abnormal sperm ratio: The sperm obtained by epididymis puncture was transferred to Eppendorf tubes with 0.5 ml Hancock solution (Norateks, Germany). At least 400 sperm samples were examined at 40x magnification to determine the ratio (Hafez and Hafez 2016).

Histopathological Examination

At the end of the experiment, necropsies of the rats were performed, testis and prostate tissue samples were taken, and the observed macroscopic changes were recorded. After the tissue pieces were fixed in 10% buffered formaldehyde solution, routine tissue follow-up was performed and embedded in paraffin blocks and 4 µm sections were taken with a microtome. They were stained with hematoxyleneosin (H&E) and examined under a light microscope, and morphological findings were photographed and evaluated.

MDA, AOPP, T-SH, CAT

Total sulphydryl content (protein and non-protein Thiols) was measured based on the method of Sedlak and Lindsay (1968). Advanced oxidation protein products, AOPP was determined via the method described by Witko-Sarsat et al. (1996). Testis tissue MDA level was measured by the method identified by Ohkava et al. (1979) and MDA level was presented as mmol/gr tissue. CAT activity was spectrophotometrically analyzed at 240 nm according to the Lartillot and Kedziora (1988) method.

Statistical Analysis

SPSS v.20 (Chicago, IL, USA) package program was used for statistical analysis. All data were expressed as mean ± standard deviation. Statistical analyzes of the groups were analyzed statistically using the One-way ANOVA followed by post hoc multiple comparisons (Tukey's test) for comparative analysis between the groups. $P<0.05$ was regarded as statistically significant.

RESULTS

Sperm Examination

Sperm motility and density were significantly decreased in geriatric group, and abnormal spermatozoa rate was significantly increased in geriatric group respectively ($p<0.001$).

Oxidative Stress

Testicular tissue MDA, AOPP and T-SH levels in geriatric rats were significantly higher when compared to testicular tissue of young rats ($p=0.023$, $p=0.000$, $p=0.000$, respectively). Testicular tissue CAT activity was found to be significantly lower in geriatric rats compared to young rats ($p=0.019$).

Histopathology

Testis

Normal histological structure of testicles was observed in the young group (Figure 2 A). In the geriatric group, tubulus seminiferus contortus were observed in an irregular manner, which lost their normal structure widely. It was determined that there was a significant decrease in tubules and spermatogenesis, and primary spermatogonium cells were hyperchromatic-pycnotic. In addition, degenerative-necrotic changes in spermatogonia and lysis were observed in some tubules (Figure 2 B, C). Enlargement of the interstitium was observed as a result of proliferation (fibromuscular hyperplasia) in smooth muscle cells in the intertubular region (Figure 2 D).

Prostate

While the prostates of the young group rats were macroscopically normal (Figure 3 A), the prostates of the geriatric group rats were found to be much larger than normal (Figure 3 B). Normal histological structure was observed in the prostates of young rats (Figure 3 C). In the geriatric group, dilatation of the prostate glands and hyperplasia in the form of papillary extensions towards the lumen were detected in some parts (Figure 3 D).

Table 1: Sperm motiliy, density and abnormal sperm rate of geriatric and young rats.

	Young Group	Geriatric Group	p
Motility (%)	82.5±4.62	26.25±9.16	<0.001
Density (x10^9 /ml)	2.34±0.21	1.06±0.16	<0.001
Abnormal sperm (%)	13.37±3.2	41.12±7.58	<0.001

Table 2: MDA, AOPP and T-SH levels and CAT activity in testicular tissue of geriatric and young rats.

	Young Group	Geriatric Group	p
MDA (nmol/gr tissue)	0.60±0.06	0.71±0.07*	0.023
AOPP (mmol/gr tissue)	16.82±1.44	21.07±1.20*	0.000
T-SH (mmol/gr tissue)	0.45±0.02	0.54±0.03*	0.000
CAT (U/L)	449.29±64.74	358.45±41.91*	0.019

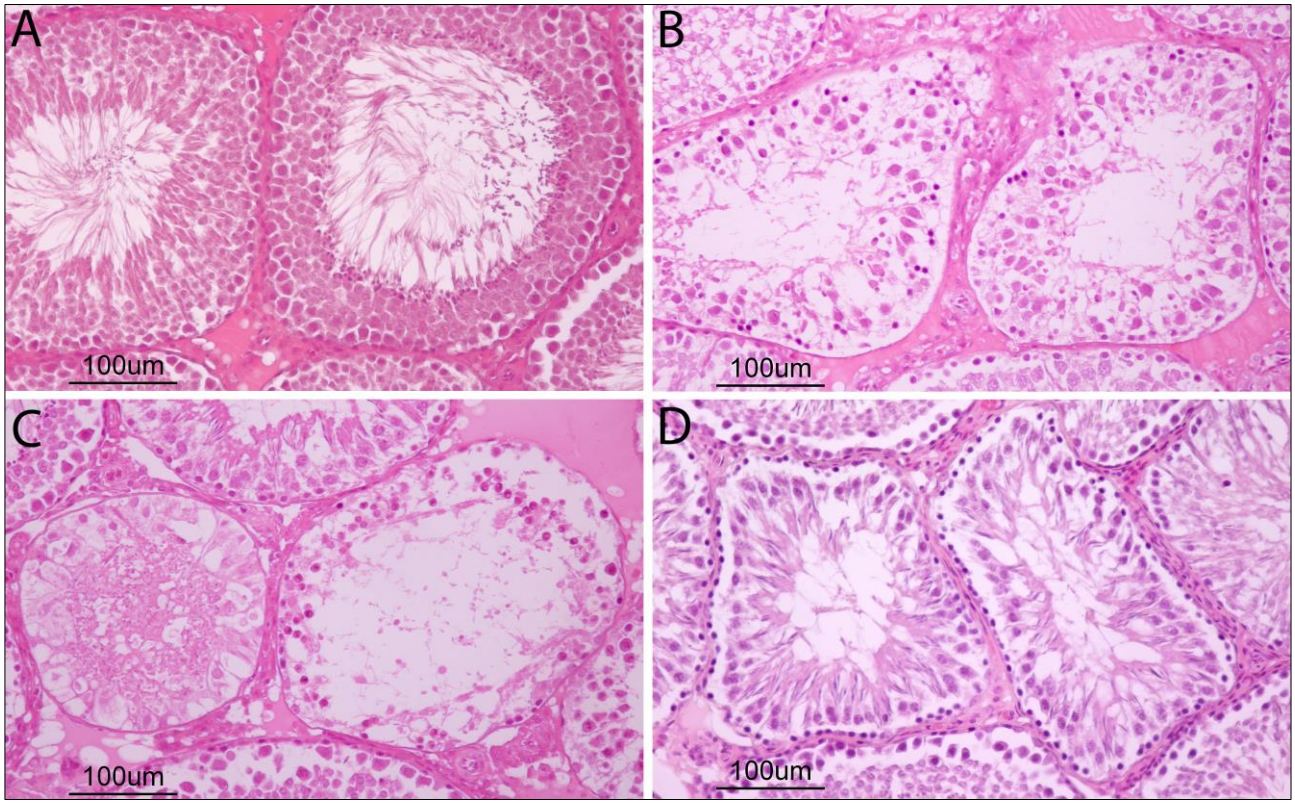


Figure 2: Testis histopatology of geriatric and young rats. A: Young Group: Normal histological appearance of testis. B-C-D: Geriatric Group: Reduction in tubules and spermatogenesis, degenerative-necrotic changes and lysis. Fibromuscular hyperplasia in the intertubular region. H.E., Bar; 100.

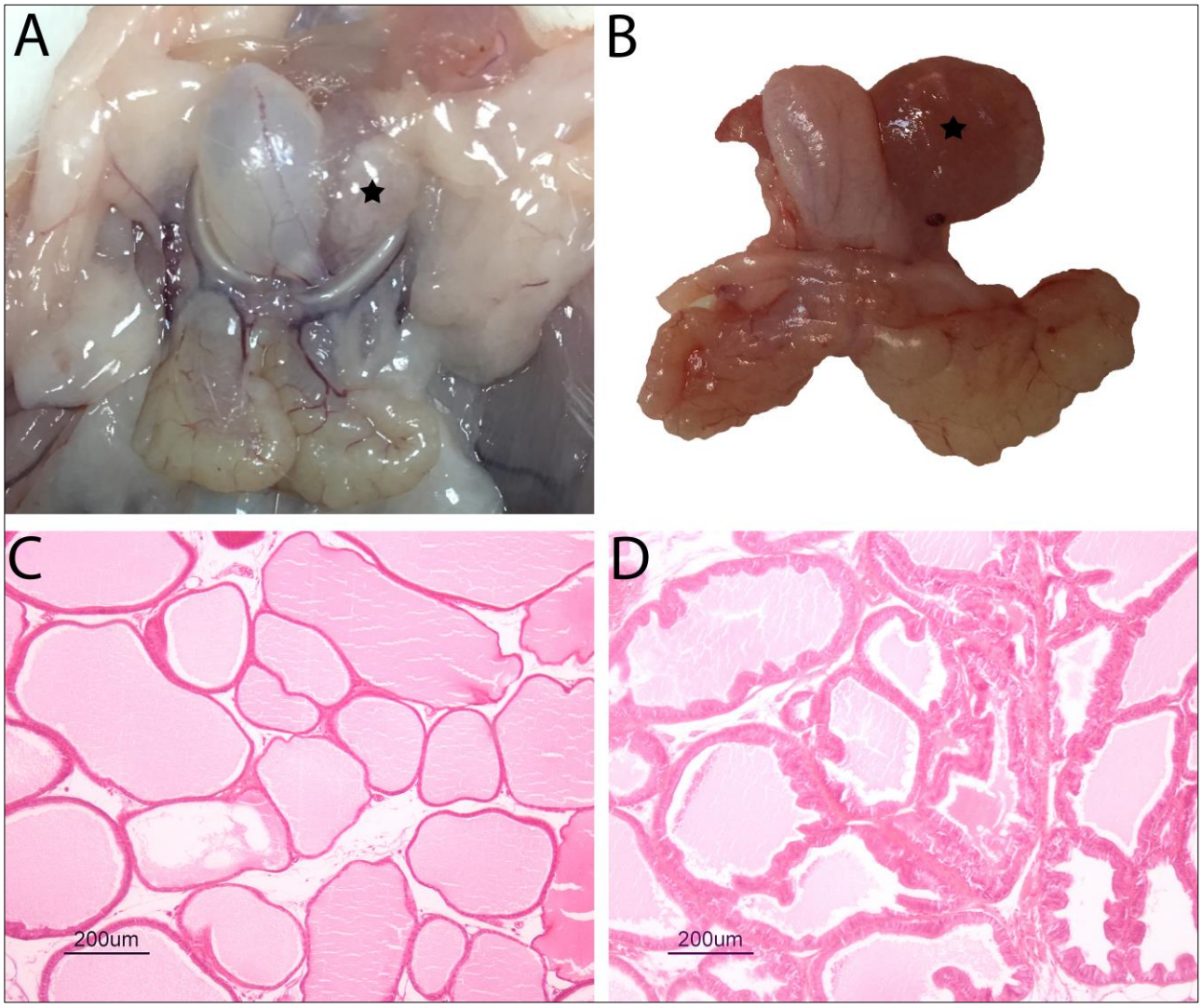


Figure 3: Prostate macroscopy and histopatology of geriatric and young rats. Macroscopy of prostates of young group (A) and Geriatric group (B) rats. C: Normal histological appearance of prostates of young rats, H.E. Bar; 200. D: Prostate hyperplasia of geriatric rats, H.E., Bar; 200.

DISCUSSION AND CONCLUSION

Aging and dying are natural and irreversible processes in all living things. Aging is often associated with a range of diseases and disorders in a healthy lifestyle. One of them is reproductive system problems.

The most important reproductive system problems are fertility problems. Reproductive problems are associated with motility, abnormal sperm and density. In this study, it was determined that motility, abnormal sperm ratio and density were negatively affected from sperm parameters ($p<0.001$). Many researchers stated that they detected a decrease in motility, an increase in the number of abnormal sperm, a decrease in density and an increase in sperm DNA damage (Berry et al. 1989; Haidl et al. 1996; Lucio et al. 2013). The increase in sperm DNA damage is caused by the increase in microdelations in the Y chromosome, telomere length, oxidative stress, gene mutations, DNA methylations and decrease in DNA repair mechanism capacity due to aging (Dong et al. 2022).

Reasons for reproductive system problems; dysfunction in Leydig cells, impaired testicular perfusion, decreased steroid synthesis, hypothalamus and pituitary dysfunction, testicular tubular membrane fibrosis, diverticula formation, decreased number of type A spermatagonia, increased number of atypical and giant spermatogonia, desquamation of immature germ cells, malformed spermatid, degeneration in sertoli cells, increased ROS level etc. (Berry et al. 1989; Gallardo et al. 1996; Haidl et al. 1996; Lucio et al. 2013; Santi et al. 2017). These problems are observed due to aging. In this study, testicular histopathology showed irregular alignment in the tubulus seminiferus contours, decreased spermatogenesis, hyperchromatic and pycnotic primary spermatogonia, degeneration and necrosis in the tubules.

Increase in oxidative stress parameters due to aging Robaire and Hales (2003), Sakamoto (2009), Ingles et al. (2014), Demir et al. (2014) stated by the researchers. In this study, the results of the histopathological examination of the testicles and the oxidative stress parameters (MDA, AOPP, T-SH, CAT) examined in the testicular tissue in the geriatric group are consistent with the negativities in the

sperm parameters. With advancing age, the level of semen ROS increases. ROS oxidizes guanine to 8-hydroxy-20-deoxyguanosine (8OHdG). 80 HdG is one of the most important markers in the capacity of spermatozoa to withstand ROS. The increase in ROS (MDA, SOD, AOPP) levels and 80 HdG oxidation cause lipid peroxidation, DNA damage, enzyme inactivation and protein oxidation, leading to infertility problems (Castleton et al. 2022).

In studies on geriatric male rats, many researchers reported that the prostate gland became hyperplasia after 24 months because of impaired testosterone metabolism. In this study, in which 36-month-old male rats were used, BPH formation due to aging was determined histopathologically. Studies have shown that the amounts of citric acid, spermine, spermidine putrescine, testosterone, protein, fructose, and PGE in the seminal plasma are decreased. These adverse events are directly related to BPH (Rui et al. 1986; Sloter et al. 2006; Smithson et al. 2019; Ross et al. 2019; Sharma et al. 2020).

In studies using geriatric rats, some researchers have stated that reproduction and sexual behaviors can continue in a healthy way. The age of the rats used in these studies was limited to 12-24 months (Berry et al. 1989; Lucio et al. 2013; Muselin et al. 2019; Felipe et al. 2019). When the rats used in this study are 36 months old and the results obtained are compared, it is observed that the probability of healthy reproduction is very low.

As a result of this study, it was determined that there were degenerations in testicular tissue, increase in oxidative stress and BPH due to aging. Due to these reasons, a decrease in sperm motility and density, and an increase in abnormal spermatozoa were detected. For these reasons, it is thought that geriatric males have very low reproductive performance, and the possibility of reproduction is very difficult.

CONFLICTS OF INTEREST

The authors report no conflicts of interest for this study.

AUTHOR CONTRIBUTIONS

Idea / Concept: VK, YB
Supervision / Consultancy: YB
Data Collection and / or Processing: ÖFK, AUK
Analysis and / or Interpretation: AUK, YB
Writing the Article: VK
Critical Review: YB, ÖFK, AUK

REFERENCES

Auerbach DI, Pearson ML, Taylor D et al. (2012). Nurse Practitioners and Sexual and Reproductive Health Services: An Analysis of Supply and Demand. *RAND*, 2 (3), 3.

Castleton PE, Deluao JC, Sharkey DJ, McPherson NO (2022). Measuring reactive oxygen species in semen for Male preconception care: A scientist perspective. *Antioxid,* 11 (2), 264.

Cole JE, Steeil JC, Sarro SJ, Kerns KL, Cartoceti A (2020). Chordoma of the sacrum of an adult naked mole-rat. *J Vet Diagn Invest*, 32 (1), 132-135.

Corona G, Lee DM, Forti G et al. (2010). Age-related changes in general and sexual health in middle-aged and older men: results from the European Male Ageing Study (EMAS). *J Sex Med*, 7 (4), 1362-1380.

de la Rosette JJ, Alivizatos G, Madersbacher S et al. (2001). European Association of Urology. EAU Guidelines on benign prostatic hyperplasia (BPH). *Eur Urol*, 40 (3), 256-264.

Demir M, Ulas T, Tutoglu A et al. (2014). Evaluation of oxidative stress parameters and urinary deoxypyridinoline levels in geriatric patients with osteoporosis. *J Phys Ther Sci*, 26 (9), 1405-1409.

Denno DM, Hoopes AJ, Chandra-Mouli V (2015). Effective strategies to provide adolescent sexual and reproductive health services and to increase demand and community support. *J Adolesc Health*, 56, 22-41.

Dong S, Chen C, Zhang J, Gao Y, Zeng X, Zhang X (2022). Testicular aging, male fertility and beyond. *Front Endocrinol*, 13, 1012119.

Duarte FCK, Hurtig M, Clark A, Simpson J, Srbely JZ (2019). Association between naturally occurring spine osteoarthritis in geriatric rats and neurogenic inflammation within neurosegmentally linked skeletal muscle. *Expl Gerontol*, 118, 31-38.

Dutton M (2020). Selected Veterinary Concerns of Geriatric Rats, Mice, Hamsters, and Gerbils. *Clin Exot Anim*, 23, 525-548.

Elsawy B, Higgins KE (2011). The geriatric assessment. *Am Fam Physician*, 83 (1), 48-56.

Gallardo E, Simón C, Levy M et al. (1996). Effect of age on sperm fertility potential: oocyte donation as a model. *Fertil Steri*l, 66 (2), 260-264.

Hafez ESE, Hafez B (2016). Reproduction in farm animals. Semen Evaluation. 7th ed. Wiley-Blackwell. 365-375.

HaidI G, Jung A, Schill WB (1996). Ageing and sperm function. *Hum Reprod*, 11 (3), 558-560.

Hou G, Lu H, Chen M, Yao H, Zhao H (2014). Oxidative stress participates in age-related changes in rat lumbar intervertebral discs. *Arch Gerontol Geriatr*, 59 (3), 665-669.

Hu Z, Shen WJ, Cortez Y et al. (2013). Hormonal Regulation of MicroRNA Expression in Steroid Producing Cells of the Ovary, Testis and Adrenal Gland. *Plos one*, 8(10), e78040.

I**nglés M, Gambini J, Carnicero JA et al. (2014).** Oxidative Stress Is Related to Frailty, Not to Age or Sex, in a Geriatric Population, Lipid and Protein Oxidation as Biomarkers of Frailty. *J Am Geriatr Soc*, 62, 1324-1328.

Kammerlander C, Roth T, Friedman SM (2010). Ortho-geriatric service a literature review comparing different models. *Osteoporos Int*, 21, 637-646.

Kramer G, Mitteregger D, Marberger M (2007). Is Benign Prostatic Hyperplasia (BPH) an Immune Inflammatory Disease?, *Eur Urol*, 51 (5), 1202-1216.

Lartillot S, Kedziora P, Athias A (1988). Purification and characterization of a new fungal catalase. *Prep Biochem*, 18 (3), 241-246.

Lindor T (2014). Geriatric Urology Springer New York Heidelberg Dordrecht London DOI 10.1007/978-1-4614-9047-0

Lopez-Marambio FA, Hutson JM (2015). The relationship between the testis and tunica vaginalis changes with age. *J Pediatr Surg*, 50 (12), 2075-2077.

Lucio RA, Tlachi-López JL, Eguibar JR, Ågmo A (2013). Sperm count and sperm motility decrease in old rats. *Physiol Behav*, 110-111, 73-79.

Mattigk A, Klein JT, Martini T (2020). Testicular torsion in geriatric 82-year-old man. *Urol Case Rep*, 33, 101258.

McVary KT (2006). BPH: epidemiology and comorbidities. *AJMC*, 12 (5), 122-128.

Muselin F, Gârban Z, Cristina RT et al. (2019). Homeostatic changes of some trace elements in geriatric rats in the condition of oxidative stress induced by aluminum and the beneficial role of resveratrol. *J Trace Elem Med Biol*, 55, 136-142.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95 (2), 351-358.

Öğüt S, Atay E (2012). Yaşlılık ve oksidatif stres. *SDÜ Tıp Fak Der*, 19 (2), 68-74.

Özcan O, Erdal H, Çakırca G, Yönden Z (2015). Oksidatif stres ve hücre içi lipit, protein ve DNA yapıları üzerine etkileri. *JCEI*, 6 (3), 331-336.

Robaire B, Hales BF (2003). Mechanisms of action of cyclophosphamide as a male-mediated developmental toxicant. *Adv Exp Med Biol*, 518, 169-180.

Rosen RC, Wei JT, Althof SE et al. (2009). BPH Registry and Patient Survey Steering Committee. Association of sexual dysfunction with lower urinary tract symptoms of BPH and BPH medical therapies: results from the BPH Registry. *URO*, 73 (3), 562-566.

Ross CN, Adams J, Gonzalez O et al. (2019). Cross-sectional comparison of health-span phenotypes in young versus geriatric marmosets. *Am J Primatol*, 81 (2), e22952.

Rui H, Thomassen Y, Oldereid NB, Purvis K (1986). Accessory sex gland function in normal young (20-25 years) and middle-aged (50-55 years) men. *J Androl*, 7 (2), 93-99.

Sabuncuoğlu S, Özgüneş H (2011). Kemoterapi, Serbest Radikaller ve Oksidatif Stres. *HUJPHARM*, 2, 37-150.

Sakamoto R, Matsubayashi K, Kimura Y et al. (2009). Comprehensive geriatric assessment of elderly highlanders in Qinghai, China, III: Oxidative stress and aging in Tibetan and Han elderly highlanders. *Geriatr Gerontol Int*, 9, 352-358.

Santi D, De Vincentis S, Magnani E, Spaggiari G (2017). Impairment of sperm DNA methylation in male infertility: a meta-analytic study. *Andrology*, 5 (4), 695-703.

Sedlak J, Lindsay RH (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem*, 25 (1), 192-205.

Sharma P, Singh S, Shukla AK (2020). Ayurvedic Preventive Health Measures in Geriatrics Care. *J Ayurveda Integr Med Sci*, 3, 125-131.

Shavlakadze T, McGeachie J, Grounds MD (2010). Delayed but excellent myogenic stem cell response of regenerating geriatric skeletal muscles in mice. *Biogerontology*, 11, 363-376.

Sloter E, Schmid TE, Marchetti F, Eskenazi B, Nat J, Wyrobek AJ (2006). Quantitative effects of male age on sperm motion. *Hum Reprod*, 21 (11), 2868-2875.

Smithson A, Ramos J, Niño E et al. (2019). Characteristics of febrile urinary tract infections in older male adults. *BMC Geriatrics*, 19, 334-344.

Spruijt BM, Meyerson BJ, Höglund U (1989). Aging and sociosexual behavior in the male rat. *Behav Brain Res*, 32, 51-61.

Tabakoğlu E, Durgut R (2013). Oxidatıve Stress in Veterınary Medicine and Effects in Some Important Diseases. *AVKAE Derg*, 3 (1), 69-75.

Thakur RP, Banerjee A, Nikumb VB (2013). Health Problems Among the Elderly: A Cross-Sectional Study. *Ann Med Health Sci Res*, 3, 19-25.

Witko-Sarsat V, Friedlander M, Capeillère-Blandin C et al. (1996). Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int*, 49 (5), 1304-1313.

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Alesawi YAK, Ergün E (2023).** The Sertoli Cell and Blood-Testis Barrier. *Van Vet J*, 34(2), 180-183.
DOI: https://doi.org/10.36483/vanvetj.1252455

ISSN: 2149-3359 **Review** e-ISSN: 2149-8644

The Sertoli Cell and Blood-Testis Barrier

Yahy Abood Kareem ALESAWI[1], Emel ERGÜN[1,*]

[1] *Ankara University, Faculty of Veterinary Medicine, Department of Histology and Embryology, 06070, Ankara, Türkiye*

Received: 18.02.2023 Accepted: 09.06.2023

ABSTRACT The Sertoli cell is a critical somatic cell that initiates the development of testicular morphology and determines important parameters for spermatogenic function. The blood-testis barrier, also known as the Sertoli cell barrier and one of the tightest tissue barriers in the mammalian body, is an immunological barrier to separate post meiotic germ cell antigens from the systemic circulation. Additionally, creating a unique microenvironment for the development of spermatocytes that exceed into the adluminal compartment from the leptotene stage. It restricts the passage of substances such as paracrine factors, electrolytes, hormones, water, and biological molecules to the apical part of the seminiferous tubule. It separates spermatogenic cells from toxic and drug-containing environmentally harmful substances, hormones, and biomolecules in the systemic circulation. This nearly impenetrable barrier prevents proteins, including antibodies, from reaching the spermatogenic cells. It also prevents protein leakage from developing spermatogenic cells and forming an immune response. This review explains Sertoli's functional properties, the testis barrier's molecular structure, the substances involved in the barrier dynamics, and their importance in realizing spermatogenesis.

Keywords: *Blood-testis barrier, Sertoli cell, Testis.*

ÖZ

Sertoli Hücresi ve Kan-Testis Bariyeri

Sertoli hücresi, testis morfolojisinin gelişimini başlatan ve spermatogenik fonksiyon için önemli parametreleri belirleyen kritik bir somatik hücredir. Sertoli hücre bariyeri olarak da bilinen ve memeli vücudundaki en sıkı doku bariyerlerinden biri olan kan testis bariyeri, postmayotik eşey hücre antijenlerini sistemik dolaşımdan ayırmak için immünolojik bir bariyerdir. Ayrıca, leptoten aşamasından adluminal bölmeye geçen spermatositlerin gelişimi için özel bir mikro ortam oluşturur. Su, elektrolitler, iyonlar, hormonlar, parakrin faktörler ve biyolojik moleküller gibi bazı maddelerin Sertoli hücrelerinin arasından apikale doğru geçişini kısıtlar. Spermatogenik hücreleri toksik ve ilaç içeren çevresel zararlı maddelerden, sistemik sirkulasyondaki hormonlar ve biyomoleküllerden ayırır. Neredeyse geçirimsiz olan bu bariyer, antikorlar da dahil olmak üzere proteinlerin spermatogenik hücrelere ulaşmasını engeller. Tersi yönde de gelişmekte olan spermatogenik hücrelerden protein sızmasını ve immun cevabı tetiklemesini engeller. Bu derlemede, Sertoli hücrelerinin fonksiyonel özellikleri ile kan-testis bariyerinin moleküler yapısı, bariyer dinamiklerine dahil olan maddeler ve spermatogenezisin gerçekleşmesindeki önemi ile ilgili bilgiler verilmesi amaçlanmıştır.

Anahtar Kelimeler: *Kan-testis bariyeri, Sertoli hücresi, Testis.*

INTRODUCTION

Sertoli cells, one of the somatic cells of the seminiferous tubules, are cells that start from the basal membrane of the tubule and extend to its lumen, and have numerous apical and lateral extensions. With these extensions, they surround the spermatogenic cells between them. Due to their close contact, they are supportive and nutritive cells for developing germ cells. These cells, which lose their ability to divide when fully specialized, do not reproduce after puberty (Skinner and Griswold 2005).

It has been reported that the formation of the blood barrier in the testis is fully formed in the postnatal period (Mok et al. 2011). The epithelium of the seminiferous is divided into two compartments by this barrier. One is the basal compartment with spermatogonium, and leptotene spermatocytes, the other one is the apical (adluminal) compartment the place of primary and secondary spermatocytes and spermatids are observed. Leptotene spermatocytes must cross this barrier to enter the adluminal compartment. The testis barrier is a structure that goes through “opening” and “closing” cycles to provide this germ cell migration (Skinner and Griswold 2005; Wong and Cheng 2005).

This barrier consists of four different cell connections.

✉ *Corresponding author: emelergun@ankara.edu.tr

These are tight junctions, desmosomes, ectoplasmic specializations, and gap junctions. Tight junctions are an important component of the barrier. It coexists and cofunctions with desmosomes, ectoplasmic specializations, and gap junctions to constitute a specific environment for completing meiosis and the subsequent spermiogenesis (Wang et al. 2022; Zhixiang et al. 2022). Ectoplasmic specializations, tight junctions, and gap junctions connect to actin microfilaments (Young et al. 2009; Mao et al. 2020), while desmosomes are associated with intermediate filaments (Delva et al. 2009; Lie et al. 2011).

Ectoplasmic specializations are a modified kind of adhesion junction observed solely in the testis. These occur in two major regions in the cell. Two types of ectoplasmic specialization have been described, apical and basal. In the basal region, they occur together with other types of junctions (tight junction, gap junction, and desmosome) to structure massive girdle-like junctional complexes between neighboring Sertoli cells (Wu et al. 2021). Unlike the adhesion belts found in other epithelia, here, on the plasma membrane of two adjacent cells, on the cytoplasmic side of the Sertoli cell, there are bundles of actin filaments sandwiched between the flattened, thinned agranular endoplasmic reticulum vesicles that run parallel along the Sertoli cell membrane and adhesion belt (Siu et al. 2003).

Ectoplasmic specializations contain many proteins such as vinculin, afadin, α-actinin, fimbrin, Eps8 (epidermal growth factor receptor pathway substrate eight), cortactin, espin, myosin VIIa, paxillin, palladin, zyxin, testin, and nectin in addition to actin filaments (Qian et al. 2013; Wen et al. 2018). In addition, several kinases (Wong et al. 2005; Lie et al. 2012) and phosphatases (Puri and Walker 2013) have been localized to regions that regulate filaments and junctions. Nectin-2, nectin-3, zyxin, n-cadherin, e-cadherin, axin, and other proteins are found in the apical ectoplasmic specialization. Ectoplasmic specializations in the basal region, along with other components of the junctional complex, are disrupted as cells migrate adluminal compartments of the epithelium. They are involved in barrier disruption and reorganization during the migration of leptotene spermatocytes into the adluminal compartment. Those in the apical region are disrupted as part of the sperm release mechanism, and new ectoplasmic specializations emerge deeper in the epithelium (Siu et al. 2003; Siu and Cheng 2004).

Structures formed between neighboring Sertoli cells and between Sertoli cells and the head of the spermatid form tubulobulbar complexes. It is a specialized adhesion-attachment complex unique to the testis. They are unique plasma membrane specializations (Young et al. 2009; Traweger et al. 2013). It helps germ cells adhere to Sertoli cell, removes excess spermatid cytoplasm, and eliminates ectoplasmic specialization so that it can remodel the Sertoli barrier before spermiation. Between 4 and 24 tubulobulbar complexes can be found in mature sperm. This indicates that it is important in germ cell movement (Magnanti et al. 2001; Siu et al. 2003). The molecular components of tubulobulbar complexes include elements such as Arp2/3 (actin-related protein), NWASP (neural wiskott-aldrich syndrome protein) (Young et al. 2009), paxillin, cofilin (Duo et al. 2013), cortactin (Young et al. 2009), Eps8, and espin. In addition, amphiphysin, dynamin 2, and dynamin 3 (Vaid et al. 2007) and focal adhesion proteins zyxin and vinculin are also present (Young et al. 2009).

The testis barrier prevents the mixture of molecules between the two compartments. This is an essential property of the Sertoli cell. Because these cells secrete several products like androgen-binding protein (ABP) and test in a polarized manner (Skinner and Griswold 2005). ABP specifically binds testosterone (T) and dihydrotestosterone (DHT). It provides to concentrate in the lumen of the tubules. Thus, the continuity of spermatogenesis is ensured. When the blood-testis barrier is dysfunctional, germ cell differentiation and development cease (Skinner and Griswold 2005; Von Engelhardt et al. 2020).

Differentiation of spermatogonium enables the formation of sperm-specific proteins. Since sexual maturity is reached after the development of the immune system, the differentiated sperm cells may be perceived as foreign and lead to an immune response that can damage the germ cells, the formation of anti-sperm antibodies (ASAs), and secondary infertility. The blood-testis barrier blocks any interaction between the developing sperm and the immune system. This barrier prevents the formation of an antibody against spermatogonium in the immune system, in other words, an autoimmune response, by cutting off the contact of spermatogonium, which are highly antigenic, with blood (Wong and Cheng 2005; Luca et al. 2018).

Molecular Structure of the Blood-Testis Barrier

Both tight junction and adhesion junction in the seminiferous epithelium consist of adaptors, signaling molecules, and integral membrane proteins. Integral membrane proteins in tight junctions; tricellulin, occludins, junctional adhesion molecules (JAM), and claudins (Wen et al. 2018).

Occludin is a 60-65 kDa intercellular adhesion molecule. They are involved in controlling the localization of proteins necessary for cell polarization. Therefore, it is thought to be involved in the arrangement of the movement of leptotene spermatocytes across the blood-testis barrier. (Lui et al. 2003a).

Claudins are another tight junction membrane protein and are thought to provide stronger adhesion than occludins (Lui et al. 2003a). In mammals, the claudin family comprises 24 members. There are seven different claudin molecules in the testis. These are claudin-1, -3, -4, -5, -7, -8, -11. Claudin-11 is expressed in both germ cells and the barrier. It plays an essential role in the integrity of the blood-testis barrier and the process of spermatogenesis. It is very important in terms of the microenvironment that must be created for spermatogenesis. Claudin 5 is expressed by cells of Sertoli, spermatogonia, and leptotene spermatocytes (Morrow et al. 2009).

Three different JAM molecules, named JAM-1, JAM-2, and JAM-3, have been identified. JAMs show a different molecular structure from occludin and claudins. Cadherins, integrins, and nectins are integral membrane proteins existing in the ectoplasmic specialization region (Takai and Nakanishi 2003). Desmosomes contain desmoglein-2 and desmocollin-2 as integral membrane proteins. Recent research has also proven that desmosomes in the blood-testis barrier structure regulate the functions of other junctional proteins such as N-cadherin and occludin in regions where basal ectoplasmic specializations and tight junctions are observed (Lie et al. 2011; Mruk and Cheng 2011a).

Gap junctions are an actin-based type of junction responsible for cell communication with each other. They

are formed as a result of the fusion of channels called conexon. Integral membrane proteins formed these channels called connexins. Connexin 43 and connexin 46 are the most studied gap junction proteins in the testis (Lie et al. 2011; Mruk and Cheng 2011a; Mruk and Cheng 2011b; Mruk and Cheng 2011c).

The communication of these proteins, which are attached to the cytoskeleton via different adapters, is regulated by signaling molecules such as kinases and phosphatases (Wong and Cheng 2005).

Adaptors are significant regulatory molecules in testicular junction dynamics. However, it is not possible to definitively define this regulation mechanism. The binding interest to different structural and signaling proteins at the junction sites will likely cause different results. Adaptors share an important function in remodeling the barrier with different proteins such as kinases and phosphatases (Wong et al. 2004; Wong and Cheng 2005; Wong et al. 2005).

Occludin, claudin, and JAM bind to the cytoskeleton via different adaptors such as afadin, zonula occludens (ZO)-1, ZO-2, and ZO-3. In addition, integral membrane proteins such as nectins, cadherins, and integrins are also associated with cytoskeletal networks through various adapters such as afadin, catenins, vinculin, cortactin, and actin (Wong et al. 2004; Wong et al. 2005).

ZO proteins belong to the membrane-associated guanylate kinase (MAGUK) family, which consists of 10 different subfamilies. These proteins also function outside the tight junctions, such as regulating cell growth and proliferation (Spadaro et al. 2012; Traweger et al. 2013; Hervé et al. 2014).

Regulation of Blood-Testis Barrier Dynamics

The blood-testicular barrier must be physically dissolved to allow the passage of leptotene spermatocytes into the adluminal compartment during spermatogenesis. In this process, there are changes in the expression of structural, binding, and signaling proteins, localization, and modifications in the communication of proteins with each other (Wang et al. 2022).

Research in rats has demonstrated that nitric oxide synthase is also a significant regulator of tight junction dynamics of Sertoli cells (Skinner and Griswold 2005).

Sertoli-germ cell coactions also regulate blood-testis barrier dynamics, sending signals to Sertoli cells to ease germ-cell migration (Wong and Cheng 2005).

Roles of Cytokines in Regulating Blood-Testis Barrier Dynamics

The blood-testis barrier restructuring to facilitate germ cell transport is arranged by cytokines like tumor necrosis factor-α and transforming growth factor-β3 (Lui et al. 2003b).

Germ and Sertoli cells secrete cytokines. They play multiple functions in spermatogenesis, such as regulating cell division, differentiation and ensuring cell survival. In addition, cytokines in the seminiferous epithelium have been shown to be crucial for regulating barrier dynamics through their influences on levels of tight junction and adhesion junction, proteases, protease inhibitors, integral membrane proteins, and extracellular matrix proteins. The most studied cytokines known to be involved in testis so far are tumor necrosis factor α (TNF-α) and transforming growth factor-β3 (TGF-β3) (Lui et al. 2003b, Siu et al. 2003).

TNF-α is a cytokine secreted by cells of Sertoli and germ cells; receptors are limited to Sertoli cells. During the in vitro montage of the blood-testis barrier, the amount of TNF-α produced by Sertoli cells was considerably reduced, suggesting that it can regulate barrier function. Although the mechanism by which TNF-α affects the blood-testis barrier in vivo is not known, proteases and protease inhibitors in the seminiferous epithelium have been shown to modulate barrier integrity by affecting homeostasis (Siu et al. 2003).

TGF-β3 exerts similar regulatory effects on the Sertoli cell tight junction barrier with different signaling pathways. TGF-β3 has the highest expression at the onset of puberty in rats. This is because the Sertoli cells produce it. In addition, spermatogonia and early spermatocytes in adult pig and rat testis also secrete this factor (Lui et al. 2003b).

TGF-β3 receptors are predominantly found in Sertoli cells. Studies show that TGF-β3 arranges the testis barrier dynamics in vivo via the p38 MAPK path and modulates the levels of tight junction and adhesion junction-related proteins (Lui et al. 2003b). These observations were confirmed in vivo using rats treated with CdCl2 in vitro as a model (Chen et al. 2018). For example, during CdCl2-induced barrier disruption, a decrease in occludin and ZO-1 levels in the blood-testis barrier region has been shown, whereas TGF-β3 was considerably induced in the testis (Wong et al. 2004). It is important to expand studies on the roles of these two cytokines in the seminiferous epithelium in the future.

Interaction of Proteases and Protease Inhibitors

Proteases are important for the tissue to regain its shape during spermatogenesis. There are a variety of proteases and protease inhibitors in the testis. They are important in the restructuring event, which includes the timely "opening" and "closing" of the blood-testis barrier during spermatogenesis. Proteases can either be directly related to eliminating junctional constituents during the passage of leptotene spermatocytes into the adluminal compartment; or indirectly by activating other molecules such as extracellular matrix components (i.e., collagen) and growth factors (Wong and Cheng 2005).

There are researches displaying that protease and protease inhibitors regulate blood-testis barrier dynamics. These include α2-macroglobulin (α2-MG, a non-specific protease inhibitor), protein C inhibitor (PCI), gelatinases (metalloproteinases), cathepsins, plasminogen activators (PAs), cystatin C (a cysteine protease inhibitor), and tissue inhibitors of metalloproteinases (TIMPs) (Wong and Cheng 2005).

Sertoli Cell Secreted Regulatory Factors

The regulatory factors are crucial for testis development, spermatogenesis, and the control of male fertility. They are factors that affect cellular function and differentiation on a molecular level. For example, while they affect Sertoli cells autocrine, they affect spermatogenic cells, Leydig, and peritubular myoid cells paracrinely. These factors are hormones and growth factors (Skinner and Griswold 2005).

Growth factors are factors that affect the cell cycle and also have effects on cellular functions and cell differentiation (Skinner and Griswold 2005). Sertoli cell-secreted growth factors such as stem cell factor (kit ligand), insulin-like growth factors (IGF-1 and 2), fibroblast growth factor (FGF), glial cell-derived neurotrophic factor, transforming growth factor alpha and beta, neurotropins, and bone morphogenetic protein

4 (Lui et al. 2003b; Skinner and Griswold 2005; Young et al. 2009; Young and Vogl 2012; Parekh et al. 2019; Hohmann and McBeath, 2022). Sertoli cells are endocrine cells that secrete hormones such as estrogen, activin, inhibin, and antimullerian hormone (Marchetti et al. 2003; Siu et al. 2003; Traweger et al. 2013; Von Engelhardt et al. 2020). Sertoli cells also produce ciliary neurotrophic factor (CNTF), erythropoietin, and leukemia inhibitory factor (LIF). First, however, these factors' specific expressions and effects must be evaluated (Magnanti et al. 2001).

In conclusion, Sertoli cell and blood-testicular barrier are critical in spermatogenesis. Infertility may occur if this barrier's structure and function are impaired. We think that this review, which examines the structure of the barrier, which provides an optimal environment by protecting the germ cells against both immunological and environmental effects, may also be a reference for studies on the biology of the barrier and male reproduction.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: YA, EE
Supervision / Consultancy: EE
Writing the Article: YA

REFERENCES

Chen E, Lui WY, Mruk DD et al. (2018). Monitoring the integrity of the blood-testis barrier (BTB): An in vivo assay. Editors: Alves MG, Oliveira PF, Methods in Molecular Biology, Sertoli Cells, 1748, 245-252, Springer Protocols, Humana Pres.

Delva E, Tucker DK, Kowalczyk AP (2009). The desmosome. *Cold Spring Harb Perspect Biol*, 1 (2), a002543.

Du M, Young J, De Asis M et al. (2013). A novel subcellular machine contributes to basal junction remodeling in the seminiferous epithelium. *Biol Reprod*, 88 (3), 1-17.

Hervé JC, Derangeon M, Sarrouilhe D, Bourmeyster N (2014). Influence of the scaffolding protein zonula occludens (ZOs) on membrane channels. *Biochim Biophys Acta*, 1838 (2), 595-604.

Hofmann MC, McBeath E (2022). Sertoli cell-germ cell interactions within the niche: Paracrine and juxtacrine molecular communications. *Front Endocrinol*, 13, 897062.

Lie PP, Cheng CY, Mruk DD (2011). The biology of the desmosome-like junction a versatile anchoring junction and signal transducer in the seminiferous epithelium. *Int Rev Cell Mol Biol*, 286, 223-269.

Lie PP, Mruk DD, Mok KW et al. (2012). Focal adhesion kinase-Tyr407 and Tyr397 exhibit antagonistic effects on blood testis barrier dynamics in the rat. *Proc Natl Acad Sci*, 109 (31), 12562-12567.

Luca G, Baroni T, Arato I et al. (2018). Role of Sertoli cell proteins in immunomodulation. *Protein and Peptide Letters*, 25 (5), 440-445.

Lui WY, Lee WM, Cheng CY (2003a). TGF-betas: Their role in testicular function and Sertoli cell tight junction dynamics. *Int J Androl*, 26 (3), 147-160.

Lui WY, Lee WM, Cheng CY (2003b). Transforming growth factor beta 3 regulates the dynamics of Sertoli cell tight junctions via the p38 mitogen-activated protein kinase pathway. *Biol Reprod*, 68 (5), 1597-1612.

Lui WY, Mruk D, Lee WM, Cheng CY (2003b). Sertoli cell tight junction dynamics: Their regulation during spermatogenesis. *Biol Reprod*, 68 (4), 1087-1097.

Magnanti M, Gandini O, Giuliani L et al. (2001). Erythropoietin expression in primary rat Sertoli and peritubular myoid cells. *Blood*, 98 (9), 2872-2874.

Mao B, Bu T, Mruk D et al. (2020). Modulating the blood-testis barrier towards increasing drug delivery. *Trends in Pharmacological Sciences*, 41 (10), 690-700.

Marchetti C, Hamdane M, Mitchell V et al. (2003). Immunolocalization of inhibin and activin alpha and betaB subunits and expression of corresponding messenger RNAs in the human adult testis. *Biol Reprod*, 68 (1), 230-235.

Mok KW, Mruk DD, Lee WM, Cheng CY (2012). Spermatogonial stem cells alone are not sufficient to re-initiate spermatogenesis in the rat testis following adjudin-induced infertility. *Int J Androl*, 35 (1), 86-101.

Morrow CM, Tyagi G, Simon L et al. (2009). Claudin 5 expression in mouse seminiferous epithelium is dependent upon the transcription factor ets variant 5 and contributes to blood-testis barrier function. *Biol Reprod*, 81 (5), 871-879.

Mruk DD, Cheng CY (2011a). Desmosomes in the testis: Moving into an unchartered territory. *Spermatogenesis*, 1 (1), 47-51.

Mruk DD, Cheng CY (2011b). The myotubularin family of lipid phosphatases in disease and in spermatogenesis. *Biochem J*, 433 (2), 253-62.

Mruk DD, Su L, Cheng CY (2011c). Emerging role for drug transporters at the blood testis barrier. *Trends Pharmacol Sci*, 32 (2), 99-106.

Parekh PA, Garcia TX, Hofmann MC (2019). Regulation of GDNF Expression in Sertoli Cells. *Reproduction*, 157 (3), R95-R107.

Puri P, Walker WH (2013). The tyrosine phosphatase SHP2 regulates Sertoli cell junction complexes. *Biol Reprod*, 88 (3), 1-11.

Qian X, Mruk DD, Wong EW, Lie PP, Cheng CY (2013). Palladin is a regulator of actin filament bundles at the ectoplasmic specialization in adult rat testes. E*ndocrinology*, 154 (5), 1907-1920.

Siu MKY, Cheng CY (2004). Interactions of proteases, protease inhibitors and the beta1 integrin/laminin gamma3 protein complex in the regulation of ectoplasmic specialization dynamics in the rat testis. *Biol Reprod*, 70 (4), 945-964.

Siu MKY, Lee WM, Cheng CY (2003). The interplay of collagen IV, tumor necrosis factor-alpha, gelatinase B (matrix metalloprotease-9), and tissue inhibitor of metalloproteases-1 in the basal lamina regulates Sertoli cell-tight junction dynamics in the rat testis. *Endocrinology*, 144 (1), 371-387.

Siu MKY, Mruk DD, Lee WM, Cheng CY (2003). Adhering junction dynamics in the testis are regulated by an interplay of beta1-integrin and focal adhesion complex-associated proteins. *Endocrinology*, 144 (5), 2141- 2163.

Skinner MK Griswold MD (2005). Sertoli Cell Biology, Elsevier Academic Press. California-USA.

Spadaro D, Tapia R, Pulimeno P, Citi S (2012). The control of gene expression and cell proliferation by the epithelial apical junctional complex. *Essays Biochem*, 53, 83-93.

Takai Y, Nakanishi H (2003). Nectin and afadin: Novel organizers of intercellular junctions. *J Cell Sci*, 116 (1), 17-27.

Traweger A, Toepfer S, Wagner RN, et al. (2013). Beyond cell-cell adhesion: Emerging roles of the tight junction scaffold ZO-2. *Tissue Barriers*, 1 (2), e25039.

Vaid KS, Guttman JA, Babyak N et al. (2007). The role of dynamin 3 in the testis. *J Cell Physiol*, 210 (3), 644-654.

Von Engelhardt W, Breves G, Diener M, Gotthold G (2020). Veteriner Fizyoloji. Çeviri editörü: Hakan Öztürk. Ankara Nobel Tıp Kitapevleri Ltd.Şti, Ankara.

Wang JM, Li ZF, Yang WX (2022). What does androgen receptor signaling pathway in Sertoli cells during normal spermatogenesis tell us? *Front Endocrinol*, 13, 838858.

Wen Q, Tang EI, Li N et al. (2018). Regulation of blood-testis barrier (BTB) Dynamics, role of actin-, and microtubule-based cytoskeletons. Editors: Alves MG, Oliveira PF, Methods in Molecular Biology, Sertoli Cells, 1748, 229-243, Springer Protocols, Humana Pres.

Wong CH, Cheng CY (2005). The blood-testis barrier: Its biology, regulation, and physiological role in spermatogenesis. *Curr Top Dev Biol*, 71, 263-296.

Wong CH, Xia W, Lee NP et al. (2005). Regulation of ectoplasmic specialization dynamics in the seminiferous epithelium by focal adhesion-associated proteins in testosterone-suppressed rat testes. *Endocrinology*, 146 (3), 1192-1204.

Wong, CH, Mruk DD, Lui WY, Cheng CY (2004). Regulation of the blood-testis barrier dynamics in the testis: An in vivo study. *J Cell Sci*, 117 (5), 783-798.

Wu S, Wang L, Tang E, Wang J, Cheng CY (2021). An in vitro assay to monitor Sertoli cell blood-testis barrier (BTB) integrity. Editor: Kursad Turksen. Methods in Molecular Biology, Permeability Barrier, 2367, 207-213, Springer Protocols, Humana Pres.

Young JS, Guttman JA, Vaid KS et al. (2009). Cortactin (CTTN), N-WASP (WASL), and clathrin (CLTC) are present at podosome-like tubulobulbar complexes in the rat testis. *Biol Reprod*, 80 (1), 153-161.

Young JS, Vogl AW (2012). Focal adhesion proteins zyxin and vinculin are co-distributed at tubulobulbar complexes. *Spermatogenesis*, 2 (1), 63-68.

Zhang J, Mruk DD, Cheng CY (2005). Myotubularin phosphoinositide phosphatases, protein phosphatases, and kinases: Their roles in junction dynamics and spermatogenesis. *J Cell Physiol*, 204 (2), 470-483.

Zhixiang M, Yawei L, Jian Z, Bo Z, Jinxing LV (2022). Drug transport across the blood-testis barrier *Am J Transl Res*, 14 (9), 6412-6423.