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ARAŞTIRMA MAKALESİ

"Köyümde Yaşamak İçin Bir Sürü Nedenim Var" Projesine Katılan Hayvancılık İşletmelerinin Genel Yapısı ve Proje Başarı Beklentisine Etkili Faktörler: Tokat Örneği

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Öz

Türkiye'nin coğrafyası, iklimi, ekonomisi ve sosyolojisine oldukça uygun olan koyunculuk, özellikle kırmızı ette ulusal arz güvenliği ve kırsal kalkınma açısından önemlidir. Bu çalışmanın amacı Tokat İl Tarım ve Orman Müdürlüğü tarafından yürütülen "Köyümde Yaşamak İçin Bir Sürü Nedenim Var" isimli bir koyunculuk projesine katılan hayvancılık işletmelerinin genel yapısını belirlemek ve proje başarı beklentisine etkili faktörleri tahmin etmektir. Veriler 2022 yılında 297 yetiştiriciyle yüz yüze yürütülen bir anket çalışmasından elde edilmiştir. Araştırmada ortalama koyun sayısı 180 baş, tarımsal arazi büyüklüğü 38 da, borç miktar ve vadesi 136835TL ve 57 ay, ilk sıradaki maliyet ve gelir kalemleri sırasıyla yem ve kasaplık satışlar olarak bulunmuştur. Proje başarı şansını "yüksek" ve "çok yüksek" görüp olumlu değerlendirenlerin oranı %70'dir. Yetiştiricilerin proje başarı beklentisi ile "sürü büyüklüğü" ($p<0.01$) ve "borçluluk düzeyi" ($p<0.05$) arasında negatif ve anlamlı bir ilişki vardır. Likert ve ikili sınıflandırma ölçeğine göre proje başarı beklentisi ile seçili diğer demografik, mesleki, teknik ve mali değişkenler arasında anlamlı bir ilişki bulunamamış, bağımsız değişkenler ikili lojistik regresyon modelini anlamlı biçimde açıklayamamıştır (>0.05). Karar alıcıların bu projeyi desteklemeye devam etmesi, nispeten büyük ölçekli işletmelerin proje yükümlükleri konusundaki kaygılarının giderilmesi, sürü yönetimi hususunda etkin bir eğitim planlanması yapılması ve proje tamamlandığında bölgeye yönelik ekonomik etkilerinin yeniden değerlendirilmesi önerilebilir.

Anahtar Kelimeler: Hayvancılık, işletme, koyun, kredi, yetiştirici

General Structure of The Livestock Enterprises Participated in the Project of "I Have Many Reasons to Live in My Village" and Factors Affecting Project Success Expectation: The Example Tokat Province

Abstract

Sheep breeding, quite suitable for Turkey's geography, climate, economy and sociology, is especially important in terms of national red meat supply security and rural development. The study aims to determine the general structure of the livestock enterprises participating in a sheep breeding project "I Have Many Reasons to Live in My Village", conducted by Tokat Provincial Directorate of Agriculture and Forestry, and to estimate the factors affecting project success expectations. The data was obtained from a face-to-face survey conducted with 297 breeders in 2022. As a result of the study, it was found that the average number of sheep in the enterprises was 180 head; the agricultural land size was 38 acres, debt amount and maturity were 136835 TL and 57 months, and the first cost and revenue items for enterprises were feed and butchery sales, respectively. The rate of those who positively evaluated the chances of the project success as "high" and "very high" was found to be 70%. There were negatively significant relationships between breeders' project success expectations with "herd size" (<0.01) and "indebtedness level" (<0.05). According to the Likert and binary classification scale, no significant relationships were found between project success expectation and other selected demographic, professional, technical and financial variables, and the independent variables could not have significantly explained the binary logistic regression model (>0.05). It can be recommended that decision makers should continue to support this project, concerns of relatively large-scale enterprises about project liabilities should be addressed, an effective training on herd management should be planned and the the project's economic impacts on region should be reassessed after the project is completed.

Key Words: Breeder, credit, enterprise, livestock, sheep

GİRİŞ

Koyunculuk yeterli ve dengeli beslenmeye verdiği katkı, yüksek üreme kabiliyeti, düşük üretim maliyeti, büyükbaş hayvancılığa göre daha ekonomik oluşu, mera ve otlakların daha iyi değerlendirilmesine olanak tanıması ve kırsal kalkınmaya verdiği destekle Türkiye'nin iklimi, ekolojisi, ekonomisi ve sosyolojisine oldukça uygun bir hayvansal alt üretim dalıdır (1-3).

Türkiye'nin 2023 yılı koyun varlığı bir önceki yıla göre %5.9 azalarak 42.06 milyon baş olup, bu değer toplam küçükbaş hayvan varlığının %80'ini oluşturmaktadır. Türkiye, FAO'ya bildirimde bulunan 142 ülke arasında koyun varlığıyla beşinci sırada yer almasına ve 2009 yılından itibaren sayısal artışlar yaşamasına rağmen, 2022 yılı ulusal kırımızı et üretimi olan 2.2 milyon ton içinde koyun eti yalnızca %22'lik bir paya sahiptir. Bu durum ülkemizde yalnızca yemde dışa bağımlılığın getirdiği yüksek üretim maliyetlerine sahip büyükbaş hayvancılık üzerinde ciddi baskı oluşturmakla kalmamakta, aynı zamanda coğrafi üstünlüğümüzden yeterince yararlanamadığımızı ve sayısal artışları verimlilikle destekleyemediğimizi de göstermektedir (4-6). Buna rağmen, Tarım ve Orman Bakanlığı'nın son yıllarda küçükbaş hayvancılığa yönelik desteklemelerde artışa gitmesi ve il bazında bazı özel projeleri başlatması koyunculuğa verilen önem ve farkındalığın arttığını göstermektedir (7,8).

Koyunculuğa yönelik yerel ve il bazındaki önemli projelerden biri de 2019 yılında Tokat İl Tarım ve Orman Müdürlüğü tarafından gerçekleştirilmiştir. Proje, küçükbaş yetiştiriciliğinin yaygınlaştırılması, kırsal gelir kaynaklarının arttırılması ve köyden kente göçün önlenmesi amacıyla hazırlanmış ve "Köyümde Yaşamak İçin Bir Sürü Nedenim Var" sloganıyla tanıtılmıştır (9). Tokat, nüfusu 612 bin, yüzölçümü 10072 km^2 olan Yeşilırmak havzasının bereketli toprakları üzerinde kurulu bir Orta-İç Karadeniz şehridir. İlin arazisinin %35.8'ini tarımsal araziler, %12.12'sini çayır ve meralar, geriye kalanını ise ormanlık alan ve tarıma elverişsiz alanlar oluşturmaktadır (10). İlde bulunan 27447 hayvancılık işletmesinde 284321 adet büyükbaş, 575014 adet küçükbaş hayvan bulunmaktadır (11). Mevcut küçükbaş hayvan sayısının 467040'ını koyun varlığı oluşturmakta olup, koyun sayısı bakımından Tokat ülkemizde 38. sıradayken, Karadeniz bölgesi illeri arasında ilk sırada yer almaktadır (12).

Bu çalışmanın amacı Tokat ilinde yürütülen "Köyümde Yaşamak İçin Bir Sürü Nedenim Var" projesine katılım sağlayan yetiştiriciler ve hayvancılık işletmelerine ait genel durumun ortaya konması (I) ve proje başarı beklentisi ve ona etkili faktörlerin belirlenmesidir (II). Çalışmadan elde edilen bulguların literatüre ve benzeri projeler için karar alıcılara yararlı bilgiler sağlayacağı düşünülmektedir.

MATERYAL VE METOT

Örneklem ve Anketler

Aşağıdaki formülde "n" örneklem hacmi, "N" çalışmaya başlarken Tokat ilinde projeye katılan yetiştirici sayısı (1020) ve "e" kabul edilen maksimum hata payı (%5) olmak üzere çalışmaya dâhil edilecek minimum yetiştirici sayısı 287 olarak hesaplanmıştır (13).

$$n = \frac{\mathrm{N}}{1+\mathrm{N}(\mathrm{e}^2)}$$

Veri temininde yaşanabilecek olası problemler dikkate alınarak belirlenen sayı önce 303'e çıkarılmış, anketler yürütülürken 6 kişinin projeden ayrılması neticesinde çalışma 297 yetiştiriciyle tamamlanmıştır. Basit tesadüfi örnekleme yöntemiyle yüz yüze Mayıs-Haziran 2022 tarihlerinde yürütülen anketler, yetiştiricilerin ve işletmelerinin bazı demografik, mesleki, teknik ve mali özelliklerine yönelik kapalı uçlu sorulardan oluşmuştur.

Hipotezler

Bu çalışmada aşağıdaki 7 hipotez test edilmiştir. Yetiştiricilerin proje başarı beklenti düzeyleri için ilk dört hipotezde 5'li Likert ölçeği (çok düşük-1, düşük-2, normal/orta-3, yüksek-4, çok yüksek-5); son üç hipotezde ise ikili sınıflandırma ölçeği (olumlu ve olumsuz) kullanılmıştır. Son üç hipotez için "normal/orta düzey" yanıtı negatif veya pozitif bir anlam içermediği için dikkate alınmayarak; olumsuz kategorisi "çok düşük ve düşük", olumlu kategorisi "çok yüksek ve yüksek" yanıtlarını kapsamıştır.

H_1: Projenin başarı beklentisine ilişkin Likert ölçeği ile yetiştiricilerin demografik özellikleri arasında istatistiki olarak anlamlı ilişkiler mevcuttur.

H_2: Projenin başarı beklentisine ilişkin Likert ölçeği ile yetiştiricilerin mesleki yetkinlikleri arasında istatistiki olarak anlamlı ilişkiler mevcuttur.

H_3: Projenin başarı beklentisine ilişkin Likert ölçeği ile işletmedeki koyun sayısı arasında istatistiki olarak anlamlı ilişki mevcuttur.

H_4: Projenin başarı beklentisine ilişkin Likert ölçeği ile işletmenin borçluluk düzeyi arasında istatistiki olarak anlamlı ilişki mevcuttur.

H_5: Projenin başarı beklentisine ilişkin ikili sınıflandırma ölçeği ile seçili kategorik mesleki, teknik ve mali özelliklerin bir veya birkaçı arasında istatistiki olarak anlamlı ilişkiler mevcuttur.

H_6: Projenin başarı beklentisini olumlu veya olumsuz bildiren yetiştiriciler arasında demografik, mesleki, teknik ve mali özelliklerin bir veya birkaçı arasında anlamlı farklılıklar mevcuttur.

H_7: Projenin başarı beklentisinin olumlu veya olumsuz oluşuna etkili faktörlerin tahmini için kurulan İkili Lojistik Regresyon modeli, seçilen bağımsız değişkenlerce anlamlı biçimde açıklanır.

İstatistiki Analizler

Demografik, mesleki, teknik ve mali bulguların tanımsal istatistikleri veri tipine uygun merkezi eğilim ve yayılım ölçüleriyle raporlanmıştır. Gruplar arası farklılık ve ilişkinin belirlenmesinden kullanılacak parametrik veya non-parametrik analiz yöntemlerinin seçiminde normal dağılıma uygunluk önce grafiklerle, ardından örneklem 35'den büyük olduğundan Kolmogorov-Smirnov ile test edilmiştir (14). H_1 den H_4 e kadarki hipotezler non-parametrik bir ilişki analizi olan Spearman korelasyon analiziyle, H_5 hipotezi Chi-square testi ile, H_6 hipotezi Mann-Whitney testi ile, H_7 hipotezi ise ikili (binary) lojistik regresyon modeliyle test edilmiştir. Nispeten kolay ve esnek olan bu lojistik modelde, iki kategoriden oluşan bağımlı değişkenin kategorilerinden birinin gerçekleşme olasılığı sürekli, ordinal ve nominal bağımsız değişkenlerce açıklanıp aralarındaki nedensellik tahmin edilmeye çalışılır (15-17). Veri giriş ve analizleri Microsoft Excel 2013 ve IBM SPSS Statistics for Windows, Version 22.0 programlarıyla gerçekleştirilmiştir.

BULGULAR

Aşağıda öncelikle tanımsal istatistiklerle özetlenen yetiştiricilere ve işletmelere dair genel bulgular verilmiş (Tablo 1-5), ardından projenin başarısına ilişkin yetiştirici beklentisi (Tablo 6) ve bu beklenti üzerine etkili faktörlere ilişkin bulgular (Tablo 7) ve hipotez testlerinin sonuçları paylaşılmıştır.

Tablo 1. Projeye katılan yetiştiricilerin demografik ve mesleki durumları

Demografik ve Mesleki Değişkenler	n (297)	X ± SD	Medyan (Min-Max)	Mod
Yaş		43.47±12.02	-	-
Eğitim düzeyi		-	1(1-3)[1]	-
Mesleki tecrübe süresi (yıl)		20.00±12.96	-	-
Mesleki eğitim alma durumu		-	-	1[2]
Proje başvurusunda hayvancılıkla meşguliyet				1[3]

[1]İlköğretim ve aşağısı 1, ortaöğretim 2, yükseköğretim 3 olarak kodlandı

Tablo 2. Projeye katılan işletmelerin hayvan varlığı

İşletmelerin Hayvan ve Tarımsal Arazi Varlığı	n	X ± SD
Koyun varlığı	295	180.26±73.43
Keçi varlığı	14	85.50±127.09
Sığır varlığı	80	13.18±14.11
Tüm işletmeler için tarımsal arazi ölçeği (dönüm)	295	34.10±39.48
Arazisi olanların tarımsal arazi ölçeği (dönüm)	249	38.18±39.37

Tablo 3. Projeye işletmelerinde sürü yönetimi, bakım ve beslemeye ilişkin bulgular

Yetiştiricilerin Sürü Yönetimi, Bakım ve Beslemesi	X ± SD	Medyan (Min-Max)	Mod
Sürü çobanlığı	-	-	1[1]
Mera kullanımı	-	-	1[2]
Merada kalış süresi (ay)	6.93±1.52	-	-
Yem bitkileri üretimi	-	-	1[3]
Koçun temin edildiği yer	-	-	1[4]
Koç katım yöntemi	-	-	3[5]
Koç katım zamanı	-	-	2[6]
Koç katımında flushing	-	-	1[7]
Kırkım yöntemi	-	-	1[8]
Kırkım ayı	-	-	3[9]
Kırkım sayısı (adet/yıl)	1.06±0.24	-	-

[1]Çobanlığın işletme sahibi tarafından yapılması 1, çobanlığın başkasına yaptırılması olarak 2 kodlandı.
[2]Kendi köyünün merasını kullananlar 1, başka köylerin merasını kullananlar 2 olarak kodlandı.
[3]Yonca 1, Arpa 2, Mısır 3, Korunga 4, Fiğ 5, Yulaf 6, Çavdar ve Tritikale 7 olarak kodlandı.
[4]Kendi işletmesinde yetiştirenler 1, köydeki başka işletmeden tedarik 2, köy dışından tedarik 3 olarak kodlandı.
[5]Elde aşım yöntemi 1, sınıf usulü aşım yöntemi 2, serbest aşım yöntemi 3 olarak kodlandı.
[6]Mevsimsel koç katımı yapanlar 1, yıl boyu koç katımı yapanlar 2 olarak kodlandı.
[7]Koç katımında flushing yapanlar 1, ilave yemleme yapmayanlar 2 olarak kodlandı.
[8]Kırkımı makasla yapanlar 1, kırkımı makineyle yapanlar 2 olarak kodlandı.
[9]Mart 1, Nisan 2, Mayıs 3, Haziran 4, Temmuz 5, Ağustos 6, Eylül 7, Ekim 8, Kasım 9 olarak kodlandı.

Tablo 4. projeye katılan işletmelerin borçluluk durumu

İşletmelerin Borçluluk Durumu		n	X ± SD	Mod
Proje Öncesi (2019)	Borcun miktarı (TL)	59	79.347±66.049	-
	Borcun vadesi (ay)		30.71±19.62	-
	Borcun kaynağı		-	1[2]
Projeye Sonrası (2021)	Borcun miktarı (TL)	297	136.835±43.997	-
	Borcun vadesi (ay)		57.48±5.64	-
	Borcun kaynağı		-	1[2]

[1]Özel şahsa borçlanma 1, bankalara borçlanma 2 olarak kodlandı.

Tablo 5. Projeye katılan yetiştiricilerin beyan ettiği gelir ve gider kalemleri

Maliyet ve Gelir Unsurları		n	%
İlk sırada beyan edilen maliyet kalemi	Yem	280	94.28
	Veteriner sağlık	8	2.69
	İşçilik	4	1.35
	Kira (arazi veya ağıl)	3	1.01
	Elektrik, akaryakıt ve su	2	0.67
	Toplam	**297**	**100**
İlk sırada beyan edilen gelir kalemi	Kasaplık satışlar	215	72.39
	Damızlık satışlar	46	15.49
	Kurbanlık satışlar	36	12.12
	Toplam	**297**	**100**

Tablo 6. Proje başarısına ilişkin yetiştirici beklentilerinin dağılımı

5'li Likert Ölçeği	Yetiştirici Sayısı (n)	Yetiştirici Oranı (%)
Çok düşük düzeyde	10	3.37
Düşük düzeyde	15	5.05
Orta/normal düzeyde	64	21.55
Yüksek düzeyde	110	37.03
Çok yüksek düzeyde	98	32.99
Toplam	297	100

Tablo 7. Proje başarı beklentisiyle seçili değişkenler arasındaki ilişkiler

Seçili Değişkenler	n	Likert sıralama ölçeğine göre proje başarı beklentisi		İkili sınıflandırma ölçeğine göre proje başarı beklentisi	
		Spearman Rho	P-value	Chi-square	P-value
Yaş	297	0.051	>0.05	-	-
Eğitim	297	0.106	>0.05	-	-
Mesleki eğitim	233	-	-	0.405	>0.05
Mesleki tecrübe	297	0.016	>0.05	-	-
Geçmişte hayvancılıkla meşguliyet	233	-	-	2.240	>0.05
Çobanlığı kimin yaptığı	233	-	-	0.005	>0.05
Sürü tipi	233	-	-	0.069	>0.05
Mera kullanımı	233	-	-	0.828	>0.05
Merada kalış süresi	297	0.445	>0.05	-	-
Sürüdeki koyun sayısı	297	0.162	<0.01	-	-
Tarımsal arazi büyüklüğü	295	0.081	>0.05	-	-
İşletmenin borçluluk düzeyi	297	0.135	<0.05	-	-

Tablo 1 incelendiğinde proje başvurusunda bulunan yetiştiricilerin kırklı yaşların başında, ilköğretim mezunu ve yirmi yıllık mesleki tecrübe sahibi kişilerden oluştuğu anlaşılmaktadır. Tabloda tepe değerleriyle özetlenen son iki satırdaki yanıtlar frekanslarıyla incelendiğinde yetiştiricilerin %54.20'sinin (161 kişi) hayvancılığa yönelik en az bir mesleki eğitimden geçtiği ve %87.87'sinin (261 kişi) bu işe yeni başlayanlar değil daha önceden hayvancılık faaliyetinde bulunan kişiler olduğu görülmektedir.

Tablo 2'de Projeye dâhil işletmelerin %27.11'inin (80 adet) aynı zamanda büyükbaş hayvancılıkla da iştigal ettiği görülmektedir. İşletmelerin %15.48'inin (46 adet) tarımsal arazi varlığı bulunmazken, tarımsal arazi sahibi olanlar için arazi büyüklüğü 38 dönüm dolayındadır. Bu tablolarda yer verilmeyen bir bulguda, yetiştiricilerin hayvanları %48 oranında il içinden, %52 oranında il dışından temin etmiş olmasıdır. Yapılan çalışmada işletmelerdeki koyun varlığının tamamına yakınının (%94.27) Akkaraman ve Karayaka ırklarından oluştuğunu görülmektedir.

Tablo 3'de işletmelerde sürü yönetimi, mera kullanımı, koç katımı ve kırkıma ilişkin bulgulara yer verilmiştir. Tablodan sürü çobanlığının çoğunlukla yetiştirici tarafından yapıldığı anlaşılırken, frekanslar incelendiğinde başkasına yaptıranların %10.43 ile (31 kişi) sınırlı kaldığı belirlenmiştir. Yetiştiricilerin yaklaşık %93'ü sürüsünü kendi köyünün merasında yayılıma çıkarırken, meradan istifade süresi ortalama 7 aydır. İlk sırada ve en çok ekimi yapılan yem bitkisi sorusuna 74 işletme "yonca", 62 işletme "arpa" ve 24 işletme "mısır" yanıtını vermiştir. Genetik ilerleme ve döl veriminde kritik role sahip koçların genellikle sürü içinden yetiştiği, çiftleştirmede "serbest aşım" yönteminin kullanıldığı, koç katım zamanının mevsimselden ziyade yıl boyu olduğu, katım öncesi ve sonrası koç ve koyunlara ilave yemleme (flushing) yapıldığı görülmektedir. Konu frekanslarla incelendiğinde aslında ilave yemleme yapmayanların sayı (127) ve oranının (%42.76) azımsanmayacak düzeyde olduğu da belirlenmiştir. Bir diğer bulgu elde aşım (9 kişi) ve sınıf usulü aşım (2 kişi) yöntemini tercih edenlerin oranının eseri kalmasıdır (%3.7). İşletmeler genelinde kırkım büyük oranda makasla yapılırken, frekansların detaylı incelenmesi neticesinde makine kullanan sadece 44 kişi (%14.81) olduğu, kırkımların ağırlıklı olarak yılda tek sefer ve mayıs ayında gerçekleştiği, iki kez kırkım yapanların (3-5 ay arayla) 18 yetiştiriciyle (%6.06) çok sınırlı kaldığı belirlenmiştir.

Tablo 4'de projeye katılan yetiştiricilerin proje öncesi ve esnasındaki borçluluk durumu özetlenmektedir. Tablo incelendiğinde, projeye katılım öncesi bankaya borçlanan yetiştiricilerin sadece 59 kişiyle sınırlı kaldığı ve katılımcıların 2019 yılı ortalama borç miktar ve vadelerinin sırasıyla 79000 TL ve 2.5 yıl dolayında olduğu görülmektedir. Projeye katılımla beraber yetiştiriciler vadesi yaklaşık 5 yıl olan 137000 TL dolayında bir borçlanma yaparken, frekanslar hiçbir yetiştiricinin özel şahıslara borcu olmadığını, yani finansmanın bütünüyle öz kaynak ve bankalara dayandığını ortaya koymuştur.

Tablo 5'te işletmeler için ilk sırada gelen yani en önemli gelir ve gider kalemleri büyükten küçüğe doğru sıralanmış olup, bu oransal dağılım ilgili unsurların toplam maliyet içindeki payı değildir. Tablodan, maliyet kalemlerinin başında açık ara farkla "yem masraflarının" geldiği ve diğer unsurları ilk sırada beyan eden yetiştirici sayısının eseri düzeyde kaldığı anlaşılmaktadır. Gelir kalemleri açısından her ne kadar ilk sırada %72 dolayında "kasaplık satışlar" gelse de pek azımsanamayacak sayıda yetiştirici "damızlık satışlar" ve "kurbanlık satışlar" yanıtını da vermiştir.

Tablo 6'da yetiştiricilerin projenin başarı düzeyine ilişkin Likert ölçeğiyle sorgulanan görüşleri ve bunların frekansları sunulmuştur. Tabloda görüleceği üzere, proje başarı şansını net olarak olumlu değerlendiren yani "yüksek ve çok yüksek" görenler, olumsuz değerlendirip "düşük ve çok düşük" görenlerin yaklaşık 9 katıdır. Başarı düzeyine ilişkin yanıtların

ortalamaları incelendiğinde medyanın 4 (yüksek düzey), aritmetik ortalama ve standart sapmanın ise sırasıyla 3.91±1.02 olduğu belirlenmiştir. Bir diğer ifadeyle, genel eğilimi gösteren ortalamalar yetiştiricilerin bu proje hakkında yüksek bir başarı beklentisine sahip olduklarını göstermektedir.

Tablo 7'nin sol kısmında Likert ölçeğine göre proje başarı beklentisi ile yedi adet seçili değişken arasındaki ilişkiler özetlenirken; sağ kısmında ikili sınıflama ölçeğine dönüştürülmüş net başarı beklentisi ile seçili beş adet değişkenin ilişkisi sunulmuştur. Projenin başarı beklentisi ile yetiştiricilerin demografik özellikleri ve mesleki yetkinlikleri arasında anlamlı ilişkiler bulunamadığından H_1 ve H_2 hipotezleri reddedilirken; "sürü büyüklüğü" ve "borçluluk düzeyi" ile bu beklenti arasında negatif ve anlamlı ilişkiler olduğundan H_3 ve H_4 hipotezleri kabul edilmiştir. Tablo 7'nin sağ tarafında ise kategorik değişkenler için 2x2 düzeninde Ki-Kare analiz sonuçları raporlanmakta olup, bulgular başarı beklentisi ile seçili kategorik değişkenlerin hiçbiri arasında anlamlı bir ilişki olmadığını ortaya koymuştur.

Bu çalışmada ayrıca, projenin başarısını olumlu ve olumsuz değerlendiren iki yetiştirici grubu arasında demografik, mesleki, teknik ve mali özellikleri arasında hiçbir anlamlı farklılık belirlenemediğinden ($p>0.05$) H_6 hipotezi reddedilmiştir. Son olarak, proje başarı beklentisine etkili faktörlerin tahmin edilmeye çalışıldığı ikili lojistik regresyon modeli istatistiki olarak anlamlı bulunmamıştır (–2 log likelihood: 155.124; Nagelkerke R^2: 0.116; $p>0.05$). Bir diğer ifadeyle Tablo 8'deki bağımsız değişkenler yetiştiricilerin proje beklentisinin niçin olumlu veya olumsuz olduğunu açıklayamadığı için H_7 hipotezi reddedilmiştir.

TARTIŞMA VE SONUÇ

Bu çalışmada, yetiştiricilerin projeden net başarı beklentisinin %70 gibi yüksek denebilecek bir oranda oluşu bir yandan projenin sürdürülebilirliği, diğer yandan projeyle amaçlanan koyunculuğun yaygınlaştırılması yoluyla kırsal gelir kaynaklarının arttırılması ve köyden kente göçün azaltılması hususunda olumlu bir işarettir. Ancak bu memnuniyetin proje süresince takip edilmesi ve somut proje çıktıları elde edildikçe teknik ve mali yönlerden yeniden değerlendirilmesi gerekir. Zira bazı istatistik ve bildirimler iş memnuniyetine ilişkin farklı sonuçlar da ortaya koymaktadır. Örneğin Türkiye'de 2009 yılından bu yana koyunculukta izlenen sayısal artış eğilimi sanki olumlu bir gelişme olarak görülmektedir (12). Elazığ'da yapılan bir çalışma koyun yetiştiricilerinin %67 oranında işlerinden memnun olduğunu göstermektedir (18). Ancak Ardahan'da üreticilerin yalnızca %28'i gelir düzeyinden memnunken (2); Van'da yetiştiricilerin %81'inin tek geçim kaynağı olan koyunculuğu farklı bir olanak çıkarsa terk edecekleri bildirilmektedir (3).

Bu projede "koyun varlığı" ve "işletme borçluluk düzeyi" ile proje beklentisi arasındaki negatif korelasyon, yani büyük ölçekli işletmelerin nispeten daha az iyimser oluşu risk ve belirsizliklere karşı daha duyarlı ve temkinli olmalarıyla açıklanabilir. Bu bulgu işletmeler küçüldükçe proje başarısına yönelik iyimserliğin arttığı anlamı da taşır. Bu durum özellikle küçük ölçekli aile işletmelerinde koyunculuğun tek geçim kaynağı olması, işletme giderlerinin büyük bir kısmını oluşturan çoban ihtiyacının sürü ölçeğinin artmasıyla dışardan temin edilmesi gibi maddi ve sosyolojik olgularla açıklanabilir. Bununla beraber daha sağlıklı değerlendirme yapabilmek için teknik ve mali nitelikteki somut proje çıktılarının ortaya çıkmasını beklemek gerekmektedir. Projenin başarılı veya başarısız olacağı görüşüne etkili faktörlerin modele dâhil edilen hiçbir demografik, mesleki, teknik ve mali değişkenle açıklanamaması ise hayvan sigorta bedelleri ve proje süresince satış yapamama gibi hukuki yükümlülüklerden kaynaklandığı düşünülmektedir. Ayrıca, proje devam ederken ülke genelinde etkileri güçlü şekilde hissedilmeye başlayan ve maliyetleri artırıp fiyat istikrarını bozan yüksek enflasyonun tepkisel veya tutarsız yanıtlara yol açabileceği de göz ardı edilmemelidir.

İşletme ve yetiştiricilikle ilgili bazı proje bulgular literatürle değerlendirildiğinde benzerlik ve farklılıklar görülmektedir. Bu çalışmada çoğunluğu Akkaraman ve Karayaka olmak üzere toplam koyun, kuzu ve koç varlığı 180 baş olarak bulunmuştur. Irklar bölgeye göre değişmekle beraber bu rakam Burdur'daki bir çalışmada ortalama 200 baş, Hatay'daki bir çalışmada 128 baş ve Van'daki bir çalışmada 150 baş olarak bildirilmiş olup bu projeyle nispeten uyumludur. Bu rakamların tamamı Türkiye'de 2022 yılı için işletme başına düşen ortalama koyun sayısından (~120) yüksektir (12).

Bitkisel üretim içinde önemli bir yere sahip olan yem bitkileri üretimi hayvansal üretimin sigortası durumundadır. Üretilen yem bitkileri öncelikle hayvan beslenmesinde kullanılmakta olup böylece hayvansal ürünlere dönüştürülerek nihai olarak insan beslenmesinde kullanılmaktadır (19). Ülkemizde yem bitkileri ekiliş alanları 2018-2022 yılları arasında %23 artarak 2022 yılında 27528380 dekara çıkmıştır. Ülkemizde en çok ekimi yapılan yem bitkileri sırasıyla yonca, silajlık mısır ve fiğ olurken bu üç yem bitkisinin ekiliş alanı, toplam yem bitkisi ekiliş alanının %55'ini oluşturmaktadır (20,21). Bu çalışmada Tokat ilinde koyunculuk işletmelerince en çok ekimi yapılan ilk üç yem bitkisi sırasıyla yonca, arpa ve mısırken; Konya'da bu durum sırasıyla yonca, fiğ ve silajlık mısırdır (22). Bu projede 38 dönüm olarak bulunan tarımsal arazi ölçeği ise, Burdur'dan bildirilen 39 dönümle örtüşürken, Van ilinde bulunan 51 dönüm sulu ve 75 dönüm kıraç araziye göre düşüktür (3,23,24).

Arazi varlığı konuyu doğrudan doğal kaba yem kaynağı olan meralara götürmektedir. Türkiye'de yapılan çalışmalar meradan zengin bölgeler hariç mera sorununu koyunculukta öncelikli problem olarak göstermektedir (18,23). Nadas, anız ve bitkisel üretime uygun olmayan alanlardan yararlanan koyunlar için en ucuz kaba yem kaynağı meralardır ki kısa boylu ve verimsiz olanları bile iyi değerlendirebilmektedir (23). Bugün ülkemizdeki toplam tarım arazisinin %37'si olan çayır ve mera alanlarının nerdeyse tamamına yakını mera olup büyük bölümü Tokat iline de komşu olan İç ve Doğu Anadolu bölgesindedir (6,21). Tokat ilinin toplam yüzölçümü 1007200 hektar olup, bunun 122101 hektar alanı (% 12.12) çayır ve mera alanlarıdır (10). Bu çalışmada yetiştiricilerin yaklaşık %93'ü sürüsünü kendi köyünün merasında yayılıma çıkarırken, Konya'da yetiştiricilerin yarısı köy, dörtte biri hazine, %14'ü her ikisi olmak üzere yaklaşık %90'ının mera kullandığı, boş arazi ve anız tarlalarında koyun otlatanların %10'dan az olduğu belirlenmiştir (22).

Koyunlarda ovulasyon ve gebelik oranının artırılarak sağlıklı ve daha fazla kuzu elde etmek amacıyla uygulanan flushing ülkemizde yaygınlaşmaya başlamıştır (25). Koç katımından iki üç hafta önce başlayan ve koç katım dönemi sonrasında belirli bir süre daha devam eden olağandan daha

yüksek ilave yemlemenin döl verimini ve ikizlik oranını artırdığı bildirilmektedir (26). Bu çalışmada damızlıkların tamamına yakını işletme içinden olmak üzere çiftleştirmede yaklaşık %96 elde aşım yöntemi kullanıldığı ve işletmelerin yarısından fazlasının koç katım öncesi ve sonrası ilave yemleme yaptığı bulunmuştur ki, bu durum literatürle uyumludur. Zira damızlıkları kendi işletmelerinden olmak üzere koç katımında %99 serbest aşım ve %25 flushing (23) veya %97 serbest aşım ve %61 flushing (22) bildiren çalışmalar mevcuttur. Yine bu çalışmada çoğunlukla mayıs ayında yapılan kırkım %86 oranında makasla, Burdur'da haziran ayında yoğunlaşmakta olan kırkımın tamamıysa makasla/elle olmaktadır (23).

2018 yılında ülkemizde koyunculuğun geliştirilmesi amacıyla Tarım ve Orman Bakanlığınca yürütülen "Üretici Şartlarında Sözleşmeli Hayvancılık Projesi" diğer adıyla ''300 Baş Koyunculuk Projesi" kapsamında Tokat ilinde 326 çiftçi müracaatta bulunmuş ancak sadece 7 üreticiye koyun teslimi yapılabilmiştir. 300 Baş Koyunculuk Projesinde yeterli müracaat alınmasına rağmen, proje kapsamında dağıtılacak hayvan sayısı, ırk özelliklerinin yerel ölçekte üretici tarafından tercih edilmemesi yine proje kapsamında yer alan yem desteği ve çoban desteğinden vazgeçilmesi projenin başarısını olumsuz etkilemiştir (27). Türkiye'de ilk defa Tokat ilinde başlayıp ''Tokat Modeli'' adıyla ulusal düzeye yayılan "Köyümde Yaşamak İçin Bir Sürü Nedenim Var Projesinde (28)" çiftçilere, Ziraat Bankası aracılığıyla küçükbaş alımı için 7 yıla kadar sıfır faizli 100000 TL yatırım kredisi, yem için 18 aya kadar 50000 TL işletme kredisi verilmiştir. Sonuç olarak 2020 yılı için toplam 150000 TL'ye kadar kredi imkânı sunulmuş olup bu proje özelinde kredilerin teminatı için hayvan rehini sistemine geçilmiştir (29). Yine proje kapsamında üreticilere koyun temini haricinde, veteriner sağlık hizmetleri, mera ıslahı, projede üretilen kuzulara alım desteği, İl Özel idaresi kaynakları ile ücretsiz koç temini, eğitim gibi geniş bir yelpazede hizmet verilmiştir (30). Projeye talebi artıran bu olanaklar projenin diğer illerde de uygulanarak yerelden ulusal düzeyde uygulanan bir proje haline gelmesine zemin hazırlamıştır. Projenin hayata geçirildiği 2019 yılından 2021 yılına kadar geçen 2 yıllık süreçte ildeki küçükbaş hayvancılığın durumunda iyileşmeler olmuştur. Zira işletme sayısı 2142 ten 2698'e (%25.9 artış), destekleme ödemesi yapılan anaç küçükbaş sayısı 224883'den 398440'a (%32 artış), anaç koyunkeçi destekleme miktarı 5622075 TL'den 9131365 TL'ye (%62 artış) yükselmiştir (31).

Tokat ilindeki gelişmelerle ulusal düzeydeki sayısal artış eğilimi ve ilgili destek kalemlerindeki artışlar koyunculuk için iyimser bir tabloya işaret etmektedir. Coğrafik, biyolojik, ekolojik ve ekonomik açıdan ülkemiz için son derece uygun bir üretim dalı olan koyunculukta, 1991 yılındaki 40.4 milyon baş olan hayvan varlığı 2009 yılına kadar azalarak 21.7 milyona gerilemiş, ardından tekrar artışa geçerek 2020 yılında 1991 seviyesini aşmış ve 2022 yılında 44.6 milyon başa kadar ulaşmıştır, ancak 2023 yılında bir önceki yıla göre %6.9 azalarak 42.06 milyon başa gerilemiştir (5). Bu durum Türkiye'yi koyunculukta dünyada beşinci sıraya yerleştirse de kırmızı et üretimindeki %22'lik pay ve ırk ıslahının yetersizliğine bağlı düşük verimlilik nicelik ve nitelik açısından yeterli olmadığını göstermektedir (4,6,22). Ancak sektörün sorunları bunlardan ibaret değildir. Örneğin ürün pazarlamada ve mesleki hakların korunmasında kritik role sahip örgütlenmede küçükbaş yetiştiricilerinin sorumluluk ve memnuniyet düzeylerinin çok düşük kalışı büyük bir dezavantajdır (24). Yine önümüzdeki yıllarda dünya koyunculuk sektörünün büyükbaş ve kanatlı gibi sektörlerle rekabette verimlilik, teknolojik gelişimlere uyum, ürün farklılaştırma ve tüketici tercihleri gibi zorlayıcı faktörlerle yüzleşeceği bildirilmektedir (32).

Bu çalışmanın zayıf yanı, projenin başarı düzeyini işletmenin dönem sonu çıktıları olan üretim, karlılık ve sermaye değişimi üzerinden değil de yetiştiricilerin başarı beklenti ölçekleriyle bir ön değerlendirme şeklinde yapmak zorunda kalışıdır. Çalışmanın güçlü yanı ise Türkiye'de ilk defa yerel düzeyde başlayıp ulusal anlamda birçok ilde uygulanan bir küçükbaş hayvancılık projesinde genel durumun ve proje başarı beklentisinin ortaya konmuş olmasıdır.

Koyunculuğun ülke ve bölge için kritik öneminin altını çizen "Köyümde Yaşamak İçin Bir Sürü Nedenim Var" projesinde net başarısızlık beklentisinin %10'un altında kalışı karar alıcıların bu projeyi desteklemeye devam etmesi gerektiğini göstermektedir. Proje yükümlükleri konusundaki kaygılarının giderilmesi, sürü yöneticisi istihdamına yönelik işletmelerin desteklenmesi, proje kapsamında sağlanan veteriner sağlık hizmetleri, mera ıslahı, pazarlama gibi destekler ile proje yararlanıcılarına yönelik eğitim faaliyetlerinin çeşitlendirilerek devamı projenin sürdürülebilirliği için oldukça önemlidir. İlerleyen yıllarda somut proje çıktıları alındıkça başarının bölgeye ekonomik etkileriyle beraber yeniden değerlendirilmesi önerilebilir. Başarı beklentisi veya memnuniyet üzerine bundan sonra yapılacak çalışmaların bankaların aradığı teminat şartlarını, işletmenin konumunu, idare ve proje personelinin yaklaşımını, Bakanlığın küçükbaşa yönelik desteklemelerini ve ilgili bazı makroekonomik değişkenleri de dikkate alması kurulacak istatistiki modelleri açıklamada daha yararlı olabilir.

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Metodoloji MFC ve SÇ; örneklem ve veri analizi SÇ, MFC, HM; makale hazırlama SÇ ve MFC; danışmanlık HM; makale düzenleme MFC ve SÇ. Tüm yazarlar makalenin yayım öncesi son halini okumuş ve kabul etmişlerdir.

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RESEARCH ARTICLE

Socio-economic Structure and Current Problems of Horse Breeding Enterprises in Mahmudiye District of Eskisehir

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Abstract

The horse has been an significant farm animal throughout history and has been used especially in agriculture, transportation and military activities. This study was conducted to ascertain the socio-economic status and current problems of horse enterprises in Mahmudiye district of Eskişehir province. The material of the study consists of the data obtained as a result of face-to-face interviews in 12 horse enterprises in Mahmudiye district of Eskişehir province in 2019. In the results of the survey, it was determined that the average age of the enterprise owners was 41.25 years and their average professional experience was 17.67 years. Of the total cost, 49.33% was feed costs, 16.87% was labor costs, 14.55% was litter costs and 8.68% was natural mating costs. The main problems identified in the region for the enterprise are marketing and feed supply (83.33%), labor supply (50%) and health problems (41.67%). Mahmudiye district is still a significant breeding region for horse breeding and racing. Encouraging tourists and children to participate in equestrian sports and activities like horseback riding will have an impact on the socio-cultural growth, career opportunities, and overall economic diversity of the local population and national income of Türkiye. Therefore, with this study, it is thought that it would be useful to establish a horse training area in Mahmudiye district in order to spread the love of horses and equestrian culture and to develop equestrianism.

Key Words: Horse breeding, livestock, Mahmudiye, socio-economic status

Eskişehir İli Mahmudiye Ilçesindeki At Yetiştiriciliği İşletmelerinin Sosyo-Ekonomik Yapısı ve Güncel Sorunları

Öz

At, tarih boyunca önemli bir çiftlik hayvanı olmuş ve özellikle tarım, ulaşım ve askeri faaliyetlerde kullanılmıştır. Bu çalışma, Eskişehir ili Mahmudiye ilçesindeki at işletmelerinin sosyo-ekonomik durumunu ve mevcut sorunlarını tespit etmek amacıyla yapılmıştır. Çalışmanın materyalini, 2019 yılında Eskişehir ili Mahmudiye ilçesinde 12 at işletmesinde yüz yüze yapılan görüşmeler sonucunda elde edilen veriler oluşturmaktadır. Anket çalışması sonuçlarında işletme sahiplerinin yaş ortalamasının 41.25, ortalama mesleki tecrübelerinin ise 17.67 yıl olduğu belirlenmiştir. Toplam maliyetin %49.33'ünü yem maliyetleri, %16.87'sini işçilik maliyetleri, %14.55'ini altlık maliyetleri, %8.68'ini ise doğal aşım maliyetleri oluşturmaktadır. İşletme açısından bölgede tespit edilen başlıca sorunlar; pazarlama ve yem temini (%83.33), işgücü temini (%50) ve sağlık sorunları (%41.67) olarak sıralanmaktadır. Mahmudiye ilçesi at yetiştiriciliği ve yarışları açısından halen önemli bir konumda bulunmaktadır. Turistlerin ve çocukların binicilik faaliyetlerine katılmalarının teşvik edilmesi, Türkiye'nin sosyo-kültürel değişimine, kariyer fırsatlarına, yerel nüfusun genel ekonomik çeşitliliğine ve milli gelirine büyük katkı sağlayacaktır. Bu nedenle bu çalışma ile at sevgisinin ve binicilik kültürünün yaygınlaştırılması ve atçılığın geliştirilmesi amacıyla Mahmudiye ilçesinde bir at eğitim alanının kurulmasının yararlı olacağı düşünülmektedir.

Anahtar Kelimeler: At yetiştiriciliği, hayvancılık, Mahmudiye, sosyo-ekonomik durum

INTRODUCTION

The horse has had an important place among farm animals throughout history. It has been actively used in jobs such as shooting, harness, people and goods transport, especially in agriculture, transportation and military operations (1-2). However, with the mechanization that emerged in the industrial revolution, the use of horses in these areas has been decreased, and horse breeding for racing and sports has become a more common activity. People in some countries and regions continue to benefit from horse traction to varying degrees in agriculture and other service sectors. Horse production and training differ significantly from other farm animals in terms of management and purpose. When comparing horses in a competitive setting, it's critical to consider the economic service provided and to plan the working rates of horses who are employed or forced to work (2).

The expenses related to horse breeding include the general upkeep and care of horses. In the long run, a horse breeding enterprise's budget must account for expenses associated with housing, feed, veterinary care, and general maintenance. Other important financial considerations of horse breeding include the expenses associated with caring for breeding stallions, mares, and foals in addition to the frequent weight assessments required to maintain the health of the horses (3-4). Although the cost of horse breeding is high, it makes significant contributions to the national economy of the countries where breeding is common (5).

The historical prominence and distinctive genetic qualities of the Arabian horse breed have made breeding them economically significant. The matrilineal side has always played a significant role in defining the purity of Arabian horses (6). However, genetic considerations are also very important in the economics of raising Arabian horses (7). Oxidative stress markers, and reproductive performance are additional aspects that affect the economic viability of Arabian horse breeding farms and can have an impact on the overall success and profitability of these enterprises (8). Horse breeding enterprises may enhance their productivity, preserve the quality and worth of their horses, and make a substantial financial impact on the equine sector by incorporating these factors into their operations.

Pure blood Arabian horse breeding has been carried out for about 200 years in enterprise of Anatolia Agriculture which was established under the name of Çiftlikat-ı Hümayun by II. Mahmut to meet the horse needs of the Ottoman army in 1815, in the Mahmudiye district of Eskişehir (9). In Mahmudiye district, there are many enterprises that are engaged in breeding and racing horse, together with horse boarding. Due to its geographical location, historical development process, trained and educated manpower etc., Mahmudiye has become one of the important centers of equestrianism and it still maintains this feature today (10). The aim of this study is to reveal the socio-economic structure of horse breeding enterprises in the Mahmudiye district of Eskişehir province and to examine their current problems.

MATERIAL AND METHODS

The material of the study consists of the primary data obtained from the enterprises engaged in Arabian horse breeding in the Mahmudiye district of Eskişehir through the data supply form. Eskişehir province is located in northwestern Türkiye (Figure 1). Eskişehir is classified as having a cold semi-arid steppe climate (BSk) according to the Köppen climate classification. This means it experiences warm to hot dry summers and cold to freezing winters. As of 2020, there are 57 private horse enterprises in Mahmudiye district and nearly 2000 horses in total (10).

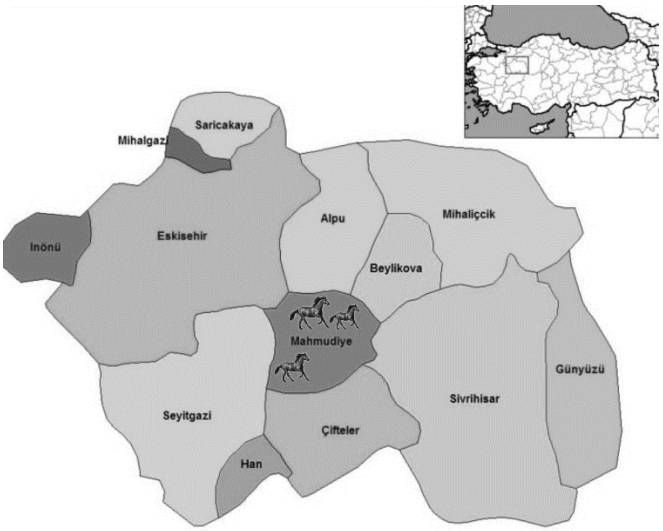

Figure 1. Display of study area.

In order to determine the socio-economic status of the enterprises, the questions such as the age of the enterprise owner, education level, experience level of owners, whether the owners have any other income, labor type, organizational structure, number of animals and technical structure, operating cost-income elements, basic problems encountered in the enterprises, etc. were asked in the data supply form. Although the data used in the study belong to 2019, 12 enterprises were visited within the scope of the study.

The data obtained from the study were transferred to the computer using Microsoft Excel 16.21 program. Descriptive statistics consisting of frequency, percentage, average and standard deviation were used in the analysis and evaluation of the data.

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RESULTS

The average age of the enterprise owners included in the study was found to be 41.25. It has been determined that the average duration of horse breeding activity in the enterprise is 17.67 years. The average number of horses available in the enterprises is 37.50. From the findings related to the land assets of the enterprises; it has been determined that 16.67% are tenants and 83.33% are property owners. The average total area of irrigable and dry land in the enterprises was 282.41 decares, the total paddock area was 58.45 decares, the total horse barn area was 4.06 decares, and the total land area was found to be 344.93 decares on average (Table 1).

Table 1. Descriptive presentation of quantitative parameters in enterprises

Parameters	Enterprises			
	Mean	Min.	Max.	Stand. Deviation
Age (years)	41.25	25	73	14.05
Experience of owner (years)	17.67	2	33	8.08
Year of establishment	7.75	1	24	7.68
Number of horses				
Land distribution of enterprises	37.50	6	109	33.09
Irrigable + dry land (da)	282.41	0	1230	405.05
Paddock area (da)	58.45	3	320	90.19
Horse barn area (da)	4.06	0.1	30	8.61
Total land area (da)	344.93	10	1246.10	422.94

Table 2 shows descriptive presentation of some categorical parameters in enterprises. When the education levels of the enterprise owners are examined; it was determined that 16.67% of them were secondary school, 33.33% were high school, 41.67% were university and 8.33% were of a post-graduate program graduates and 50% of the owners of the enterprises receive training in this field of activity.

Table 2. Descriptive presentation of qualitative parameters in enterprises

Parameters	Enterprises	
	n	Mean
Education Level (%)		
Secondary School	2	16.67
High School	4	33.33
University	5	41.67
Post-graduate	1	8.33
Main Economic Activity (%)		
Yes	10	83.33
No	2	16.67
Scale-up Targets (%)		
Continue with current scale	6	50.00
Increase the scale	4	33.33
Continue with current capacity for 5 years	2	16.67
Labor Type (%)		
Family Labor	7	58.33
Hired Labor	1	8.33
Family and Hired Labor	4	33.33
Membership of any associations (%)		
Yes	3	25.00
No	9	75.00
Satisfaction of state supports (%)		
Yes	7	58.33
No	5	41.67
Working with contracted veterinarian (%)		
Yes	6	50.00
No	6	50.00
Receiving training in horse breeding (%)		
Yes	6	50.00
No	6	50.00
Insurance presence (%)		
Yes	1	8.33
No	11	91.67
Land ownership status (%)		
Owner	10	83.33
Tenant	2	16.67

The enterprises are asked about their future enterprise scale targets and 50% of the enterprises declared that they want to ensure continuity with the current herd size, 33.33% plan to increase the scale of the enterprise (to make foal production places, etc.), 16.67% of them want to continue at the same capacity for at least 5 years.

The ratio of enterprises that carry out horse breeding as the main source of income has been determined as 83.33%. The remaining enterprises declared that they also earned income from agricultural activities and retirement. When the labour structure in the enterprises is examined; it has been determined that 58.33% of the enterprises use family labor. The number of enterprises that do not use family labor in the enterprise is only 1, and other enterprises use both family and hired labor together. However, 50% of the enterprises work with a veterinarian on a contractual basis (Table 2).

When the organizational structure of the enterprises is examined; it was observed that 75% of them were not members of any organization. When asked about their opinions on support and credit adequacy, 58.33% of the enterprises stated that the facilities were sufficient, and 41.67% of the enterprises stated that the facilities were not sufficient.

Table 3 shows cost and income factors disctribution among total cost and income in horse enterprises. Among the elements that make up the costs of the enterprises; while the feed cost is in the first place with a rate of 49.33%, it is followed by; labor cost with 16.87%, litter cost with 14.55%, natural mating cost with 8.68%, water-electricity-fuel cost with 5.88%, veterinary-health cost with 1.54% and other costs (such as equipment maintenance, blacksmith cost, riding supplies cost) with 3.14%. When the factors that make up the total income of the enterprises are examined; it has been determined that 43.09% of the total income consists of foal sales income, 21.02% income from races, 16.63% breeding income, 19.26% other income elements such as breeder sale and horse riding-training activities (Table 3).

Table 3. Cost and income factors distribution in total cost and income in %

	Factors	Distribution rate (%)
Expenses of enterprises	Feed cost	49.33
	Labor cost	16.87
	Litter cost	14.55
	Natural mating cost	8.68
	Water- Electricity- Fuel cost	5.88
	Veterinary-Health cost	1.54
	Other costs (Equipment-maintenance cost- Blacksmith cost- Riding supplies cost)	3.14
Incomes of enterprises	Foal sales income	43.09
	Racing income	21.02
	Breeding income	16.63
	Other incomes (Breeder sales, Horse riding and training activities)	19.26

When the main problems encountered in enterprises are examined; it has been observed that the increase in input costs is the main problem in all enterprises. While 83.33% of the enterprises had problems in both marketing and supplying feed, 50% in the supply of labor, and 41.67% of them stated that they faced health problems (Table 4).

Table 4. Current problems in horse enterprises

	Enterprises	
Current Problems (%)	**n**	**Mean**
Marketing	10	83.33
Feed supply	10	83.33
Labor supply	6	50.00
Education/Training	2	16.67
Credit supply	1	8.33
Health issues	5	41.67

*Some owners has reported more than one problem

DISCUSSION AND CONCLUSION

The findings from this study were rather evaluated and interpreted independently because the quantitative and qualitative scientific research on the socio economic analysis of horse breeding enterprises was lacking in the literature. The present study found that the average age was 41.25 while the average working experience was 17.67 years among enterprise owners.

When the education levels of owners were examined, it was revealed that almost more than half of them had a university or higher degree. It is noteworthy that the average age of those engaged in horse breeding is lower than other livestock sub-sectors, but their education level is higher compared to other sectors (11-13).

This situation may cause some positive effects in the sector. Although older horse enterprise owners may have accumulated years of experience and knowledge about breeding practices, horse care, and managing the enterprise, on the other side younger owners may bring fresh perspectives, education in modern practices, and a willingness to adopt innovative approaches. Some researches indicate that there is a favorable correlation between education level and the ability to adjust to new developments and technological advancements (14-16).

In one of the study conducted in Bangladesh, it is reported that horse pulling cart was only and main source of earning for their livelihood in the society (17). Similar to this study, it has been observed that horse breeding is carried out as the main economic activity in most of the enterprises and this is provided mostly by family workforce (81.66%) in our study. The average number of horses was 37.50 in enterprises. When the scale-up goals of enterprises are evaluated, it is seen that the majority of enterprises want to stay at their current scale. It's essential to recognize that the decision to scale up or stay at a certain scale is complex and influenced by a combination of personal preferences, market conditions, and strategic considerations. However, large-scale horse breeding operations can have heavy workloads and responsibilities. Owners may evaluate their time, energy, and experience when determining if they can effectively oversee a larger enterprise. Therefore, to improve work-life balance and minimize risks, some could decide to maintain a smaller scale enterprises.

In this study, it was determined that only 3 of the enterprises included in the research were members of any organization, and the remaining was not members of any organization. One of the most important problems in livestock enterprises in Türkiye is the marketing problem that arises as a result of the insufficient level of organization of the producers. This was also the main problem faced by enterprise owners in our study findings.

In our study, when the main problems faced by enterprises are examined, it is seen that marketing comes first and followed by feed and labor supply respectively. In a study conducted in Çanakkale province, it was stated that a significant portion of horse breeding enterprises had difficulties with feed prices. However, similar to our study, it was observed that there were also problems in labor supply (18). In another study conducted in Western Ethiopia, it was determined that feed shortage was also one of the problem that the horse enterprises faced (19). The study conducted in Central Ethiopia, the constraints were ranked from higher to lower rank based on their impact on equines; accordingly, diseases were the first major constraints (46.67%), followed by feed shortage (26%), water shortage (18%), and marketing problem (9.33%) (20). The results of this study are similar to the results of our study in terms of problems encountered in enterprises.

It is frequently necessary to combine strategic planning, good management techniques, keeping up with market developments, and situational flexibility to overcome these obstacles. Horse enterprise owners can create plans to deal with these difficulties by regularly evaluating the enterprise's strengths and limitations.

The cost factors are important in enterprises. In this study, feed costs with a ratio of 49.33% rank first among the cost factors that constitute the total cost in enterprises. This was followed by 16.87% labour costs, 14.55% litter costs, 8.68% natural mating cost and 5.88% water- electricity- fuel costs, respectively. In a study conducted in Hungary, it was determined that the highest share of costs in horse keeping is labour costs, followed by other costs, feeding costs and finally veterinary costs (21).

The cost of feed and labor in horse breeding enterprises can be higher due to a combination of specialized nutritional needs of race horses, individual horse care requirements, labor-intensive jobs, and the requirement for professional workers. Therefore, effective cost management is essential to the enterprises's capacity to remain financially viable.

On the other hand, for horse breeding enterprises to be successful overall and to remain sustainable, income considerations must be taken into consideration. In a study which is conducted in Hungary, it is stated that horse enterprises get their revenue from mostly renting out their board and providing riding training (21).

Our study results showed that, foal sales income and racing income were two important income factors in total income distribution with the ratio of 43.09% and 21.02% respectively. Since the Arabian horses and racing income have a complex relationship, with each horse's performance on the track affecting both its breeding value and the possible revenue streams available to their owners. The general financial picture of the industry is also significantly influenced by the level of support and popularity for Arabian horse racing.

The market for horses and associated services is subject to fluctuations, since they are impacted by various variables like prevailing equestrian sports and leisure pursuits. Especially in regions with numerous horse enterprises such as our study area, agro-tourism should be more at the

forefront to provide tourists with opportunities to engage in and learn about horse-related activities within a farm or rural setting. Agro-tourism with an equine focus benefits horse enterprises financially and increases tourists' admiration for horses and rural living. In an engaging and instructive way, it may be a mutually beneficial experience that links people to the worlds of agriculture and equestrian sports (22-24).

Horse breeding and training activities in the world, is gradually developing as a more modern and conscious livestock sub-sector, especially in developed countries. On the other hand, equestrianism is on its way to reaching an important industrial level in the development of horse races and other equestrian activities in Türkiye. However, the sector's contribution to the country's economy is also increasing.

From the findings of this study, which was based on horse breeding, it was concluded that horse breeding is the main source of income for most enterprise owners and this activity still maintains its importance for Mahmudiye district. As in many livestock sub-sectors, it is seen that feed cost constitutes the highest share in input costs in horse breeding. The increase in input and especially feed prices and the difficulties experienced in the supply of feed form the basis of the problems faced by the enterprises. In order to provide profitable and productive feeding in horse enterprises, the nutritional needs of horses must be known very well and the ration content must be determined in a balanced and adequate manner accordingly. Therefore, the measures to be taken and the policies to be applied to reduce input costs are important for the sustainability of this livestock activity.

Mahmudiye district is an important location for horse breeding, but also offers important opportunities especially within the scope of agro-tourism activities. The dissemination of activities such as horse riding, equestrian sports etc. to spread the love of horses and equistrian culture, will contribute significantly to the increase in income levels and socio-cultural development with the employment and economic diversity to the local people.

On the other hand, two important recommendations can be given for enterprises operating in Mahmudiye district: i) to create a fieldhouse in Mahmudiye district where horses can train in order to reach a certain condition before the race and to ensure their physical and mental development. ii) to establish a foal sales area in Mahmudiye district in order to shorten the travel time of horses during horse sales transactions and to prevent horses from being injured or traumatized. The work to be carried out by public institutions to implement these suggestions is important for the horse breeders of Mahmudiye district.

In conclusion, it should be taken into consideration that financial and economic researches to be carried out at different levels and areas of activity in the horse industry will make a significant contribution to enterprises and the sector in general.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

AUTHOR CONTRIBUTIONS

In this study, the research design and methodology were determined by AP, AEÜ and YA. Data collection and fieldwork were conducted by AEÜ, AP and OA, and data analysis was conducted by AP, OA, ŞO and YA. The writing of the article was carried out by AP, AEÜ and ŞO, and the editing and control of the article was completed by all authors. All authors read and approved the final version of the article.

ETHICAL STATEMENT

The authors declare that this study does not require the ethical statement.

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ARAŞTIRMA MAKALESİ

Diyarbakır Yöresinde Mastitisli Keçi Sütlerinde Etken İzolasyonu ve Duyarlı Antibiyotiklerin Belirlenmesi

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Öz

Keçi sütünün, laktoz intoleransı olan insanlarda inek sütüne alternatif olmasından dolayı son yıllarda dünya genelinde tüketiminde artışı görülmektedir. Keçi mastitisi, süt kalitesini olumsuz etkilemekte ve süt veriminde azalmalara yol açmaktadır. Bu nedenle mastitis patojenlerinin belirlenmesi enfeksiyonun önlenmesi açısından önemlidir. Bu çalışmanın amacı Diyarbakır ve ilçelerinde bulunan keçilerde, subklinik mastitis etkenlerinin izolasyonu ve identifikasyonu ile bunlara etkili antibiyotiklerin minimal inhibitör konsantrasyonu (MİK) yöntemi ile belirlenmesidir. Bu amaçla toplam 358 adet keçi süt örneğine California Mastitis Testi (CMT) ile mastitis yönünden bakıldı. CMT (+2,+3) pozitif olan toplam 106 süt örneğinin mikrobiyoloji incelemesinde 79'undan (%74.5) bakteri izole edilirken, 27'sinden (%25.5) herhangi bir üreme gözlenmedi. Üreme olan süt örneklerinin 30'undan (%37.97) *Staphylococcus aureus* ve 26'sından (%32.91) *Koagülaz-negatif Staphylococcus* (KNS), 3'ünden (%3.80) *Streptococcus agalactiae*, 1'inden (%1.27) *Enterococcus* spp., 18'inden (%22.78) *Escherichia coli* ve 1'inden de (%1.27) *Acitenobacter* spp. izole ve identifiye edildi. KNS pozitif olan bakterilerin orantısal olarak dağılımı %20.25 *Staphylococcus xylosus*, %5.06 *Staphylococcus warneri*, %2.53 *Staphylococcus lugdunensis*, %1.27 *Staphylococcus capitis*, %2.53 *Staphylococcus chromogenes* ve %1.27 *Staphylococcus hominis* olarak identifiye edildi. Çalışmada izole ve identifiye edilen bu bakterilere karşı yapılan MİK sonuçlarına göre siprofloksasin, tigesiklin, benzilpenisilin, oksasilin ve seftazidimin mastitis tedavisinde etkili olabileceği belirlendi.

Anahtar Kelimeler: İdentifikasyon, izolasyon, keçi, mastitis, prevalans

The Isolation of Mastitis Agents and Determination of Sensitive Antibiotics in Goat Milks with Subclinical Mastitis in Diyarbakır Region

Abstract

There has been an increase in the consumption of goat milk worldwide in recent years, as it is an alternative to cow milk for people with lactose intolerance. Mastitis in goats negatively affects the quality of milk and leads to decreases in milk yield. Therefore, identifying mastitis pathogens is important to prevent infection. The aim of this study is to isolate and identify subclinical mastitis agents in goats in Diyarbakır and its districts and to determine effective antibiotics by the minimum inhibitory concentration (MIC) method. A total of 358 goat milk samples were examined for mastitis with the California Mastitis Test (CMT). In the microbiology examination of a total of 106 milk samples that were CMT (+2, +3) positive, while bacteria were isolated in 79 (74.5%) samples, no growth was observed in 27 (25.5%) samples.. Of the milk samples with bacterial growth, 30 (37.97%) were *Staphylococcus aureus*, 26 (32.91%) were *Coagulase-negative Staphylococci* (CNS), 3 (3.80%) were *Streptococcus agalactiae*, 1 (1.27%) *Enterococcus* spp., 18 (22.78%) *Escherichia coli* and 1 (1.27%) *Acitenobacter* spp. was isolated and identified. Proportional distribution of CNS positive bacteria: 20.25% *Staphylococcus xylosus*, 5.06% *Staphylococcus warneri*, 2.53% *Staphylococcus lugdunensis*, 1.27% *Staphylococcus capitis*, 2.53% *Staphylococcus chromogenes* and 1.27% *Staphylococcus hominis* were identified. According to the MIC results against these bacteria isolated and identified in the study, it was determined that ciprofloxacin, tigecycline, benzylpenicillin, oxacillin and ceftazidimine could be effective in the treatment of mastitis.

Key Words: Goat, identification, isolation, mastitis, prevalence

GİRİŞ

Keçi yetiştiriciliği, hayvansal protein kaynağı sunması, laktoz intoleransı olan insanlarda inek sütüne alternatif olması ve keçi sütünden üretilen süt ürünlerinin popüler olması dolayı son yıllarda dünya genelinde artış göstermektedir (1-3). Ancak, keçi yetiştiriciliğinde mastitis, sütün kalitesini ve verimini olumsuz etkilemektedir. Mastitis meme bezlerinin enfeksiyonu olup klinik ve subklinik formlarda görülebilmektedir (2,4,5). Subklinik mastitislerde meme bezi normal görünümündedir ve klinik mastitislere göre daha yaygındır (5,6). Subklinik mastitis teşhisi, somatik hücre sayımı ve sütün bakteriyolojik kültürü ile yapılabilmektedir. Subklinik mastitis teşhisinde California Mastitis Test (CMT) yaygın bir biçimde kullanılmaktadır (7,8). Ancak, fizyolojik olarak birim bazında keçi sütü, koyun ve inek sütüne göre daha fazla miktarlarda somatik hücre bulundurur (ml'de 600.000-800.000 somatik hücre) (9-11).

Keçilerde klinik ve subklinik mastitisin bakteriyel patojen etkenleri arasında *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*S. agalactiae*) ve *Pseudomonas aeruginosa* ve koagulaz negatif *Staphylococcus* (KNS), *Pasteurella (Mannheimia) haemolytica, Corynebacterium pseudotuberculosis, Mycoplasma* spp. ve nadiren *Salmonella* spp. ve *Listeria* spp patojenleri olduğu bildirilmektedir (11,12,13,14). Ancak, keçilerde tüm mastitis formlarında görülen başlıca etiyolojik etken *Staphylococc*us türleri olmakla beraber klinik mastitiste buna ilaveten koagulaz-pozitif stafilokoklar, subklinik mastitiste ise koagülaz-negatif Stafilokok (KNS) etkenlerine daha yoğun rastlanmaktadır (5,6,8,15,16,17). Keçilerde mastitis vakalarında nadiren mantar, maya, virüs ve mikoplazma etkenleri de tespit edilmektedir (6,11,18,19).

İneklerde ve koyunlarda mastitis ile ilgili dünyada birçok çalışma yapılmasına rağmen keçi mastitisleri ile ilgili çalışmaların inek ve koyuna göre daha az olduğu görülmektedir. Bu amaçla; Diyarbakır ve çevresinde keçi yetiştiriciliğinin önemli sorunlarından biri olan mastitis olgularında subklinik mastitise neden olan etkenlerin belirlenmesi ve bunlara karşı etkili antibiyotik türlerinin saptanması hususları bu çalışmada araştırılmıştır.

MATERYAL VE METOT

Saha Çalışması Aşaması

Bu araştırmada, Diyarbakır ve yöresinde 20 farklı aile işletmesinde yetiştirilen 179 kıl keçisinden süt örnekleri alınmıştır. Örneklemede subklinik mastitis teşhisi amacıyla 358 adet süt örneği CMT yöntemi kullanılarak test edilmiştir (20). Subklinik mastitis teşhisi konulmasında CMT skorlaması araştırıcıların (20-23) ifade ettiği şekilde yapılıp pozitif olanlar (CMT; +2 ve +3) belirlenerek çalışmaya dahil edilmiştir. CMT pozitif sonuç veren 106 hayvandan alınan aynı sayıdaki süt örneği, çalışmanın materyalini oluşturmuştur. Örnekler alınmadan önce meme başları %70'lik alkolle temizlenmiş ve önsüt atıldıktan sonra sağılan süt, steril tüplere (8-10 ml) alındıktan sonra kısa sürede soğuk zincir ile Dicle Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı laboratuvarına getirilmiştir.

Laboratuvar Analizleri Aşaması

Bakteriyel izolasyon ve identifikasyon

Süt örnekleri homojenize edildikten sonra her biri %5 koyun kanlı agar (Oxoid, CM0055, İngiltere) ve Eosin Methylen Blue agarlara (Oxoid, CM0069, İngiltere) ekilmiş ve 37°C'de aerobik koşullarda 24-48 saat inkübe edildi. İnkübasyon sonrasında koloniler nutrient agara (Condalab, 1060, İspanya) pasajlanıp ve aynı koşullarda inkübe edilmiştir (24). Gram boyama ile G (+) ve G (-) özellikleri belirlenen suşlar VITEK® 2 GP ID ve VITEK® 2 GN ID kartları ile VITEK® 2 Compact Sistem (Biomerieux®, Fransa) ile identifiye edildi.

Mycoplasma spp. izolasyonu için süt örnekleri, Mycoplasma selektif suplement G (Oxoid, SR0059, İngiltere) eklenmiş Mycoplasma sıvı besiyerine (Oxoid, CM0403, İngiltere) ekildi ve 37°C'de mikroaerofilik koşullarda 72 saat inkübe edildi. İnkübasyon sonrası sıvı kültürler Mycoplasma selektif suplement G ilave edilmiş Mycoplasma agara (Oxoid, CM0401, İngiltere) pasajlanarak aynı koşullarda inkübe edildi. İnkübasyonun 48. saatinden itibaren agarlar her gün tipik *Mycoplasma* spp. koloni morfolojisi yönünden ışık mikroskobunda 4-10x (stereomikroskopta 20-60x) büyütmede görüntülendi. 14. günden sonra tipik koloni belirlenmeyen örnekler *Mycoplasma* spp. negatif olarak kaydedilmiştir (25).

Antibiyotik Duyarlılık Özelliklerinin Saptanması

Bakteri ve MİK belirleme

Toplam 358 süt örneği, en az 3 hafta antibakteriyel tedaviye maruz kalmayan laktasyondaki keçilerden toplandı. Subklinik mastitis teşhisi açısından CMT pozitif sonuç veren 106 hayvandan alınan süt örneğinden 79 süt numunesinde bakteriyel üreme oldu. Üreme görülen 79 bakteri izolatı MIC için kullanıldı. Bu süt örneklerinden izole edilen bakteriler için belirlenen kartlar kullanılarak antibiyotiklere olan direncini ve moleküler özelliklerini VİTEK-2 sistemi (BioMerieux, Fransa) ile belirlendi. Minimum inhibitör konsantrasyon (MİK) (arama aralığı, ≤0.5) sistemin tescilli yazılımı (sürüm 9.02) tarafından üreticinin talimatlarına göre VITEK 2 AST-P640 (sefoksitin, benzilpenisilin, oksasilin, gentamisin, siprofloksasin, eritromisin, klindamisin, linezolid, daptomisin, teikoplanin, vankomisin, tetrasiklin, tigesiklin, fosfomisin, fusudik asit ve trimetoprim/sülfametoksazol) ve AST-N327 (ampisilin, amoksisilin klavunik asit, piperasilin/tazobaktam, sefuroksim, sefuroksim aksetil, sefoksitin, sefiksim, seftazidim, seftriakson, ertapenem, imipenem, meropenem, amikasin, gentamisin, siprofloksasin, fosfomisin, nitrofurantoin ve trimetoprim/sülfametoksazol) duyarlılık kartları (bioMérieux) kullanılarak otomatik olarak ilişkilendirilmiştir. Duyarlılık testi sonuçlarına ilişkin veriler Microsoft Excel 2013 ile analiz edildi. Bu MİK'ler daha sonra dirençli veya duyarlı olarak sınıflandırılmıştır.

BULGULAR

Subklinik mastitis açısından kontrol edilen toplam 358 örnekten, 106'sının (%29.6) CMT pozitif sonuç verdiği saptanmıştır. Pozitif kabul edilen 106 süt örneğinin 79'undan (%74.5) bakteri izole edilirken, 27'sinden (%25.5) herhangi bir üreme gözlenmemiştir. Kültür pozitif örneklerin 30'undan (%37.97) *S. aureus* ve 26'sından (%32.91) KNS izole edilmiştir. Bunların

da orantısal olarak dağılımı %20.25 *S. xylosus*, %5.06 *S. warneri*, %2.53 *S. lugdunensi*, %1.27 *S. capitis*, %2.53 *S. chromogenes* ve %1.27 *S. hominis* şeklinde bulunmuştur. Bunların dışında 3'ünden (%3.80) *S. agalactiae*, 1'inden (%1.27) *Enterococcus* spp., 18'inden (%22.78) *E. coli* ve 1'inden de (%1.27) *Acitenobacter* spp. izole ve identifiye edilmiştir (Tablo 1).

MİK Analizi

Laktasyondaki keçilerden toplanan ve subklinik mastitis testi pozitif olarak bildirilen 79 süt numunesinden toplam 11 izolat elde edilmiştir. Elde edilen bu izolatlara karşı test edilen bazı antimikrobiyal bileşiklerin MİK'leri Tablo 2 ve Tablo 3'de gösterilmektedir.

Tablo 1. CMT pozitif olan süt örneklerinden izole edilen mikroorganizmalar

İzole edilen bakteriler	Pozitif Örnek Sayısı % (n)
S. aureus	%37.97 (30)
S. xylosus	%20.25 (16)
S. warnerii	%5.06 (4)
S. lugdunensis	%2.53 (2)
S. capitis	%1.27 (1)
S. chromogenes	%2.53 (2)
S. hominis	%1.27 (1)
S. agalctiae	%3.80 (3)
Enterococcus spp.	%1.27 (1)
E. coli	%22.78 (18)
Acitenobacter spp.	%1.27 (1)
Toplam	**100 (79)**

Tablo 2. VITEK 2 kullanılarak subklinik mastitis pozitif izolat olarak bildirilen 60 süt örneğinin antimikrobiyal duyarlılık profilleri (bioMérieux, Marcy-l'Étoile, France)

	MİK (µg/mL)														
İzolatlar (n)	BP	OX	GEN	CIP	E	DA	LNZ	DAP	TP	VA	TE	TGC	FF	FA	SXT
Staphylococcus aureus (30)	≤0.03	≤0.25	≤0.5	≤0.5	≤0.25	≤0.12	1	≤0.12	≤0.5	≤0.5	≤1	≤0.12	≤8	≤0.5	≤10
Strept. agalactiae (3)	≥64	-	-	-	-	≥8	≥8	≥8	≥32	≥32	2	≤0.12	-	-	≤0.10
Staphylococcus xylosus (16)	-	≤0.25	≤0.5	≤0.5	≤0.25	≤0.12	1	≤0.12	1	≤0.5	≤1	≤0.12	≤8	≤0.5	≤10
Staphylococcus warneri (4)		≤0.25	≤0.5	≤0.5	1	≤0.12	1	≤0.12	2	1	≥16	≤0.12	≥128	≤0.5	≤10
Staphylococcus lugdunensis(2)	≤0.03	≤0.25	≤0.5	≤0.5	1	0.5	1	0.25	-	-	≤1	≤0.12	≤8	≤0.5	≤10
Staphylococcus capitis (1)		≤0.25	≤0.5	≤0.5	≤0.25	≤0.12	1	0.5	≤0.5	≤0.5	≤1	≤0.12	≥128	≤0.5	≤10
Staphylococcus chromogenes (2)		≤0.25	≤0.5	≤0.5	1	≤0.12	1	≤0.12	1	≤0.5	≤1	≤0.12	≤8	≤0.5	≤10
Staphylococcus hominis (1)		≤0.25	≤0.5	≤0.5	0.5	0.25	2	≤0.12		≤0.5	≥16	≤0.12	64	≤0.5	≤10
Enterococcus spp.(1)				1			2		≤0.5	≤0.5		≤0.12			≤10

BP: Benzilpenisilin, OX: Oksasilin, GEN: Gentamisin, CIP: Siprofloksasinin, E: Eritromisin, DA: Klindamisin, LNZ: Linezolid, DAP: Daptomisin, TP: Teikoplanin, VA: Vankomisin;TE: Tetrasiklin TGC: Tigesiklin, FF: Fosfomisin, FA: Fusidik asit, SXT: Trimetoprim + Sülfametoksazol

Tablo 3. VITEK 2 kullanılarak subklinik mastitis pozitif izolat olarak bildirilen 19 süt örneğinin antimikrobiyal duyarlılık profilleri (bioMérieux, Marcy-l'Étoile, France)

	MİK (µg/mL)																	
İzolatlar (n)	AMP	AMK	TZP	CXM	ACE	CFT	CFX	CAZ	CTX	ERT	IPM	MEM	AMC	GEN	CİP	FF	NF	SXT
E. coli (18)	≤2	≤2	≤4	≤1	≤1	≤4	≤0.25	≤0.12	≤0.25	≤0.12	≤0.25	≤0.25	≤2	≤1	≤0.25	≤16	≤16	≤20
Acitenobacter (1)	-	-	8	-	-	-	-	0,5	-	-	≤0.25	≤0.25	≤2	≤1	≤0.25	-	-	≤20

AMP: Ampisilin, AMK: Amoksisilin/klavulanat, TZP: Piperasilin/tazobaktam, CXM: Sefuroksim, ACE: Sefuroksim/asetil, CFT: Sefoksitin, CFX: Sefoksitin, CAZ: Ceftazidime, ERT: Ertapenem, Imipenem (IPM), Meropenem (MEM), Amikasin; AMC, Gentamisin (GEN), CIP: Siprofloksasin, FF: Fosfomisin, NF: Nitrofurantoin, SXT: Trimetoprim + Sülfametoksazol

TARTIŞMA VE SONUÇ

Küçükbaş ruminantlarda subklinik mastitis görülme aralığı %6.5 ile %40.2 arasında değiştiği ifade edilmektedir (1,8,26,28). Yapılan çalışmada subklinik mastitis prevalans oranının (%29.6), araştırıcıların bildirdiği oranların aralığında olduğu görülmektedir.

Çalışmalarda etken değişkenliği ve görülme oranındaki farklılıklar ile bunların duyarlı oldukları antibiyotikler bölgelere göre değişiklik gösterebilmektedir. Keçi mastitislerinde en çok izole edilen bakterilerin stafilokoklardır. Doğruer ve ark. (8)'nın Hatay ve çevresindeki keçilerde tüm *Staphylococcus* spp. oranı %71.5 ve bunlar arasında en fazla olanın da %23.7 ile *S. intermedius* olduğu bunun dışında %8 *Streptococccus spp.,* %5 *Bacillus*, %4 *Escherichia coli,* %3.4 *Corynobacterium* spp., %2.3'er oranda *Acinetobacter* ve *Pseudomonas* ile %2.3 miks enfeksiyon tespit ettiklerini bildirmektedirler. İşnel ve Kırkan (29)'ın Aydın bölgesindeki keçilerde yapmış oldukları çalışmada ise %69.6 oranında *S. aureus,* %7.8 oranında *S. epidermidis*, %4.9 oranında *S. intermedius,* %3.9 oranında *Klebsiella pneumoniae* ve düşük oranlarda *Corynebacterium* spp., *Pseudomonas* spp., *E. coli*, ve *Mannheimia haemolytica* etkenlerinin izole edildiği bildirmektedirler. Cantekin ve ark.'ı (3) Hatay bölgesinde en fazla KNS (%50), *S. aureus* (%26.67) ve *S. uberis* (%16.67) etkenleri olduğunu belirtmektedirler. Aydın ve ark. (23)'nın Konya'da yapmış oldukları çalışmada %17.7 *Staphylococcus aureus* ve %69 KNS olarak izole edildiği rapor edilmiştir. İtalya ve Bulgaristanda yapılan çalışmalarda en çok izole edilen bakteri türünün *Staphylococcus* spp. olduğu ifade edilmektedir (30,31). Pirzada ve ark. (11)'nın Pakistan'da keçilerde yaptıkları subklinik mastitis çalışmasında %38 oranında pozitif sonuç elde edildiği ve bunlardan da en fazla *S. aureus* (%36.84) izole edildiğini belirtmektedirler. Zhao ve ark. (6)'nın Çin'de yaptıkları çalışmada en fazla oranda KNS (%59.2) ve *S. aureus* (%15.24) etkenlerinin izole edildiğini bildirilmektedir.

Bu çalışmada 106 örnekten 79'unda üreme elde edildiği, bunlar arasında en fazla izole edilen bakteri türünün ise %70.88 ile *Staphylococcus* spp.*,* oluşturduğu saptanmıştır. Bunun da %37.97'si *S. aureus,* %20.25 *S. xylosus,* %5.06 *S. warnerii,* %2.53 *S. lugdunensis,* %2.53 *S. chromogenes,* %1.27 *S. capitis,* ve %1.27 *S. hominis* şeklinde dağılım gösterdiği görülmüştür. Çalışmada KNS toplam oranı %32.91 olarak hesaplanmıştır. *Staphylococcus* spp.*,*'den ayrı olarak *Enterococcus* spp.*, E. coli* ve *Acinobacter* etkenleri de örneklerden izole edilen diğer mikroorganizmalardır. Yapılan benzer çalışmalarda keçilerde subklinik mastitislerde en fazla *Staphylococcus* spp.*,* etkenlerinin izole edildiği sonuçları çalışmamızda bulunan veriler ile benzerlik gösterdiği görülmektedir. Ancak araştırıcıların değişik bölgelerde elde ettikleri KNS oranları ile karşılaştırıldığında sunulan çalışmada bu oranın daha az, *S. aureus* ve *E. coli* oranının ise daha fazla olduğu görülmüştür (3,6,8,23). Bunun da yukarıda belirtilen araştırmalarda olduğu gibi bazı bölgelere göre bu oranların değişebildiği veya etkenlerin görülme oranlarında farklılıkların olduğu benzer birçok çalışma ile ortaya konmuştur. Değişik bölgelerde keçilerde yapılan subklinik mastitis etken izolasyonuna yönelik çalışmalardaki mikroorganizmalar arasındaki çeşitlilik ve görülme oranları arasındaki bu farklılıkların coğrafi bölge değişiklikleri, bakım ve beslenme koşulları gibi faktörlerden etkilendiği düşünülmektedir.

Mastistis tedavisinde doğru tanı ve eş zamanlı uygun ilaç seçimi önemli bir yer tutmaktadır (32). Son yıllarda dünya genelinde hatalı teşhis, antibiyotiklerin aşırı veya uygunsuz kullanımı uygun olmayan antibiyotik seçimi ve yetersiz doz ayarlaması gibi durumlar antibiyotiklere direncin artmasına katkıda bulunmuş ve insanlarda ciddi halk sağlığı sorunlarının ortaya çıkmasına neden olmaktadır (33). Bu gibi nedenlerden dolayı klinik antimikrobiyal ilaçlara karşı duyarlılık testlerinin yapılması gereklilik haline gelmiştir. Bu amaçla olası patojenlerin belirlenmesi ve hastalığa neden olan patojenlere karşı gerçekleştirilen duyarlılık testlerinin yapılması tedavide elde edilen başarı oranında artışa neden olmaktadır (34,35). Minimum inhibitör konsantrasyon (MİK), patojenlerin antimikrobiyal duyarlılığında yaygın olarak kullanılmaktadır (36). Minimum inhibitör konsantrasyonu belirlemek için genellikle disk difüzyon prosedürü, mikrodilüsyon metodu, E testi ve otomatik ticari antimikrobiyal duyarlılık testleri kullanılır. Hızlı tanımlama ve antimikrobiyal duyarlılık testi yapan otomatik sistemler giderek daha fazla kullanılmaktadır (37,38). Daha önce keçilerde yayınlanmış çalışmaların çoğu az sayıda izolat ile ve otomatik metodlar kullanılarak birkaç antibiyotiğin MİK değerlerine odaklanmaktadır (39,40). Bu çalışma ise çok sayıda mastitis izolatının MİK değerlerinin VİTEK II sistemi kullanılarak değerlendirildiği ilk çalışmalardan biri olma özelliğini taşımaktadır.

Elde edilen sonuçlara benzer şekilde *Staphlococcus* spp. ve *E. coli* patojenleri mastitisli keçilerde izole edilen ana mastitis patojenleri olarak kabul edilir ve bu bakterilere bağlı meydana gelen enfeksiyonlar hastalıkla ilgili önemli ekonomik kayıplara neden olmaktadır. (6,41). Bu nedenle çalışmamızın sonuçları, veteriner hekimlerin mastitis ile enfekte keçileri tedavi etmek için antibiyotik kullanmaya karar verirken *Staphlococcus* spp. ve *E. coli* gibi patojenleri dikkate almaları gerektiğini göstermektedir.

Tüm mastit izolatlarının, uzun süredir mastitis tedavisinde tercih edilen florokinolonların grubunda bulunan siprofloksasinin bu çalışmada belirlenen tüm izolatlara karşı MİK değerlerinin ≤0.25 ve ≤0.5 µg/mL olarak düşük veya eşit olduğu belirlenmiştir. Bu konsantrasyon önceki raporlarda elde edilen siprofloksasin konsantrasyonlarına benzemektedir (42). Tigesiklinin MİK değerleri tüm staphlococcus, streptococcus ve enterococcus izolatlarına karşı ≤0.12 µg/mL'ye eşit veya daha düşük oranla en etkili MİK konsantrasyonunu sağlamıştır. Tigesiklin bazı dirençli enfeksiyonların tedavisinde kulllanımı önerilen glisiklin grubu bir antibiyotiktir (43,44). Benzilpenisilin'in *S. aureus* ve *S. lugdunensis'e* karşı yüksek etkinliğini bu araştırmada gözlemlenmiştir (MİK≤0.03 µg /mL). Penisilin grubunda bulunan oksasilin, MİK ≤0.25 µg /mL ile bu çalışmada izole edilen staphylococcuslara karşı güçlü aktivite gösterirken enterococcusa karşı MİK değeri belirlenemedi. Seftazidim ise *E. coli* ve *Acitenobacter* izolatlarına karşı ≤0.12 ve 0.5 µg/mL MİK göstermiştir. Bu sonuçlara göre ilgili antimikrobiyaller ile tedaviye başlamadan önce mastitise neden olan ajan kesin olarak tanımlanmadan tedavi protokolü oluşturulabilir. Ancak siprofloksasin, tigesiklin, benzilpenisilin, oksasilin ve seftazidimin farmakolojik özellikleri ve yan etkileri nedeniyle gıda değeri olan hayvanlarda kullanımı ile ilgili sınırlı bilgiler bulunmaktadır.

Sonuç olarak Diyarbakır ve çevresinde kıl keçilerinde subklinik mastitis etken izolasyonuna yönelik yapılan çalışmada koagulaz pozitif stafilokokların izolasyon oranının yük-

sek olduğu ve özellikle *S. aureus* bakterilerinin ağırlıkta görüldüğü tespit edilmiştir. Bu çalışmada sunulan MİK değerleri, mastitisli keçilerde antimikrobiyal direncin gelişimini izlemek ve klinik sınır değerlerinin belirlenmesine yardımcı olmak için kullanılabilir. Ancak keçilerde ilgili ilaçların tedavide kullanımının önerilmesi için detaylı farmakokinetik, farmakodinamik ve klinik çalışmalarının yapılmasına ihtiyaç olduğu sonucuna varılmıştır.

TEŞEKKÜR

Maddi desteklerinden dolayı Dicle Üniversitesi Bilimsel Araştırma Projeleri Koordinatörlüğüne teşekkür ederiz.

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Bu çalışma Dicle Üniversitesi Bilimsel Araştırma Projeleri Koordinatörlüğü tarafından desteklenen "VETERİNER17.015" nolu projeden hazırlanmıştır.

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ETİK BEYAN

Dicle Üniversitesi Sağlık Bilimleri Uygulama Ve Araştırma Merkezi, Sayı No: 700193

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RESEARCH ARTICLE

Distribution of Some Heat Shock Proteins in the Tongue Tissues of Sheep of Different Ages

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Abstract

Heat shock proteins are molecular chaperones that regulate and modulate a multitude of cellular and physiological processes. This study was designed to determine the immunoexpression of HSP27 and HSP90 in tongue tissues throughout the development of sheep. Tongue tissues were collected from sheep aged 6-12 months (G1,n:6), 1-2 years (G2,n:6), and 3-5 years (G3,n:6). Immunohistochemical staining was performed after the tissue samples were subjected to routine histological procedures. Immunoreactivity for HSP27 and HSP90 was not observed in peripheral nerves, serous Von Ebner's glands, and mucous glands. While HSP27 immunoreactivity was not observed in the gland duct epithelium, HSP90 immunoreactivity was detected. HSP27 and HSP90 immunoreactivity was seen in the epithelial layer, skeletal muscle cells, vascular endothelium, and vascular smooth muscle cells. There were no statistically significant differences in HSP90 immunoreactivity in the gland duct epithelium, epithelial layer, blood vessels, and skeletal muscle cells throughout the development of the sheep ($p>0.05$). While HSP27 immunoreactivity in blood vessels and the epithelial layer was not statistically changed between groups ($p>0.05$), HSP27 immunoreactivity in skeletal muscle cells was statistically higher in G1 compared to G3 ($p<0.01$). The results of this study demonstrated that HSP27 and HSP90 were expressed in the luminal epithelium, blood vessels, and skeletal muscle cells of the tongue tissues throughout the development of the sheep. This study shows that HSP27 and HSP90 are essential for sheep tongue development and play critical roles in cellular events.

Key Words: Heat shock protein, HSP27, HSP90, immunohistochemistry, sheep, tongue

Farklı Yaşlardaki Koyunların Dil Dokusunda Bazı Isı Şok Proteinlerinin Dağılımı

Öz

Isı şok proteinleri çok sayıda hücresel ve fizyolojik süreci düzenleyen ve modüle eden moleküler şaperonlardır. Bu çalışma, koyunların gelişimi boyunca dil dokularında HSP27 ve HSP90' nın immüneksp-resyonunu belirlemek için tasarlandı. 6-12 aylık (G1,n:6), 1-2 yaşındaki (G2,n:6) ve 3-5 yaşındaki (G3,n:6) koyunlardan dil dokuları toplandı. Doku örnekleri rutin histolojik işlemlere tabi tutulduktan sonra immünohistokimyasal boyama yapıldı. Periferik sinirlerde, seröz Von Ebner bezlerinde ve müköz bezlerinde HSP27 ve HSP90 immünoreaktivitesi gözlenmedi. Kanal epitelinde HSP27 immünoreaktivitesi görülmezken, HSP90 immünoreaktivitesi tespit edildi. Epitel katmanda, iskelet kası hücreleri, damar endoteli ve damar düz kas hücrelerinde HSP27 ve HSP90 immünoreaktivitesi görüldü. Epitel katman, iskelet kası hücreleri, kanal epiteli ve kan damarlarındaki HSP90 immünoreaktivitesinde koyunların gelişimi boyunca istatistiksel olarak anlamlı bir farklılık saptanmadı ($p>0.05$). Kan damarları ve epitel katmandaki HSP27 immünoreaktivitesi gruplar arasında istatistiksel olarak değişmezken ($p>0.05$), iskelet kası hücrelerindeki HSP27 immünoreaktivitesi G3'e kıyasla G1'de istatistiksel olarak daha yüksekti ($p<0.01$). Bu çalışmanın bulguları HSP27 ve HSP90'nın koyunların gelişimi boyunca dil dokularının epitel katmanından, kan damarları ve iskelet kası hücrelerinden eksprese olduğunu göstermiştir. Bu çalışma, HSP27 ve HSP90'nın koyunların dil gelişimi için gerekli olduğunu ve hücresel olaylarda kritik roller oynadığını göstermektedir.

Anahtar Kelimeler: Dil, HSP27, HSP90, ısı şoku proteini, immünohistokimya, koyun

INTRODUCTION

The manner in which vertebrates are fed and their dietary habits are significant factors in determining their capacity to adapt to their environment. The tongue, in conjunction with other oral cavity organs, plays a pivotal role in the process of feeding. The structural differences observed in the tongue are indicative of the specific food sources and the particular habitat of each species of mammal (1,2).

The tongue is covered with stratified squamous keratinized epithelium. The keratinized stratified squamous epithelium is divided into several layers: the stratum basale, the stratum spinosum, the stratum granulosum, and the stratum corneum (3). The tongue's mucosa comprises various papillary systems that serve gustatory and mechanical functions (1,4). The majority of the mammalian tongue is composed of longitudinal, transverse, and vertical skeletal muscle cells (3). The submucosa and connective tissue between skeletal muscle cells contain serous Von Ebner's, mucous, and seromucous lingual glands. The connective tissue beneath the epithelium includes adipose tissue, blood vessels, and nerve plexuses (5).

Heat shock proteins (HSPs), also known as molecular chaperones, are highly conserved proteins (6). These proteins are essential for the normal functioning of cells and play a significant role in protecting cells against damage in stressful conditions (7,8). HSPs are overexpressed in a variety of environmental conditions, including temperature changes, oxidants, ethanol, radiation, viral infections, heavy metal ions, and anoxia (8). HSPs are known to perform several cell-protective functions, including protein assembly and disassembly, interaction with surface receptors, and antigen presentation (6,7). HSPs are categorized according to their molecular weight and function. This classification includes the small HSPs, HSP40, HSP60, HSP70, HSP90, and HSP110 (7,9,10).

HSP27 is a small molecular weight HSP family member and an ATP-independent chaperone (11,12). HSP27 prevents the aggregation of misfolded proteins and assists in correctly folding proteins. HSP27 is involved in cellular functions such as proliferation, differentiation, migration, and signal transduction (11). HSP27 is associated with several intermediate filament networks. HSP27 regulates apoptosis by interacting with key components of the apoptotic signaling pathway. HSP27 is associated with actin and regulates the polymerization of actin (12).

HSP90 forms 1-2% of cellular protein under physiological conditions. HSP90 is an ATP-dependent molecular chaperone that regulates the activation, late maturation, and stability of a variety of proteins. HSP90 interacts with nuclear or cytoplasmic proteins, including transforming or regulatory tyrosine kinases, some serine/threonine kinases, transcription factors, cytoskeletal proteins, or calmodulin. HSP90 is involved in fundamental cellular processes and regulatory pathways such as apoptosis, cell signaling, cell cycle control, and cell viability by interacting with other proteins and co-chaperones in the cells (13). HSP90 plays a role in antigen presentation, and activation of lymphocytes, macrophages, and dendritic cells (14).

The tongue is an important organ that can provide information about the general health status of animals. Therefore, studies on the tongue of sheep are of great importance both for applied veterinary medicine and animal health and for basic biological research. Changes in the expression of molecular factors in tongue tissue can be used to understand sheep's responses to temperature changes, malnutrition, or other stress factors. Knowing the expression patterns of molecular factors in the tongue tissue, which undergoes structural and morphological changes during the developmental process of sheep, may contribute to the understanding of the functional properties and adaptation mechanisms of the tongue. HSP27 and HSP90 play important roles in metabolic events in cells under normal physiological conditions, while under various stress conditions, the expression of these proteins in cells is increased and they play important roles in cellular repair and protection mechanisms. There are a limited number of studies on the expression of HSP27 and HSP90 in tongue tissue. Therefore, this study aimed to show the expression of HSP27 and HSP90 in the tongue tissues of sheep of different ages by immunohistochemistry.

MATERIAL AND METHODS

Animal Material and Tissue Processing

The tissue material used in this study was obtained from sheep that were brought to the abattoirs in the province of Siirt for slaughter. Tongue tissues from a total of 18 sheep aged 6-12 months (G1,n=6), 1-2 years (G2,n=6), and 3-5 years (G3,n=6) were used as tissue material in the study. Small pieces of tissue taken from the tongue of each animal were fixed in 10% formaldehyde (pH=6.9-7.1) for 24 hours at room temperature. Tissue samples were embedded in the paraffin after routine histological processing. Tissue blocks were cut at a thickness of 5 microns. Sections were transferred to poly-L-lysine-coated slides.

Immunohistochemical Staining

Immunohistochemical staining was performed using the streptavidin-biotin-peroxidase complex (Strept-ABC) to determine the immunoreactivities of HSP27 and HSP90 in sheep tongue tissues of different ages. The sections were passed through the xylol-alcohol series. Afterward, they were placed in citrate buffer (pH=6) and subjected to boiling in a microwave oven at 600W for 20 minutes to facilitate antigen retrieval. To inhibit the endogenous peroxidase activity, the sections were incubated in 3% H_2O_2 in PBS for 20 min. The sections were encircled with a hydrophobic PAP pen and maintained in a blocking solution (Large Volume Ultra V Blok, TA-125-UB, Thermo Fisher Scientific) for 10 minutes to prevent non-specific antigenic binding. Afterward, the sections were incubated with HSP27 (Santa Cruz Biotechnology, sc-13132, dilution: 1/150) and HSP90 (Santa Cruz Biotechnology, sc-13119, dilution:1/200) primary antibodies at 4°C overnight. The next day, a biotinylated secondary antibody (Biotinylated Goat Anti-Polyvalent, Thermo Fisher Scientific, TP-125-BN) was applied to the sections for 30 minutes. Then, they were kept with enzyme-conjugated streptavidin solution (Streptavidin Peroxidase, Thermo Fisher Scientific, TS-125-HR) for 30 minutes. To visualize the immunostaining, the sections were incubated in 3,3' diaminobenzidine tetrahydrochloride (Large Volume DAB Substrate System, Thermo Fisher Scientific, TA-125-HD) for 1 minute. Nuc-

lear counterstaining was conducted using Harris' hematoxylin. The sections were then subjected to the alcohol-xylol series and covered with Entellan. To ascertain the specificity of the results, negative control sections were performed by the same procedure, but treated with PBS in place of the primary antibody. The prepared sections were examined under a light microscope (DM750, Leica) connected to a digital camera (MC170, Leica).

Semiquantitative Evaluation

The immunostaining for HSP27 and HSP90 was evaluated semiquantitatively based on intensity scores (ISs) (15). The grading of the IS was based on a four-point scale: 0, no immunoreaction; 1, weak immunoreaction; 2, moderate immunoreaction; and 3, strong immunoreaction. The immunostaining of HSP27 and HSP90 in the sheep tongues was examined microscopically at 10x, 20x, and 40x objective magnification. Ten areas per section were randomly selected for evaluation and the average of these results was taken as a value.

Statistical Analysis

Statistical analysis of the data was performed using Minitab® (v21.4.1). To assess the normal distribution of the data, the Anderson-Darling normality test was performed. Non-parametric tests were preferred as the data were not normally distributed. The Kruskal-Wallis test was used to evaluate HSP90 and HSP27 immunoreactivity between age groups in sheep tongue tissue. The statistical significance level was evaluated as $p<0.05$.

RESULTS

HSP27

During the development of the sheep, no immunoreactivity for HSP27 was observed in the peripheral nerves (Figure 1d), gland duct epithelium (Figure 1e), serous Von Ebner's glands (Figure 1f), and mucous glands (Figure 1e) in the tongue tissues. However, skeletal muscle cells (Figure 1c,d), vascular endothelium, and vascular smooth muscle cells (Figure 1c) exhibited cytoplasmic HSP27 immunostaining. In addition, cytoplasmic HSP27 immunoreactivity was detected in the stratum basale, stratum spinosum, and stratum granulosum of the epithelial layer, excluding the stratum corneum. In the stratum basale, some cells showed negative HSP27 immunoreactivity, while some cells showed very weak HSP27 immunoreactivity. HSP27 immunostaining in the epithelial layer gradually increased from stratum basale to granulosum (Figure 1b).

There was no statistically significant difference in HSP27 immunoreactivity in the luminal epithelium, vascular endothelium, and vascular smooth muscle cells throughout the development of the sheep ($p>0.05$) (Table 1). However, HSP27 immunostaining in skeletal muscle cells was statistically higher in G1 (Figure 1c) compared to G3 (Figure 1d) ($p<0.01$) (Table 1).

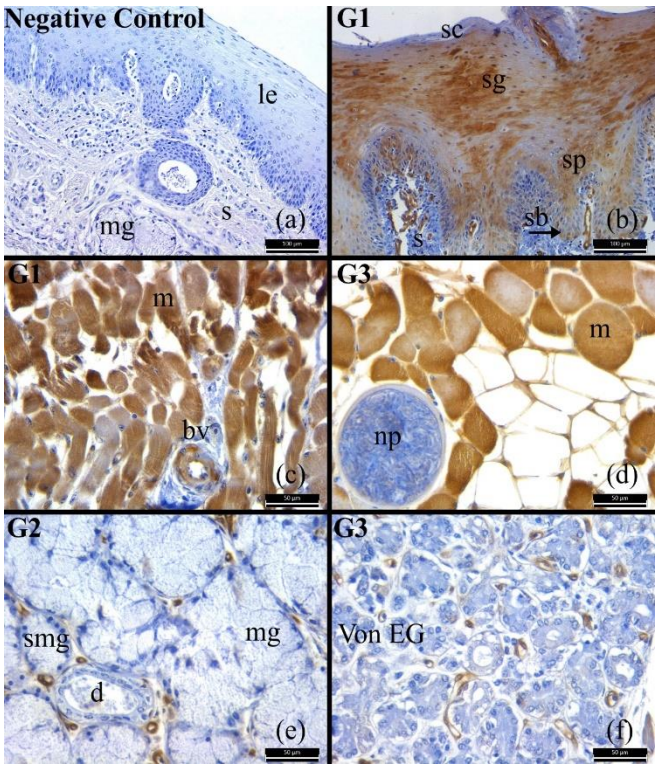

Figure 1. Immunostaining of HSP27 in the tongues of sheep aged 6-12 months (G1), 1-2 years (G2), and 3-5 years (G3). bv: blood vessel, d: excretory ducts, le: luminal epithelium, m: skeletal muscle cell, mg: mucous gland, np: nerve plexus, s: stroma, sb: stratum basale, sc: stratum corneum, sg: stratum granulosum, smg: seromucous gland, sp: stratum spinosum, Von EG: serous von Ebner's gland. Bar: a-b: 100 µm; c-f: 50 µm.

Table 1. Intensity scores for HSP27 and HSP90 expression in the tongues of sheep aged 6-12 months (G1), 1-2 years (G2), and 3-5 years (G3)

	G1	G2	G3	p-value
HSP27				
Luminal epithelium	2.00^{a}	1.50^{a}	1.00^{a}	>0.05
Skeletal muscle cell	3.00^{a}	2.50^{ab}	2.00^{b}	0.003**
Mucous gland	0	0	0	-
Serous Von Ebner's gland	0	0	0	-
Gland duct epithelium	0	0	0	-
Peripheral nerve	0	0	0	-
Blood vessel endothelium	3.00^{a}	3.00^{a}	3.00^{a}	>0.05
Blood vessel smooth muscle cell	3.00^{a}	3.00^{a}	3.00^{a}	>0.05
HSP90				
Luminal epithelium	2.00^{a}	1.50^{a}	2.00^{a}	>0.05
Skeletal muscle cell	2.50^{a}	3.00^{a}	3.00^{a}	>0.05
Mucous gland	0	0	0	-
Serous Von Ebner's gland	0	0	0	-
Gland duct epithelium	1.50^{a}	1.00^{a}	1.00^{a}	>0.05
Peripheral nerve	0	0	0	-
Blood vessel endothelium	1.00^{a}	1.00^{a}	1.00^{a}	>0.05
Blood vessel smooth muscle cell	1.00^{a}	1.00^{a}	1.00^{a}	>0.05

**p<0.01; a, b; different letters indicate that the differences between the median values of the age groups are significant.

HSP90

No immunoreactivity for HSP90 was observed in the peripheral nerves (Figure 2c), serous Von Ebner's glands (Figure 2d), and mucous glands (Figure 2e) in the tongue tissues of sheep of different ages. In the luminal epithelium of the sheep tongues, HSP90 positivity was detected in the cytoplasm of epithelial cells in the stratum basale, stratum spinosum, and stratum granulosum excluding the stratum corneum (Figure 2b). Some gland duct epithelium cells showed nuclear HSP90 positivity (Figure 2e). In addition, cytoplasmic HSP90 immunoreactivity was found in skeletal muscle cells (Figure 2c,f), vascular endothelium, and vascular smooth muscle cells (Figure 2f).

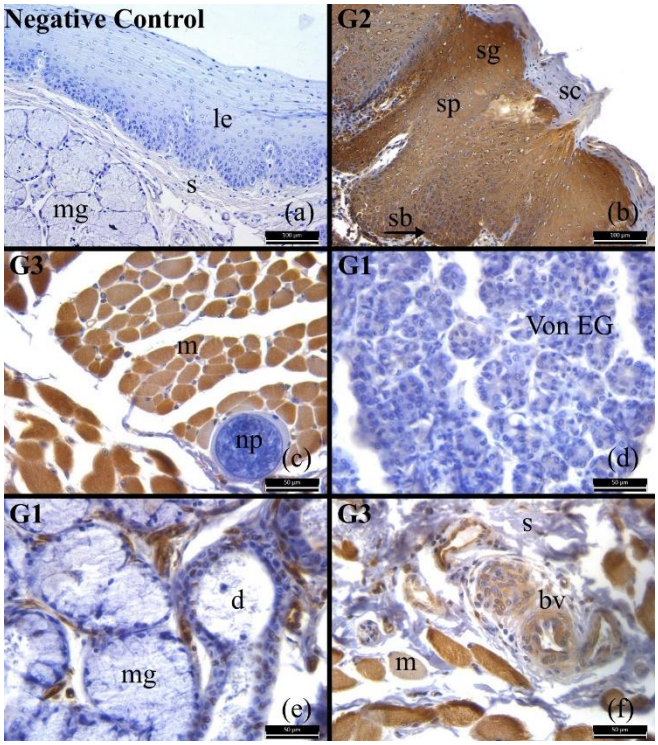

Figure 2. Immunostaining of HSP90 in the tongues of sheep aged 6-12 months (G1), 1-2 years (G2), and 3-5 years (G3). bv: blood vessel, d: excretory ducts, le: luminal epithelium, m: skeletal muscle cell, mg: mucous gland, np: nerve plexus, s: stroma, sb: stratum basale, sc: stratum corneum, sg: stratum granulosum, sp: stratum spinosum, Von EG: serous von Ebner's gland. Bar: a-b: 100 µm; c-f: 50 µm.

HSP90 immunoreactivity in the epithelial layer, skeletal muscle cells, gland duct epithelium, vascular endothelium, and vascular smooth muscle cells did not change statistically throughout the development of the sheep ($p>0.05$) (Table 1).

DISCUSSION AND CONCLUSION

HSPs play a critical role in maintaining cellular homeostasis, promoting cell survival under stress conditions, and modulating various cellular processes that are essential for normal cell function and organismal health. It is very important to know the localization and expression in tissues of these proteins, which are very important for organisms. Therefore, in this study, the localization and expression of HSP27 and HSP90 were demonstrated in the tongue tissue, which is an important organ of the digestive system, throughout the developmental process of sheep.

Tekkesin et al. (16) reported that HSP27 was not expressed in healthy human tongue. In normal human skin, Wilson et al. (17) determined that HSP27 was expressed in the basal and suprabasal layers of the epidermis. In a study carried out in rats, Zheng et al. (18) showed that HSP27 was present in the cytoplasm of the epithelial cells of the esophagus. In normal mouse epidermis, Laplante et al. (19) indicated that HSP27 had a suprabasal pattern of expression, but the stratum corneum was not labeled. In a study conducted on normal human skin, Gandour-Edwards et al. (20) reported that HSP27 was not expressed in the basal layer and stratum corneum layer of the epidermis, but that HSP27 expression in the suprabasal layer gradually increased from the stratum spinosum to the stratum granulosum. In the canine epidermis, Romanucci et al. (21) observed that HSP27 was expressed in the stratum spinosum and granulosum, whereas the stratum basale exhibited negative or only weakly positive immunoreactivity. The researchers also reported that the intensity of immunolabeling in the upper layers showed a gradual increase from positive to strongly positive in the stratum granulosum, while the stratum corneum was negative. In the present study, a similar HSP27 immunostaining pattern was detected in the epithelial layer of tongue tissues throughout the development of sheep. HSP27 involves many cellular processes, including signal transduction, differentiation, proliferation, and cellular movements. It plays a role in keratinocyte differentiation and epidermis development and acts as a chaperone for keratinization (11,20,21). Based on this information and the results obtained, it can be said that HSP27 plays a role in epithelial cell proliferation and keratinocyte differentiation in sheep tongues.

In normal human skin, Wilson et al. (17) demonstrated that HSP90 was present in the basal and suprabasal layers of the epidermis. In a study on the normal mouse epidermis, Laplante et al. (19) reported that HSP90 was mainly present in the high suprabasal cells, with basal and low suprabasal cells showing very weak labeling. Yang et al. (22) detected that HSP90 was present in the epidermis of yak skin. Similarly, HSP90 was shown to be expressed in the epithelial layer of the sheep tongues in this study. It has been observed that HSP90 immunoreactivity in the epithelial layer, like HSP27, did not change statistically throughout the development of the sheep. However, in contrast to HSP27, the HSP90 immunostaining pattern in the epithelial layer was homogeneous. These findings suggest that HSP90 is constitutively expressed in the epithelial layer, excluding the stratum corneum, throughout the developmental process of the sheep and that HSP90 functions as a chaperone protein to ensure the homeostasis of epithelial cells.

HSP27 is an actin-associated protein that regulates the polymerization of actin in the cells (12). HSP27 is expressed in the tongue and cardiac muscles during mouse embryogenesis (23) and plays a critical role in developing cardiac and skeletal muscle tissues (24). In a study in rats (25), HSP27 immunoreactivity was found in cardiac and skeletal muscle cells, as well as in the smooth muscle cells of blood vessels and hollow organs. In adult vertebrate muscle tissue, HSP27 is expressed at high levels in slow-twitch skeletal and cardiac muscle, with lower but still significant expression in fast-twitch skeletal muscle (26-28). Sun et al. (29) detected that

HSP 27 showed a down-regulation of expression in the tibialis anterior muscle of the rat during postnatal growth and development. Similarly, the present study showed that HSP27 was expressed in skeletal muscle cells in the sheep tongues and that HSP27 immunoreactivity statistically decreased during sheep development. After birth, skeletal muscle cells continue to grow and develop into adulthood. The growth and development of skeletal muscle cells involve many different processes that are controlled by many factors, including growth factors, hormones, kinases, and transcription factors. Although the growth and development of skeletal muscle cells have been the focus of many researchers, relatively little is known about these events (29). The decrease in HSP27 immunoreactivity in the skeletal muscle cells of the tongue due to the development of sheep shows that HSP27 plays a role in important events during the growth and development of skeletal muscle cells. However, more detailed studies need to be conducted on this subject.

HSP90 is expressed in a wide variety of tissues, including skeletal muscle cells, where it functions as a myosin chaperone (30). HSP90 participates in the modification of myosin in myofibrils (31). Moreover, HSP90 plays a pivotal role in the correct folding of the motor domain, which is also referred to as myosin subfragment-1 (S1) in skeletal muscle cells (30,32). Srikakulam and Winkelmann (30) suggest that HSP90 is involved in the initial folding of striated muscle myosin. Bornman et al. (33) detected moderate levels of HSP90 immunoreactivity in the sarcoplasm and nucleus of mature muscle cells. In this study, HSP90 positivity was detected in the skeletal muscle cells in the tongue of sheep and it was found that HSP90 reactivity in skeletal muscle cells did not change statistically throughout the development of the sheep. These results indicate that HSP90 is constitutively expressed in the skeletal muscle cells of the tongue throughout the developmental process of sheep.

In humans, Basset et al. (34) reported that HSP27 and HSP90 were present in the acini and ducts of embryonic salivary glands, but in adult glands, HSP27 was only present in the ducts and HSP90 was completely absent. In a study conducted on adult human salivary glands, Vanmuylder et al. (35) showed positive expression of HSP27 and HSP90 in epithelial cells of striated and excretory ducts, whereas HSP27 and HSP90 were not expressed in acinic cells. Takahashi-Horiuchi et al. (36) indicate that HSP27 was only expressed in the vascular endothelium, nerve fibers, and parts of the interlobular duct in normal rat submandibular glands. In this study, no immunostaining for HSP27 and HSP90 was observed in the serous Von Ebner's glands, mucous glands, and nerve plexuses of the sheep tongues. However, in contrast to HSP27, HSP90 immunostaining was seen in gland ductal epithelium cells.

In the rat uterus during the involution process, Liman (37) reported that blood vessels had moderate cytoplasmic and strong nuclear HSP90 immunoreactivity in endothelial and smooth muscle cells. Zheng et al. (18) found that HSP27 was present in smooth muscle cells and vascular endothelial cells in the esophagus of rats. Bao et al. (38) indicated that HSP27 and HSP90 were consistently present in the endothelium of the glomerular capillaries in pigs. Similarly, in this study, constitutive expression of HSP27 and HSP90 were determined in the vascular endothelium and vascular smooth muscle cells of the tongue throughout the developmental process of sheep.

In conclusion, this study demonstrated the localization and expression of HSP27 and HSP90 in tongue tissue throughout the developmental process of sheep. In all study groups, HSP27 and HSP90 were not expressed in peripheral nerves, serous Von Ebner's glands, and mucous glands, but were expressed in the skeletal muscle cells, epithelial layer, vascular endothelium, and vascular smooth muscle cells. Throughout the development of the sheep, HSP27 immunoreactivity decreased in skeletal muscle cells, whereas HSP90 immunoreactivity did not change. In the luminal epithelium of the tongue of sheep of all ages, while HSP90 showed a homogeneous staining pattern, HSP27 immunostaining gradually increased from the basal layer to the granulosum. HSP27 and HSP90 immunoreactivity in the luminal epithelium and blood vessels did not change throughout the developmental process of the sheep. In addition, in contrast to HSP27, HSP90 immunoreactivity was detected in the gland duct epithelium.

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CONFLICTS OF INTEREST

The author declares that she has no conflict of interest.

AUTHOR CONTRIBUTIONS

All processes, such as the design of the study, tissue collection, laboratory procedures, data analysis, preparation of the original draft of the article and the revision process, were conducted by Banu KANDİL.

ETHICAL STATEMENT

This study was approved by Siirt University Local Ethics Committee for Animal Experiments (File no: 2024/11, Decision no: 2024/03/11).

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RESEARCH ARTICLE

Association of Distal Extremity Thermographic Temperatures with Lameness in Cattle

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Abstract

Early diagnosis of foot diseases with lameness in cattle farms contributes to animal welfare and economy. Since early diagnosis of foot diseases characterized by lameness is very important, this study aimed to compare lameness scores and thermal temperatures in cattle. While lameness scoring was performed in ten cows (3-5 years old), thermal temperatures were measured from 280 points (right and left limbs) in the foot regions. Before lameness scoring, the gait of the cows was video recorded. Then, the recordings were watched and scored by two observers. Animals were taken to a shaded area for thermographic examination, and thermal records were kept. The temperature values obtained from the thermal camera represent the highest temperature in the anatomical regions. Pearson's correlation coefficient (r) through a bivariate linear regression model was used to investigate the relationship between lameness score and hoof temperature. In addition, the correlation between different anatomical regions was also analyzed. The analysis revealed no significant correlation between the lameness score and any specific region on the hoof or limb. However, significant correlations were found between anatomical areas. According to the data obtained from the Forelimb, the dorsal surface of the lateral hoof (R1) had a high correlation with the dorsal surface of the interdigital area (R5). Similarly, a high correlation was found between the dorsal surface of the medial hoof (R3) and the posterior surface of the interdigital area (R7). In the data obtained from the Hindlimb, two regions were identified where the dorsal surface of the lateral hoof (R2) had a high correlation: the dorsal surface of the medial hoof (R4) and the dorsal surface of the interdigital area (R6). In conclusion, if thermography detects temperature increases before illnesses occur, appropriate measures can be taken. According to the study's findings, it was determined that thermography could be a guide in methods such as lameness scoring and can play an effective role in taking precautions in preventive medicine.

Key Words: Cattle, lameness, score, thermography

Sığırlarda Distal Ekstremite Termografik Sıcaklıklarının Topallık ile İlişkisi

Öz

Sığır çiftliklerinde topallıkla seyreden ayak hastalıklarının erken teşhisi hem hayvan refahına hem de ekonomiye katkı sağlar. Topallıkla karakterize ayak hastalıklarının erken teşhisi çok önemli olduğundan, bu çalışma sığırlarda topallık skorlarını ve termal sıcaklıkları karşılaştırmayı amaçlamıştır. Toplam on inekte (3-5 yaş arası) topallık skorlaması yapılırken, ayak bölgelerinde toplam 280 noktadan (sağ ve sol uzuvlar) termal sıcaklıklar ölçülmüştür. Topallık skorlamasından önce ineklerin yürüyüşü videoya kaydedilmiştir. Daha sonra kayıtlar iki gözlemci tarafından izlenmiş ve puanlanmıştır. Termografik inceleme için hayvanlar gölgelik bir alana alınmış ve termal kayıtlar tutulmuştur. Termal kameradan elde edilen sıcaklık değerleri, anatomik bölgelerdeki en yüksek sıcaklığı temsil etmektedir. Topallık skoru ile tırnak sıcaklığı arasındaki ilişkiyi araştırmak için iki değişkenli doğrusal regresyon modeli aracılığıyla Pearson korelasyon katsayısı (r) kullanılmıştır. Ayrıca, farklı anatomik bölgeler arasındaki korelasyon da analiz edilmiştir. Analiz, topallık skoru ile tırnak veya distal ekstremite üzerindeki herhangi bir spesifik bölge arasında anlamlı bir korelasyon olmadığını ortaya koymuştur. Bununla birlikte, anatomik bölgeler arasında anlamlı korelasyonlar bulunmuştur. Ön ayaklardan elde edilen verilere göre lateral tırnağın dorsal yüzeyi (R1) interdigital alanın dorsal yüzeyi (R5) ile yüksek bir korelasyona sahipti. Aynı şekilde medial tırnağın dorsal yüzeyi (R3) ile interdigital alanın arka yüzeyi (R7) arasında yüksek bir korelasyon belirlendi. Arka ayaklardan elde edilen verilerde lateral tırnağın dorsal yüzeyinin (R2) yüksek korelasyona sahip olduğu iki bölge belirlendi; medial tırnağın dorsal yüzeyi (R4) ve interdigital bölgenin dorsal yüzeyi (R6). Sonuç olarak, termografi hastalıklar oluşmadan önce sıcaklık artışını belirleyebilmek için kullanılırsa gerekli önlemler alınabilir. Çalışma bulgularına göre Termografinin topallık skorlaması gibi yöntemlerde yol gösterici olabildiği ve koruyucu hekimlikte önlemlerin alınmasında etkin bir rol oynayabileceği belirlenmiştir.

Anahtar Kelimeler: Sığır, skor, termografi, topallık

INTRODUCTION

Lameness in ruminants is a significant problem affecting health and welfare in the livestock industry. Lameness, which has a multifactorial etiology, deficient hygiene, and high humidity in stables, can be effective by creating ideal conditions for bacterial growth (1). Early diagnosis is crucial for preventing lameness and immediate treatment of clinical signs (2). Diagnosis is usually made during hoof trimming after the cow has started to limp (3), which is economically inefficient. There are several methods to diagnose foot diseases. Several ways have been used to diagnose cases of lameness in dairy cattle, such as mobility scoring, which refers to a structured subjective assessment of a cow's gait (4). However, besides being time-consuming, they are personal, and there is no standard for evaluation (5). This method is inexpensive and does not require direct contact with the animal. Therefore, it is used as a screening method for lameness assessment in cattle herds. Individual clinical examination may then be necessary (6).

Infrared thermography is a non-invasive, radiation-free, rapidly developing diagnostic method that measures surface temperature and represents it as a color scale. It is used to detect local temperature abnormalities on the skin surface by capturing infrared radiation that can directly correlate with the surface temperature of a part of the body. It has been reported that the lesioned hoof has a higher surface temperature than the healthy one (7). It has been shown that there is almost no temperature difference between an animal's right and left limbs. Some authors have reported that a difference of 1 °C may indicate a pathological process (8). This technique helps assess inflammatory changes and is particularly useful for diagnosing lameness and localization of lesions. Therefore, using it for early control before clinical symptoms appear is useful (9).

Based on the given literature information, this study aimed to investigate the benefits of using digital infrared thermal imaging as an early detection tool in a small-scale dairy farm to correlate lameness scores of cattle with thermographic data from different regions and to provide early treatment or control since the increase in temperature indicates an inflammatory condition at that point, even if there is no clinical symptom, the cause of lameness can be determined in advance. Thus, by comparing the thermographic imaging method with the clinical lameness scoring method, it was hypothesized that thermography may be advantageous.

MATERIAL AND METHODS

Animals and Lameness Scoring

The study was conducted in a small-scale dairy cattle enterprise in Kastamonu province in September 2023. According to the legislation of our country, local ethics committee permission is not required for studies conducted without contact with animals. Therefore, our study did not obtain permission from the local ethics committee. However, the study followed European regulations (Directive 2010/63/EU).

Ten cows in a dairy farm (Holstein, 3-5 years old, mean body weight 450-650 kg) were enrolled in the study. First, the animals were filmed from different viewing positions while walking on a concrete floor (20 meters) inside the semi-open enclosure. GoPro Hero 12 Black camera (GoPro Inc., San Mateo, California) was used for video recordings. Two observers examined the recordings to determine lameness according to the scale defined by Sprecher et al. (10) (Score 1: Normal, Score 2: Mildly Lame, Score 3: Moderately Lame, Score 4: Lame, Score 5: Severely Lame).

Infrared Thermography Examination

Animals were taken to a covered area (ambient temperature 25°C) for evaluation in the shade, and thermal camera images were taken (Flir E6390, Sweden). A total of 280 thermographic images (right and left limbs, clean hoof with no coarse dirt) were taken from 10 cows. The emissivity value for subjects was 0.93, and all images were taken at the same distance (2 meters) (11). The points where thermographic images were taken are given in Figure 1, and their abbreviations are shown in Table 1.

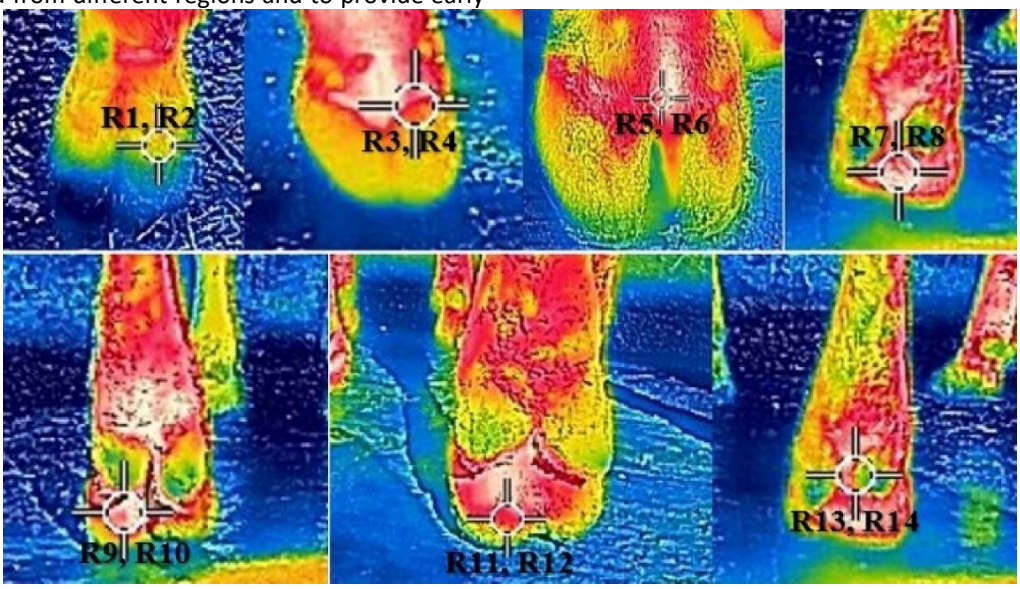

Figure 1. Anatomical points where thermographic temperatures are measured. Odd numbers refer to the forelimbs, and even numbers refer to the hindlimbs. **R1, R2;** Dorsal surface, lateral hoof, **R3, R4;** Dorsal surface medial hoof, **R5, R6;** Interdigital dorsal surface, **R7, R8;** Interdigital posterior surface, **R9, R10;** Heel lateral, **R11, R12;** Heel medial, **R13, R14;** Between the dew claw

Table 1. Thermography image points and abbreviations

Abbreviation	Thermographic image point
R1	Dorsal surface, lateral hoof forelimb
R2	Dorsal surface, lateral hoof, hind limb
R3	Dorsal surface medial hoof forelimb
R4	Dorsal surface medial hoof hind limb
R5	Interdigital dorsal surface, forelimb
R6	Interdigital dorsal surface, hind limb
R7	Interdigital posterior surface forelimb
R8	Interdigital posterior surface hind limb
R9	Heel lateral forelimb
R10	Heel lateral hind limb
R11	Heel medial forelimb
R12	Heel medial hind limb
R13	Between the dew claw forelimb
R14	Between the dew claw hind limb

In the study, the arithmetic mean of temperature measurements obtained from the right and left front hooves was taken as the data for the forelimb temperature, and the arithmetic mean of temperature measurements obtained from the right and left hind hooves was taken as the data for the hindlimb temperature.

Statistical Analysis

Pearson's correlation coefficient (r) was employed through a bivariate linear regression model to investigate the relationship between lameness score and hoof temperature. All correlation coefficients were reported with an alpha level set at 0.05, indicating the significance level of the findings. The statistical analysis was conducted using SPSS software (Version 22, IBM Corp., Armonk, NY, USA).

RESULTS

The study was completed with all the values in the dataset. The analysis revealed no significant correlation between lameness score and any specific location on the hoof or leg ($p > 0.05$). However, noteworthy correlations were identified between certain aspects of the foot and the legs. The lameness scores and measured thermographic values are given in Table 2.

Table 2. Lameness scores and thermographic temperatures from anatomical regions

			Dorsal Surface				Interdigital Dorsal Surface		Interdigital Posterior Surface		Heel				Between the dew claws	
			Lateral Hoof		Medial Hoof						Lateral Hoof		Medial Hoof			
			Fore limb	Hind limb	Fore limb	Hind limb	Fore limb	Hind limb	Fore limb	Hind limb	Fore limb	Hind limb	Fore limb	Hind limb	Fore limb	Hind limb
Animal	Lameness score		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14
1	1	Right	21.68	21.65	21.54	21.73	22.76	22.76	23.41	22.60	22.14	24.30	21.78	26.10	21.93	26.00
1	1	Left	21.68	21.65	21.54	21.73	22.76	22.76	23.41	27.60	22.14	28.40	21.78	21.90	21.93	28.20
2	2	Right	26.30	21.65	21.54	21.73	28.30	22.76	23.41	31.90	22.14	30.40	21.78	32.40	21.93	22.00
2	2	Left	23.30	21.65	21.54	21.73	30.30	22.76	23.41	28.80	22.14	30.30	21.78	33.30	21.93	23.90
3	1	Right	27.10	29.60	29.20	28.80	29.00	29.80	28.70	20.14	26.20	20.01	26.00	20.11	22.00	19.39
3	1	Left	26.90	23.90	28.00	25.10	31.10	29.50	26.10	20.14	25.40	20.01	21.90	20.11	16.80	19.39
4	2	Right	15.40	26.00	14.00	24.30	15.90	30.90	23.40	14.30	27.30	13.40	20.90	13.50	12.80	15.10
4	2	Left	15.50	25.70	24.00	28.80	12.70	32.10	21.20	12.30	18.90	12.70	24.00	12.00	22.90	12.60
5	1	Right	14.00	14.10	15.10	13.30	15.60	13.60	16.40	14.70	15.10	13.90	16.50	14.10	17.30	14.80
5	1	Left	15.70	14.40	14.50	15.20	16.00	15.60	16.40	14.40	15.10	15.20	14.90	15.00	17.10	12.90
6	1	Right	21.40	21.80	24.00	22.80	25.80	24.90	26.00	22.80	23.70	19.50	24.50	22.90	25.80	22.40
6	1	Left	29.00	21.60	21.00	24.50	21.10	23.20	24.00	21.70	22.10	21.60	25.20	22.60	26.30	22.30
7	3	Right	21.60	14.80	24.00	16.50	24.30	15.10	27.30	16.20	24.40	13.90	22.80	15.80	25.00	15.70
7	3	Left	21.30	20.50	19.00	20.40	20.40	19.70	23.70	16.40	23.30	16.80	22.00	16.40	23.50	17.80
8	1	Right	26.00	21.30	25.80	20.20	27.30	17.90	29.40	18.90	23.30	17.60	27.30	19.50	26.80	16.90
8	1	Left	20.90	21.50	22.60	22.40	21.50	21.80	23.80	21.80	23.30	19.90	21.80	17.10	22.90	18.80
9	2	Right	22.10	21.70	17.90	20.90	23.00	22.80	18.50	17.80	19.00	17.70	18.30	16.40	21.40	18.30
9	2	Left	21.50	22.10	20.20	18.30	20.40	22.70	20.30	17.70	20.00	19.40	18.60	20.70	20.10	16.80
10	3	Right	20.00	23.50	21.90	21.60	23.90	23.30	25.80	20.70	24.00	21.90	21.30	20.40	25.20	21.80
10	3	Left	22.30	23.90	23.40	24.50	23.00	21.20	23.60	22.00	23.10	23.20	22.40	21.80	24.90	22.80

In the Forelimb, the dorsal surface of the lateral hoof (R1) had a high correlation with the dorsal surface of the interdigital area (R5) (R=0.803), a moderate correlation with the dorsal surface of the medial hoof (R3) (R=0.662), the posterior surface of the interdigital area (R7) (R=0.629) and the medial heel (R11) (R=0.610) and a weak correlation with the lateral heel (R9) (R=0.483) and between the dew claws (R13) (R=0.475). The dorsal surface of the medial hoof (R3) had a high correlation with the posterior surface of the interdigital area (R7) (R=0.670) and the medial heel (R11) (R=0.760), a moderate correlation with the lateral heel (R9) (R=0.528), between the dew claws (R13) (R=0.525).

The dorsal surface of the interdigital area (R5) had a moderate correlation with the posterior surface of the interdigital area (R7) (R=0.656), the lateral heel (R9) (R=0.531), and the medial heel (R11) (R=0.448). The posterior surface of the interdigital area (R7) had a high correlation with the lateral heel (R9) (R=0.857) and the medial heel (R11) (R=0.868), a moderate correlation between the dew claws (R13) (R=0.503).

Finally, a moderate correlation was found between the temperature of the lateral (R9) and medial heels (R11) (R=0.667), as well as between the temperature of the medial heel (R11) and the temperature between the dew claws

(R13) (R=0.634). The correlation coefficients for the Forelimb are shown in Table 3.

Table 3. Forelimb correlations

Anatomical Location 1	Anatomical Location 2	Correlation coefficient	P value
R1	R3	0.662	p = 0.001
R1	R5	0.803	p < 0.01
R1	R7	0.629	p = 0.003
R1	R9	0.483	p = 0.031
R1	R11	0.610	p = 0.004
R1	R13	0.475	p = 0.034
R3	R7	0.670	p = 0.001
R3	R8	0.779	p < 0.01
R3	R9	0.528	p = 0.017
R3	R11	0.760	p < 0.01
R3	R13	0.525	p = 0.018
R5	R7	0.656	p = 0.002
R5	R9	0.531	p = 0.016
R5	R11	0.448	p = 0.047
R7	R9	0.857	p < 0.05
R7	R11	0.868	p < 0.01
R7	R13	0.503	p = 0.024
R9	R11	0.667	p = 0.001
R11	R13	0.634	p = 0.003

In Hindlimb, the dorsal surface of the lateral hoof (R2) had a high correlation with the dorsal surface of the medial hoof (R4) (R=0.913) and the dorsal surface of the interdigital area (R6) (R=0.888). Similarly, a high correlation was observed between the temperature of the dorsal surface of the medial hoof (R4) and the temperature of the interdigital dorsal surface (R6) (R=0.887). The posterior surface of the interdigital area (R8) had a high correlation with the medial heel (R12) (R=0.913) and between the dew claws (R14) (R=0.839), a moderate correlation with the dorsal surface of the lateral hoof (R2) (R=0.584) and the dorsal surface of the interdigital area (R6) (R=0.680). A high correlation was observed between the temperature of the lateral heel (R10) and the temperature of the posterior interdigital surface of the hoof (R8) (R=0.965).

Lastly, a moderate correlation was observed between the temperature between the dew claws (R14) and the medial heel (R12) (R=0.747). Correlation coefficients for the Hindlimb are shown in Table 4.

Table 4. Hindlimb correlations

Anatomical Location 1	Anatomical Location 2	Correlation coefficient	P value
R2	R4	0.913	p < 0.01
R2	R6	0.888	p < 0.01
R4	R6	0.887	p < 0.01
R8	R12	0.913	p < 0.001
R8	R14	0.839	p < 0.001
R8	R2	0.584	p = 0.007
R8	R6	0.680	p = 0.001
R10	R8	0.965	p < 0.001
R14	R12	0.747	p < 0.001

DISCUSSION AND CONCLUSION

The use of infrared thermography to identify lameness and foot lesions in cattle has increased due to its non-invasiveness and cost reduction. However, these studies were conducted by correlating thermographic data with the disease when it progressed and manifested in lameness. Thermographic temperatures rise considerably in conditions that cause lameness (9). The present study used thermographic images with lameness scoring to identify tissues with increased temperature before clinical symptoms appeared. The relationship between lameness scoring and thermography was also examined. We found no significant correlation between scoring and temperatures. This result may be affected by the low scores of the cattle in the study. Low scores are also due to the absence of apparent symptoms of foot disease. However, as mentioned earlier, it is necessary to identify temperature increases and take appropriate measures before clinical symptoms appear. At this point, the importance of including thermography in the scoring becomes apparent.

When scoring lameness, the observer's experience and method are important. Danscher et al. (12) showed that movement scores obtained by live observation differed from those obtained by filming and reported that fine movements could be verified by video recording when performing multiple assessments. Therefore, in our study, video recordings were taken during lameness scoring and then evaluated by two observers. Factors such as differences in how cows walk, udder size, age, and environment can be ignored when scoring lameness (13), and scoring may not be effective in the early stages of diseases. However, clinical examination and other diagnostic methods, such as thermography, may be more effective. Infrared thermography is a non-invasive method for early detection of lesions before the appearance of clinical symptoms (14). When comparing anatomical regions, a 1 °C or more difference may indicate a possible pathology (15). Although the number of subjects in our study was small, thermographic measurements were made from different points (a total of 280 anatomical regions) on the feet of cows to determine the temperature differences, as stated in the reference above.

Stokes et al. (16) reported that the temperature threshold for dirty feet is 27°C. When this parameter was evaluated, four animals with a lameness score of 1, i.e., considered normal, had a temperature above 27°C at least one point. Even in 10 anatomical regions of animal 3, temperatures above 27°C were detected. Conversely, an animal with a lameness score of 3, i.e., considered moderately lame, does not exceed 27°C in any anatomical region. More subjects are needed to evaluate the parameter as a threshold for early lameness detection and compare it with scoring. To make such a comparison, many animals must be scored for lameness and temperature measurements from many anatomical sites.

Studies have reported that most lameness originates from the hindfoot, especially the lateral hoof (17-19). Muray et al. (18) even reported a lesion rate of 65% in the lateral hoof of the Hindlimb. Our study found no significant difference between the temperature values obtained from the forelimbs and hindlimbs. However, the highest value was 3 in the scored animals. In the case of severely lame animals, i.e., animals with a score of 4 or 5 were identified and included in the study, we think that the temperature differences detected from the forelimbs and hindlimbs would be revealed.

Alsaaod and Buscher (9) found that mean skin temperatures for healthy hoof ranged between 29.9-32.1°C

when considered within an ambient temperature of 20.3°C. We noted that the ambient temperature was 25°C during the thermographic measurements. When skin temperatures were evaluated under these conditions, it was determined that the temperatures ranged between 14-33.30°C, although they were higher than the ambient temperature stated in the literature. It may not be correct to give a precise temperature value for healthy feet based on the possibility that thermographically measured temperatures may be affected by many other factors, not only the ambient temperature. The feet measured in our study were not dirty. Any waste on the foot can change the temperature. Therefore, many factors must be examined and eliminated to give an average value for healthy feet.

Stokes et al. (16) reported that the mean plantar temperature of dirty-footed animals without lesions was 22.2°C. In the present study, the temperature was measured at R8, R10, R12, and R14 on the plantar aspect of the hindlimbs. The average of the measurements taken from R8 was 20.14°C (12,30-28,80°C). The average was 20.10°C at R12 (12-33.30°C), 20.06°C at R10 (12.70-30.40°C) and 19.30°C at R14(12.60-28.20°C).

In their study, Stokes et al. (16) only reported the temperature on the plantar aspect of the feet without specifying the anatomical region. Our study measured temperature at 4 points on the plantar surface. Even if these temperatures are averaged, they are lower than the temperature reported by Stokes et al. (16). Moreover, a lower temperature was found even though the feet we measured were clean. Therefore, different measurements should be taken by detailing the anatomical regions in the studies.

The results of Alsaaod and Buscher (9) showed an increase in the surface temperature of the coronary band of the affected foot compared to the healthy contralateral foot. When determining coronary band temperatures, we took measurements from both the medial and lateral hoof. When the measures taken from the right and left (forelimb-hindlimb) feet were evaluated in general, the coronary band temperatures ranged between 13.30-29.20°C, the interdigital dorsal surface 14.40-31.90°C, the lateral and medial heel 12.70-33.30°C and the between the dew claw 12.60-28.20°C. In this case, making comparisons between regions may not be very accurate. Still, we believe that it would be more accurate to make thermographic measurements to determine which area is most exposed to temperature increases in diagnosed foot diseases.

In their literature research, the authors found that lateral hoofs were warmer in hindlimbs (20,21), while there was no difference between medial and lateral hoofs in forelimbs (9,20). Our study found a high correlation between the dorsal surface and the dorsal surface of the medial hoof and the dorsal surface of the interdigital region. However, higher temperatures were not found in the lateral hoof of the hindlimbs. Instead of separating the medial and lateral hoof, we saw a diffuse temperature increase on the dorsal surface of the area. Comparisons between affected and healthy contralateral anatomical structures on multiple scan images help define the consistency of an abnormality (9,15,21). Instead of detecting increased temperature after the onset of diseases, it would be better to measure it before clinical signs appear and compare it with other methods, such as lameness scoring. Thermography may have great potential in the early diagnosis of foot diseases. Taking non-invasive and accurate measurements in multiple animals in rapid succession (9,22) is one of the advantages we have seen in our study.

In conclusion, although no disease was diagnosed in our study, high temperatures were determined at some anatomical points. We think that these regions should be followed up by examining them later. Because thermography may indicate damaged tissue. In this way, measures can be taken in the early period, contributing to both the enterprise's economy and the animals' welfare. Since lameness scoring alone may be insufficient before lameness progresses, we think thermography can be a guide. In addition, thermography may be advantageous in preventing loss of productivity or economic costs due to early diagnosis of diseases that cause lameness.

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CONFLICT OF INTEREST

There is no conflict of interest to be declared by the authors.

AUTHOR CONTRIBUTIONS

Elif Dogan, Mumin Gokhan Senocak and Ayse Basak Kapcak were involved in the original study design. All three authors wrote and revised the manuscript. Data analysis/interpretation: Elif Dogan, Mumin Gokhan Senocak. Statistical analysis, manuscript drafting, and literature research: all authors. All authors have critically revised and approved the final version of the manuscript.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. According to the Turkey legislation, there is no need to obtain local ethics committee permission for studies conducted without contact with animals. Therefore, local ethics committee permission was not obtained for our study. However, the study was conducted in accordance with European regulations (Directive 2010/63/EU).

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RESEARCH ARTICLE

Preliminary Study on the Genetic Diversity of *Hepatozoon canis* in Dogs and *Rhipicephalus sanguineus* Sensu Lato Ticks

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Abstract

Hepatozoon canis, a protozoan parasite, is the primary cause of canine hepatozoonosis worldwide, typically causing subclinical infection in dogs but potentially leading to severe illness when accompanied by other pathogens. This study investigates the genetic diversity of *H. canis* in dogs and *Rhipicephalus sanguineus* sensu lato ticks using bioinformatics analysis. Archived DNA samples from dogs and ticks were analyzed through PCR amplification of the *18S rRNA gene*, followed by sequence comparison using BLAST analysis and phylogenetic analysis using bioinformatics tools. The results revealed genetic variability, identifying several single nucleotide polymorphisms (SNPs) critical for distinguishing between different haplotypes. Minimum Spanning Networks created in PopART identified 18 distinct haplotypes across a broad geographical distribution. The study highlights the extensive genetic diversity of *H. canis*, with implications for understanding its evolutionary dynamics, transmission, pathogenicity, and resistance. Future studies should employ more variable genomic regions to further elucidate the genetic landscape of *H. canis*, aiding in the development of targeted control strategies and enhancing epidemiological knowledge.

Key Words: *18S rRNA*, Bioinformatics, genetic diversity, haplotype, *Hepatozoon canis*, *Rhipicephalus sanguineus* sensu lato

Köpeklerde ve *Rhipicephalus sanguineus* Sensu Lato Kenelerinde *Hepatozoon canis*'in Genetik Çeşitliliği Üzerine Ön Çalışma

Öz

Hepatozoon canis, köpeklerde tipik olarak subklinik enfeksiyona neden olan ancak diğer patojenlerle birlikte olduğunda ciddi hastalıklara yol açabilen bir protozoon paraziti, dünya çapında köpeklerde hepatozoonozun birincil sebebidir. Bu çalışma, köpeklerde ve *Rhipicephalus sanguineus* sensu lato kenelerinde *H. canis*'in genetik çeşitliliğini biyoinformatik analiz kullanarak araştırmaktadır. Köpeklerden ve kenelerden alınan arşiv DNA örnekleri, *18S rRNA* geninin PCR amplifikasyonu ile analiz edilmiş ve ardından BLAST analizi ile dizi karşılaştırması ve biyoinformatik araçlar kullanılarak filogenetik analiz yapılmıştır. Sonuçlar, farklı haplotipleri ayırt etmek için kritik olan çeşitli tek nükleotid polimorfizmlerini (SNP'ler) tanımlayarak genetik değişkenliği ortaya koymuştur. PopART'da oluşturulan Minimum Yayılma Ağları, geniş bir coğrafi dağılıma sahip 18 farklı haplotipi belirlemiştir. Çalışma, *H. canis*'in geniş genetik çeşitliliğini vurgulamakta ve evrimsel dinamiklerini, bulaşma yollarını, patojenitesini ve direncini anlamak için önemli çıkarımlarda bulunmaktadır. Gelecekteki çalışmalar, *H. canis*'in genetik yapısını daha ayrıntılı bir şekilde açıklığa kavuşturmak, hedeflenmiş kontrol stratejilerinin geliştirilmesine ve epidemiyolojik bilginin artırılmasına yardımcı olmak için daha değişken genom bölgelerini kullanmalıdır.

Anahtar Kelimeler: *18S rRNA*, Biyoinformatik, genetik çeşitlilik, haplotip, *Hepatozoon canis*, *Rhipicephalus sanguineus* sensu lato

INTRODUCTION

Hepatozoon species, which are blood parasites belonging to the class Apicomplexa, infect a wide range of vertebrate hosts including amphibians, reptiles, birds, marsupials, and mammals (1). Among these, *Hepatozoon canis* is a protozoan that primarily infects dogs and other wild carnivores, and it is recognized as the most common cause of canine hepatozoonosis worldwide (2). While infections are typically subclinical, they can lead to moderate to severe illness characterized by cachexia and anemia, especially when accompanied by other pathogens (3). Though dogs are the primary intermediate hosts for *H. canis*, various wild canine species, foxes, and other carnivores have also been reported to be infected with *H. canis* or other *Hepatozoon* species (4).

Dogs become infected with *H. canis* by ingesting *Rhipicephalus sanguineus* sensu lato ticks that contain mature oocysts with infective sporozoites. Once ingested, these sporozoites are released in the intestine, entering the bloodstream and lymphatic system. They enter to tissues such as the liver, kidneys, spleen, bone marrow, and lymph nodes, where they undergo merogony, an asexual replication process, forming meronts. The micro and macromerozoites within these meronts are then released and invade neutrophils and monocytes in the bloodstream, where they develop into gamonts through sexual reproduction. When a tick feeds on the host's blood, these infected blood cells are ingested and broken down in the tick's gut. The free gamonts divide to form macrogametes and microgametes, which fuse to form a zygote. The zygote then develops into an oocyst, within which sporozoites are produced through sporogony (5,6).

Hepatozoon canis infection is predominantly reported in tropical, subtropical, and temperate regions where vector tick species are abundant. In Europe, the infection is mainly observed in areas near the Mediterranean basin. Molecular studies in Türkiye have reported high rates of *H. canis* infection in dogs and in *R. sanguineus* sensu lato ticks, the main known vector (7–11). *Hepatozoon canis* is transmitted transstadially among the developmental stages of ticks (larva, nymph, adult) (5,10). A study conducted in Chile analyzed haplotypes of *Hepatozoon* spp. *18S rRNA* sequences from rodents and their associated ticks, revealing significant genetic diversity in the haplotypes found in these hosts (12). While there are studies on the genetic diversity of *H. canis* in various vertebrate hosts, there is a lack of research on the genetic diversity of this parasite in infected dogs and vector ticks. This preliminary study aims to investigate the genetic diversity of *H. canis* in infected dogs and *R. sanguineus* s.l. ticks using bioinformatics analysis.

MATERIAL AND METHODS

Amplification of *H. canis 18S rRNA* Gene

In this study, archived DNA samples obtained from dogs and ticks in 2015 were used (10). DNA samples were extracted from 2 engorged nymphs (AYN1 and AYN8) and 2 engorged adults (AEG4-2 and AEG2) of *R. sanguineus* sensu lato, as well as from 3 dogs (Nimf2, Nimf4, Nimf5). To investigate the genetic diversity of *H. canis*, the *18S rRNA* gene was amplified by PCR. Primers HEPF and HEPR were used to amplify the *Hepatozoon* sp. *18S rRNA* gene fragment (13). PCR amplification was carried out using Phusion® High-Fidelity PCR Master Mix with GC Buffer (#M0532S; NEB). The PCR reaction was performed in a total volume of 20 µL, containing; 10 µL of 2X Phusion Master Mix, 1 µL of each forward and reverse primer, 1 µL of template DNA, 7 µL of nuclease-free water. All samples (n=7) were subjected to sequence analysis.

Bioinformatics Analyses

The obtained nucleotide sequences were compared to those in the NCBI database using BLAST analysis. Phylogenetic analysis was conducted using the MEGA X program (14). Sequence data from different geographic regions reported in GenBank, obtained from dogs and ticks infected with *H. canis*, were used for comparison. All sequences were aligned using the MEGA X and CLC Sequence Viewer 8.0 program and adjusted to equalize the ends of the sequences. For data analysis, sequences were converted to Nexus format for use in the PopART (Population Analysis with Reticulate Trees) software (15). Haplotypes were created using Minimum Spanning Networks in PopART, and relationships between haplotypes were analyzed. Nucleotide content, haplotype numbers, haplotype and nucleotide diversity values, and the amount of mutation among molecular haplotypes were determined using the DnaSP 6 program (16).

RESULTS

The sequence analysis of the *18S rRNA* gene from *H. canis* in both dogs and *R. sanguineus* sensu lato ticks revealed genetic diversity. Many regions of the sequences were highly conserved across all samples, which indicates a high degree of similarity in the *18S rRNA* gene among these samples. However, several single nucleotide polymorphisms (SNPs) were identified, which are critical for distinguishing between different haplotypes. Using BLAST analysis, the obtained nucleotide sequences were compared to those in the NCBI database, confirming the presence of *H. canis*. The phylogenetic analysis conducted using MEGA X and CLC Sequence Viewer 8.0 aligned the sequences from different geographic regions reported in GenBank. This alignment revealed the evolutionary relationships and geographical distribution of *H. canis*. The alignment results showed that while the majority of the *18S rRNA* gene sequences were conserved, the identified SNPs contributed to the genetic variability observed among the samples (Figure 1). In addition, phylogenetic analysis showed that *H. canis* sequences from various parts of the world formed different clades (Figure 2).

In PopART, haplotypes were created using Minimum Spanning Networks to analyze relationships between them. The analysis revealed 18 distinct haplotypes, indicating significant genetic variation within *H. canis* populations in both dogs and ticks (Table 1). Haplotype 3, which had the highest number of samples (8), suggests a widespread or common genetic variant. Haplotypes 1, 2, 10, 14, 17, and 18 also had multiple samples, though fewer than Haplotype 3. The samples originated from various regions, including Spain, India, Portugal, Croatia, Nigeria, Germany, Iran, Israel, Türkiye, Italy, Brazil, Hungary, Egypt, Japan, and Taiwan, indicating a broad geographical distribution of these haplotypes. The samples included both dogs and ticks (*R. sanguineus* sensu

lato*, Ixodes ricinus, Dermacentor marginatus, Amblyomma cajennense, Haemaphysalis longicornis, Haemaphysalis bispinosa*), highlighting potential host diversity or transmission dynamics. Among the 38 aligned nucleotide sites, 49 variable sites were detected (Figure 3). The analysis showed a notable degree of haplotype diversity, with several unique haplotypes identified in the samples.

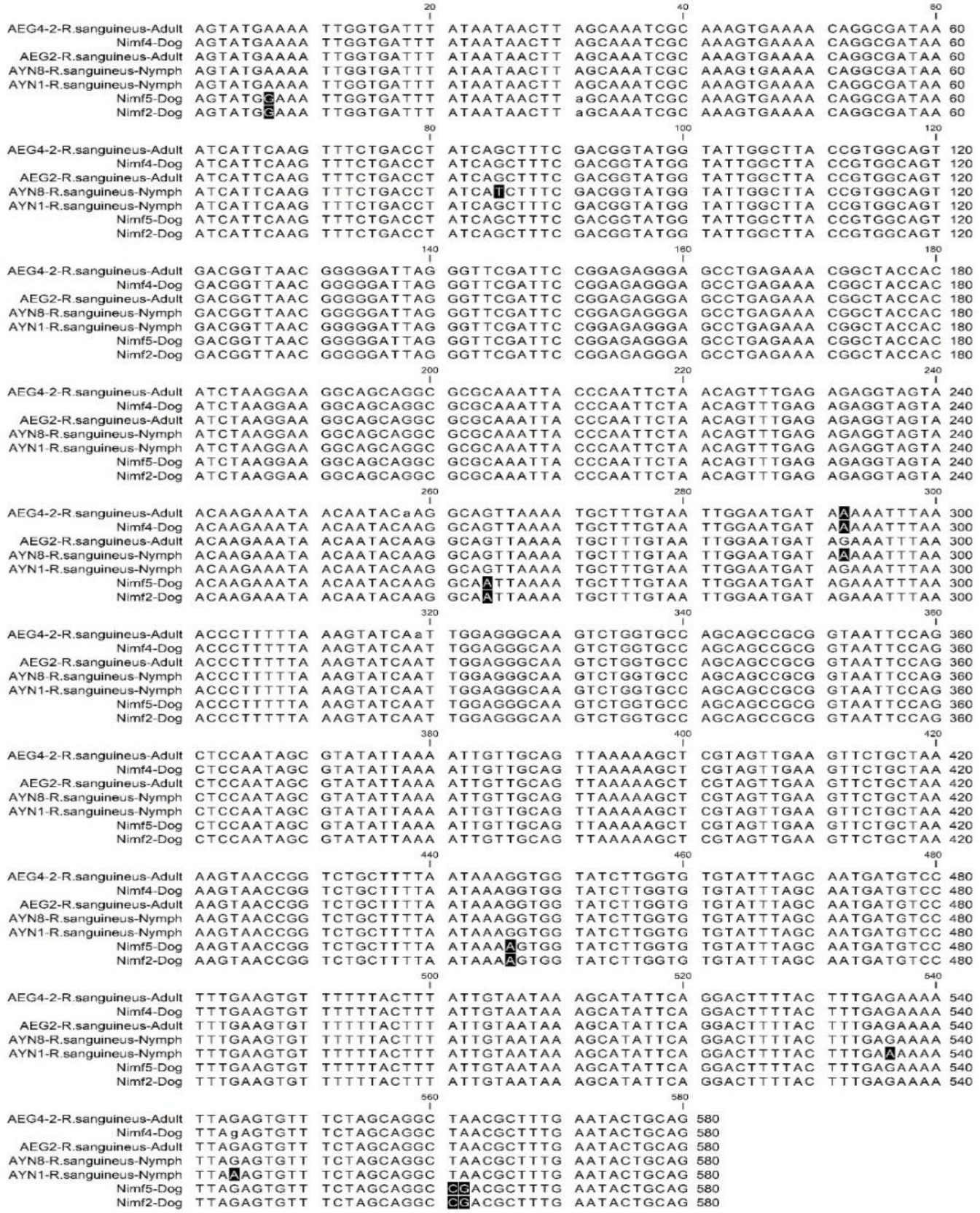

Figure 1. Sequence alignment of the *18S rRNA* gene from *H. canis* in dogs and *R. sanguineus* sensu lato

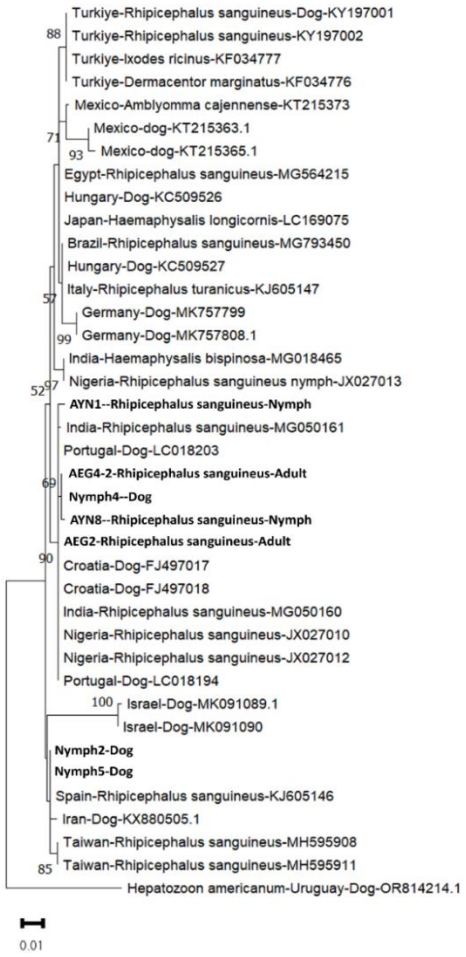

Figure 2. The phylogenetic tree created using the Mega X program shows the phylogenetic relationship of *H. canis* (in bold), identified in this study, with *H. canis* sequences reported in different regions in ticks and dogs obtained from GenBank. The evolutionary history was inferred based on the Tamura 3 model. Next to each branch is the percentage of replicate trees in which the associated taxa are clustered together in the bootstrap test (100 replicates). Only bootstrap values higher than 50 are displayed next to the branches. As an outgroup, *H. americanum* (OR814214) was utilized. The scale bar indicates the evolutionary distance in terms of nucleotide substitutions per site.

Table 1: Distribution of haplotype samples and geographic origins

Haplotype	Number of Samples	Sample Details
Hap_1	2	**AEG4-2- *Rhipicephalus_sanguineus*-Adult, Nimf4-Dog**
Hap_2	3	**Nimf5-Dog, Nimf2-Dog,** Spain-*Rhipicephalus_sanguineus*-KJ605146
Hap_3	8	AEG2-*Rhipicephalus_sanguineus*-Adult India-*Rhipicephalus_sanguineus*-MG050160, Portugal-Dog-LC018203, Portugal-Dog-LC018194, Croatia-Dog-FJ497017, Croatia-Dog-FJ497018, Nigeria-*Rhipicephalus_sanguineus*-JX027012, Nigeria-*Rhipicephalus_sanguineus*-JX027010
Hap_4	1	**AYN8-*Rhipicephalus_sanguineus*-Nymph**
Hap_5	1	**AYN1-*Rhipicephalus_sanguineus*-Nymph**
Hap_6	1	Mexico-dog-KT215365.1
Hap_7	1	Mexico-dog-KT215363.1
Hap_8	1	Mexico-*Amblyomma_cajennense*-KT215373
Hap_9	1	India-*Rhipicephalus_sanguineus*-MG050161
Hap_10	2	Germany-Dog-MK757799, Germany-Dog-MK757808.1
Hap_11	1	Iran-Dog-KX880505.1
Hap_12	1	Israel-Dog-MK091089.1
Hap_13	1	Israel-Dog-MK091090
Hap_14	4	Turkiye-*Rhipicephalus_sanguineus*-Dog-KY197001, Turkiye-*Rhipicephalus_sanguineus*-KY197002, Turkiye-*Ixodes_ricinus*-KF034777, Turkiye-*Dermacentor_marginatus*-KF034776
Hap_15	3	Italy-*Rhipicephalus_turanicus*-KJ605147, Brazil-*Rhipicephalus_sanguineus*-MG793450, Hungary-Dog-KC509527
Hap_16	3	Egypt-*Rhipicephalus_sanguineus*-MG564215, Hungary-Dog-KC509526, Japan-*Haemaphysalis_longicornis*-LC169075
Hap_17	2	India-*Haemaphysalis_bispinosa*-MG018465, Nigeria-*Rhipicephalus_sanguineus*_nymph-JX027013
Hap_18	2	Taiwan-*Rhipicephalus_sanguineus*-MH595911 Taiwan-*Rhipicephalus_sanguineus*-MH595908

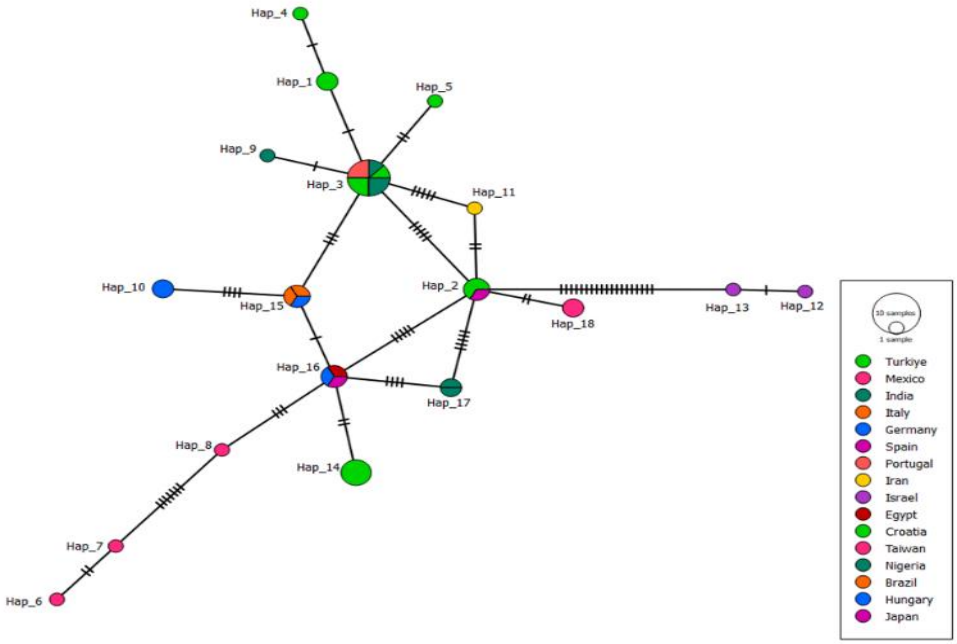

Figure 3: Haplotype network of *H. canis* dog and tick host. The size of the circle represents the frequency of each haplotype. The different colored dots represent haplotypes from the different populations.

DISCUSSION AND CONCLUSION

In this study the observation of multiple haplotypes with varying degrees of relatedness suggests that *H. canis* exhibits significant genetic diversity within the studied populations. This genetic variation, observed across different geographic regions, plays a crucial role in understanding the evolutionary dynamics of this parasite. The identified SNPs in the sequence alignment offer valuable insights into genetic variations that may be associated with geographical adaptations or host-specific interactions. Such diversity could impact the parasite's transmission dynamics, pathogenicity, and resistance to environmental pressures.

Studies on the genetic diversity of *Hepatozoon* spp. across various hosts and regions have revealed a complex and rich genetic landscape for these parasites. Research on snakes from North Africa and the Mediterranean Basin demonstrates significant patterns of genetic diversity, suggesting a complex evolutionary history in reptilian hosts (17). Similarly, substantial genetic diversity has been observed in *Hepatozoon* spp. infecting coyotes from the South-Central United States, indicating a high adaptability and local environmental influences on parasite genetics (18). In Chile, the genetic variability of *Hepatozoon* spp. in rodents emphasizes the importance of regional studies to understand their genetic structure (12). The first molecular detection and genetic analysis of *Hepatozoon* sp. in a crocodile monitor in Thailand provides new insights into the host range and genetic variability in reptilian species (19). In the eastern Amazon, studies on *Hydrochoerus hydrochaeris* and *Pecari tajacu* highlight the genetic richness and host-specific adaptations of these parasites (20). Investigations in South Africa and globally on domestic cats revealed significant genetic variations in *Hepatozoon felis*, indicating a broad host range and extensive diversity (21). Additionally, the molecular prevalence and genetic diversity of *Hepatozoon* spp. in stray cats of İzmir, Türkiye, underscore their widespread presence and variability in feline populations (22). Genetic studies of *H. canis* in golden jackals and grey wolves in Serbia show high degrees of genetic variation, reflecting the dynamic epidemiology of these parasites in wild canid populations (23). Furthermore, research on dogs and foxes in Brandenburg, Germany, identified identical *18S rRNA* haplotypes of *H. canis*, highlighting the genetic similarities and potential transmission pathways between domestic and wild canids in this region (24). These findings collectively illustrate the extensive genetic diversity and adaptability of *Hepatozoon* spp. across different hosts and regions.

This preliminary study highlights the genetic diversity of *H. canis* in both dogs and *R. sanguineus* sensu lato ticks, emphasizing the importance of understanding the evolutionary relationships and geographical distribution of this parasite. The presence of distinct haplotypes and SNPs within the *18S rRNA* gene underscores the genetic variability of *H. canis*, which is crucial for developing targeted control strategies and enhancing our understanding of the parasite's biology and epidemiology. To further refine our insights, haplotype networks using more variable target regions, such as recently published mitochondrial and apicoplast genomes, should be employed in future epidemiological studies (25). This approach could significantly improve resolution and provide deeper insights into the genetic landscape of *H. canis*.

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There is no conflicts of interest.

AUTHOR CONTRIBUTIONS

All analyses and writing of the study and final checks were carried out by SO.

ETHICAL STATEMENT

Not applicable

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RESEARCH ARTICLE

Evaluation of Serum Iron, Total Iron Binding Capacity, Transferrin Saturation, Haptoglobin and Ceruloplasmin Levels in Cows with Reticuloperitonitis Traumatica (RPT) and Pericarditis Traumatica (PT)

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Abstract

The aim of this study was to evaluate serum iron (Fe), total iron binding capacity (TIBC), transferrin saturation (TD), haptoglobin and ceruloplasmin levels in cows with reticuloperitonitis traumatica (RPT) and pericarditis traumatica (PT). Swiss brunettes and crossbreds between 2-6 years of age were used in the study. A total of 60 cows, 30 cows diagnosed clinically and radiologically as RPT and 30 cows diagnosed as PT, constituted the patient groups. The control group consisted of 20 healthy cows in the same age range. Serum Fe, TD and TDBK levels were significantly lower in PT and RPT groups compared to the control group (P<0.001); haptoglobin levels were significantly higher in PT and RPT groups compared to the control group (P<0.001); ceruloplasmin levels were significantly higher in RPT and PT groups compared to the control group (P<0.05). As a result, serum Fe level, total iron binding capacity, transferrin saturation played a role as negative acute phase reactivators while haptoglobin and ceruloplasmin changed as positive acute phase reactivators in cows with RPT and PT. These parameters are very important in determining the severity of inflammation.

Key Words: Acute phase response, cow, iron, pericarditis, reticuloperitonitis traumatica, transferrin saturation

Reticuloperitonitis Travmatika (RPT) ve Pericarditis Travmatika (PT)'lı İneklerde Serum Demir, Total Demir Bağlama Kapasitesi, Transferrin Satürasyonu, Haptoglobin ve Seruloplazmin Düzeylerinin Değerlendirilmesi

Öz

Sunulan bu çalışmada retikuloperitonitis travmatika (RPT) ve perikarditis travmatika (PT) teşhisi konulan ineklerde serum demir (Fe), total demir bağlama kapasitesi (TDBK) ve transferrin doyumu (TD) ile haptoglobin ve seruloplazmin seviyelerinin değerlendirilmesi amaçlanmıştır. Çalışmada 2-6 yaş aralığında, İsviçre esmeri ve melezleri kullanılmıştır. Klinik ve radyolojik olarak RPT teşhisi konulan 30, PT teşhisi konulan 30 toplam 60 inek hasta gruplarını oluşturmuştur. Kontrol grubunu ise aynı yaş aralığında ve sağlıklı olan 20 inek oluşturmuştur. Serum Fe, TD ve TDBK seviyesinin kontrole göre sırasıyla PT ve RPT'li grupta istatistiksel açıdan çok önemli düzeyde düşük olduğu (P<0,001); haptoglobin seviyesinin kontrol grubuna göre sırasıyla PT ve RPT'li grupta istatistiksel açıdan çok önemli düzeyde yüksek (P<0,001) olduğu; seruloplazmin seviyesinin ise kontrol grubuna göre sırasıyla RPT' li ve PT'li grupta önemli derecede (P<0,05) yüksek olduğu tespit edilmiştir. Sonuç olarak retikuloperitonitis travmatikalı ve perikarditis travmatikalı ineklerde serum Fe seviyesi, total demir bağlama kapasitesi, transferrin doyumu negatif akut faz reaktivatörü olarak rol oynarken; haptoglobin ve seruloplazmin ise pozitif akut faz reaktivatörü olarak değişim göstermiştir. Bakılan bu parametreler yangının şiddetinin tespit edilmesinde oldukça önemlidir.

Anahtar Kelimeler: Akut faz yanıt, demir, inek, perikarditis, retikuloperitonitis travmatika, transferrin saturasyonu

INTRODUCTION

Reticuloperitonitis traumatica (RPT) is a disease seen in cattle that occurs when sharp, pointed and piercing foreign bodies taken with feed penetrate the reticulum and cause inflammation and damage in various organs after leaving this organ. Factors that facilitate the ingestion of objects include poor sense of taste in cattle, gluttony of cattle, insufficient chewing of food, pica state that occurs as a result of mineral and trace element deficiencies, long lactation period, contamination of barns and pastures by foreign objects, and prolonged stay of animals in pasture. Pointed and sharp objects pierce the reticulum wall and cause peritonitis or penetrate the liver, kidney, spleen, lung and heart, causing damage to these organs (1). Inflammation of the pericardial sac occurs when objects in the reticulum penetrate the diaphragm and sink into the heart, a disease called pericarditis traumatica (PT) (2,3). Symptoms include fever, anorexia, rumen atony and recurrent tympani, abdominal distension, groaning due to pain, cachexia, tachypnea, tachycardia, fullness in the vena jugularis and positive venous pulse, friction and churning sound in the heart, swelling in the ventral part of the body due to circulatory failure, dull sound on heart percussion and cardiac arrhythmia (4-8). Clinical findings, ferroscopic and radiographic examinations, pericardiocentesis and ultrasonographic examinations are used in the diagnosis of the disease. (8,9).

Acute phase proteins (AFP) are proteins synthesized by the liver in response to an acute inflammatory response. These proteins are negligible in healthy animals but increase rapidly during inflammation and act as an indicator of inflammation (10). The main function of haptoglobin, an important acute phase protein for cattle, is to prevent Fe loss by forming stable complexes with free hemoglobin in the blood. Another important acute phase protein for cattle is ceruloplasmin, which is particularly useful in monitoring the inflammatory process (1).

The aim of the study was to evaluate serum iron, total iron binding capacity, transferrin saturation, haptoglobin and ceruloplasmin levels in cows with RPT and PT.

MATERIAL AND METHODS

Animal Material

The animal material of this study consisted of a total of 60 cows between 2-6 years of age, including 30 cows diagnosed clinically and radiologically with RPT and 30 cows diagnosed with PT. The control group consisted of 20 healthy cows in the same age range.

Blood Sampling and Biochemical Measurements

Blood samples were collected from sick cows after diagnosis and from healthy cows after diagnosis using a sterile needle tip (Vacuette®, Greiner Bio-One GmbH, Austria) compatible with the holder into vacuum gel serum tubes (BD Vacutainer®, BD, UK). Blood samples taken in vacuum tubes were centrifuged at 3000 rpm for 10 minutes (Hettich Rotina 380R®, Hettich, Germany) to obtain serum samples. Before blood samples were taken, the animals were also clinically examined and vital signs were evaluated and noted. Biochemical measurements were made from the serum samples obtained. Total iron binding capacity (TIBC) was calculated by summing serum iron (Fe) and unsaturated iron binding capacity (UIBC) levels. Serum transferrin saturation (TS) was determined by calculating the formula (TS (%)=Fe/TIBC x 100) from serum Fe and TIBC levels (11). Fe and UIBC were measured colorimetrically (Epoch, Biotek, USA) with a commercial test kit (Biolabo, France). Haptoglobin was determined as reported by Skinner et al. (12), ceruloplasmin was measured by the method of Colombo and Richterich (13).

Glutaraldehyde Test Procedure

Glutaraldehyde (GLA) testing is routinely used to assess the severity of inflammation and prognosis in patients diagnosed with RPT and PT. The advantages of the test are that it is easy to administer, inexpensive and provides results in a short time. The test is done using whole blood and provides information about the amount of fibriogen. In a sterile empty tube, a 1/1 ratio of blood and GLA solution is placed and mixed, then the clotting time is interpreted by looking at the duration of clotting by turning upside down every 30 s. If the clotting time is between 0-5 minutes, the inflammation is strongly positive, if it is between 6-10 minutes, the inflammation is moderately positive, if it is between 11-15 minutes, the inflammation is mildly positive, and if no clotting occurs within 15 minutes, the test is interpreted as negative (14-16).

Radiographic Imaging Procedure

The radiological evaluation was performed in the Department of Radiology at XXX University School of Veterinary Medicine. The reticulum and diaphragm border were evaluated in the radiologic examination. A Dynamic brand ceiling static x-ray device and an FCR Prima brand (Fujifilm FCR T2 Veterinary Set, Medical Technology, Türkiye) imaging unit were used in the radiologic evaluation. For this purpose, irradiation doses between 35-40 mA and 85-90 kW were adjusted to the size of each cow. Radiographic images were taken by irradiation at a distance of 80 cm between the tube and the cassette (35x43 size).

Statistical Analysis

Data were presented as mean ± standard error of mean (SEM). The groups were showed normal distribution according to the Shapiro-Wilk test. The one-way ANOVA test was used for multiple comparisons, and the Tukey HSD test was used for post-hoc comparisons. The SPSS (SPSS Version 26.0®, Chicago, IL, USA) program was used for all statistical analyses. The differences between the groups in terms of the parameters were considered significant at the $p<0.05$ level.

RESULTS

Clinical Findings

In the clinical examination of the cows with RPT and PT, fever, anorexia, rumen atony and chronic tympani, abdominal tension, groaning, kyphosis posture, cachexia, loss of efficiency and positive ferroscopic examination were noted. However, it was also found that the group with PT had fullness and positive venous pulse in the vena jugularis, friction or churning sound in the heart on auscultation of the heart,

swelling in the ventral part of the body due to circulatory failure, dull sound on percussion of the heart and rhythm disturbance in the heart. Figure 1 shows the edema and positive venous pulse in the gerd region of 2 cows from the PT group of the present study. The severity of the inflammation was evaluated by GLA test on cows diagnosed with RPT and PT after clinical and radiological examinations.

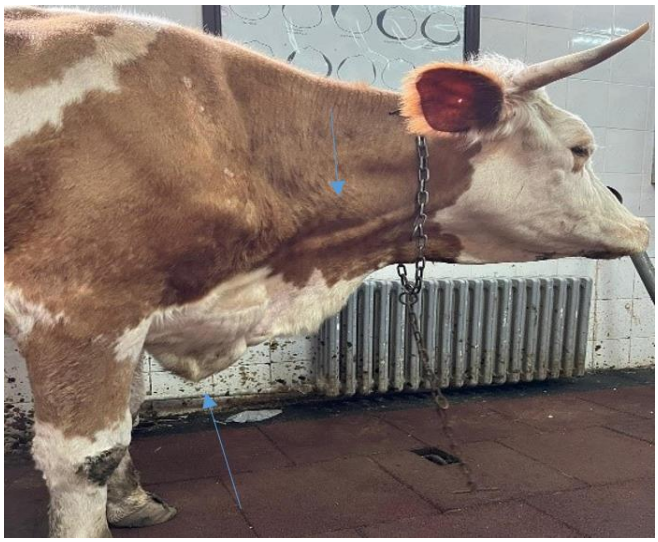

Figure 1. Show the edema and positive venous pulse in the gerd region of cows from the PT group of the present study

Biochemical Findings

The vital and biochemical findings of the sick and healthy cows are given in Table 1 and Table 2, respectively. Among the biochemical parameters, Fe, TS and TIBC levels were statistically significantly lower in PT and RPT groups compared to the control group (P<0.001); haptoglobin level was statistically significantly higher in PT and RPT groups compared to the control group (P<0.001); ceruloplasmin level was significantly higher in RPT and PT groups compared to the control group (P<0.05).

Parameters	Groups (Mean ± SEM)			P value
	TRP (n:30)	TP (n:30)	Control (n:20)	
Rectal temperature (°C)	38.57 ± 0.28^{ab}	39.28 ± 0.38^{b}	37.89 ± 0.14^{a}	0.008
Breaths/min	25.78 ± 1.43	26.45 ± 1.27	22.30 ± 1.12	0.362
Heart beats/min	71.62 ± 2.47^{b}	61.31 ± 2.25^{a}	68.50 ± 2.26^{ab}	<0.001

a-b: The mean values with different letters in the same line represent the difference between patient and control groups (p<0.05). **n:** The number of cow in groups. **SEM:** Standard error of mean. **RPT:** Reticuloperitonitis traumatic group. **PT:** Pericarditis traumatic group.

Table 2. Biochemical findings of RPT, PT and control groups

Parameters	Groups (Mean ± SEM)			P value
	RPT (n:30)	PT (n:30)	Control (n:20)	
Iron (mg/dL)	78.80 ± 1.83^{b}	61.14 ± 1.92^{a}	112.36 ± 5.69^{c}	<0.001
Total iron-binding capacity (g/dL)	212.81 ± 3.84^{a}	200.14 ± 5.03^{a}	264.17 ± 6.21^{b}	<0.001
Transferrin saturation (%)	37.46 ± 1.13^{b}	31.37 ± 1.39^{a}	42.69 ± 2.05^{c}	<0.001
Haptoglobin (g/L)	0.29 ± 0.02^{b}	0.31 ± 0.02^{b}	0.09 ± 0.01^{a}	<0.001
Ceruloplasmin (mg/dL)	14.11 ± 0.84^{b}	13.92 ± 0.90^{b}	10.17 ± 0.86^{a}	0.011

a-c: The mean values with different letters in the same line represent the difference between patient and control groups (p<0.05). **n:** The number of cow in groups. **SEM:** Standard error of mean. **RPT:** Reticuloperitonitis traumatic group. **PT:** Pericarditis traumatic group.

Apart from this, the correlation of biochemical parameters in the study is given in Table 3. Total iron binding capacity and transferrin saturation were positively correlated with serum iron level. It was found that the level of haptoglobin was negatively correlated with serum iron level and total iron binding capacity. It was noted that ceruloplasmin level was negatively correlated with serum iron level and total iron binding capacity.

Table 3. Correlation of biochemical parameters in the study

Parameters	Iron (mg/dL)	Total iron-binding capacity (g/dL)	Transferrin saturation (%)	Haptoglobin (g/L)
Total iron-binding capacity (g/dL)	0,564**			
Transferrin saturation (%)	0,794**	-0,038		
Haptoglobin (g/L)	-0,397**	-0,441**	-0,182	
Ceruloplasmin (mg/dL)	-0,293**	-0,256*	-0,148	0,183

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Radiological Findings

In cases with RPT, foreign bodies sinking into the reticulum may have different orientations and the problem in the abdomen is usually related to the direction of sinking and whether or not they pass into the abdominal cavity after sinking. In most of our cases, the objects that penetrated the reticulum were either oriented towards the diaphragm or craniovetrale. In cases with RPT, it was observed that the foreign

body penetrated into the base of the reticulum and formed a lesion in this region and the reticulum border was regular. (Figure 2). However, in cases with pericarditis, it was observed that most of the foreign body left the reticulum, moved towards the chest cavity and the diaphragmatic border became more irregular (Figure 3).

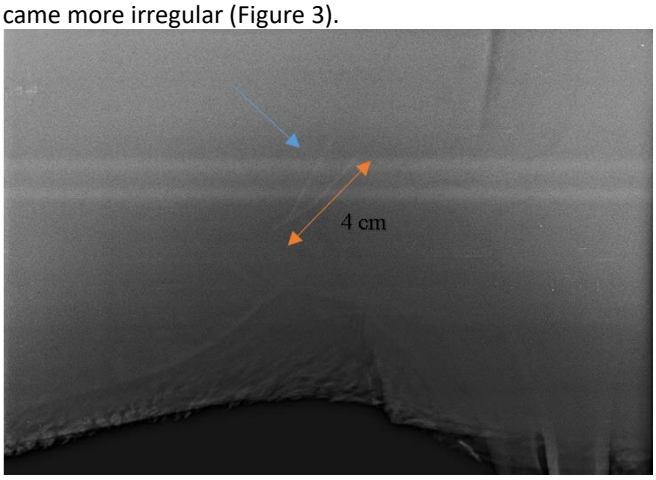

Figure 2 In cases with RPT, it was observed that the 4 cm long foreign body penetrated into the base of the reticulum and formed a lesion in this region and the reticulum border was regular.

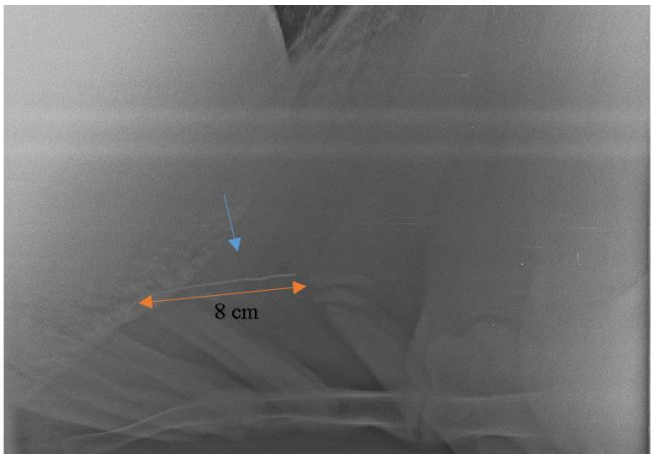

Figure 3. In cases with pericarditis, it was observed that most of the 8 cm long foreign body left the reticulum, moved towards the chest cavity and the diaphragmatic border became more irregular.

Glutaraldehyde Test Findings

In the group diagnosed with RPT and PT, the clotting time of the glutaraldehyde test was between 0-5 minutes, while this time was longer than 15 minutes in the control group (Table 4). This is an indication that the inflammation is severe in the patient group.

Table 4. Comparison of GLA test times between PT, RPT and control group

Parameters	Groups (Mean ± SEM)			P value
	RPT (n:30)	PT (n:30)	Control (n:20)	
GLA (minute)	2 ± 0.20	4.5 ± 0.32	16.5	<0.001

In the group diagnosed with RPT and PT, the clotting time of the glutaraldehyde test was between 0-5 minutes, while this time was longer than 15 minutes in the control group. **n:** The number of cow in groups. **SEM:** Standard error of mean. **RPT:** Reticuloperitonitis traumatic group. **PT:** Pericarditis traumatic group.

DISCUSSION AND CONCLUSION

Reticuloperitonitis traumatica and its complications are among the important digestive system diseases in cattle (17). In studies conducted in cattle with RPT and PT, fever, anorexia, rumen atony and chronic tympani, abdominal tension, groaning and kyphotic posture due to pain, cachexia, loss of yield and positive ferroscopic examination; However, in the group with PT, fullness in the vena jugularis and positive venous pulse, friction or churning sound in the heart on auscultation of the heart, swelling in the ventral part of the body due to circulatory failure, dull sound on percussion of the heart and cardiac arrhythmia are among the clinical symptoms reported (2,18,19). In this study, similar clinical findings were observed in the RPT and PT groups in accordance with the literature.

GLA Test is used to detect the severity of inflammation in many diseases, especially RPT and PT in cattle. This test is a practical method that can be applied very quickly. It is based on the detection of increases in serum fibrinogen and globulin concentrations. Glutaraldehyde forms a clot by primarily reacting chemically with free amino groups in fibrinogen and immunoglobulin. The clotting time in this test allows estimating the amount of protein produced in response to the inflammatory process (19). In studies conducted in cattle with RPT and PT, it was reported that blood clotted between 0-5 minutes in the GLA test due to increased fibrinogen concentration in patients with severe inflammation and the test was strongly positive (1,16). In this study, which was conducted in accordance with the aforementioned literature, blood clotting within the first 5 minutes in the GLA test performed in the patient group is an indication of the severe course of the inflammation.

Acute phase proteins are very useful in the evaluation of infection, inflammation, trauma, etc. occurring in the organism (10). Haptoglobin and ceruloplasmin are among the positive acute phase proteins synthesized by the liver in cattle (20-22). Studies have reported that haptoglobin and ceruloplasmin levels increase in severe inflammation, trauma and infectious conditions (19,22). In this study, haptoglobin and ceruloplasmin levels were significantly higher in cows diagnosed with RPT and PT compared to the control group. Authors think that the probable cause of this is trauma due to foreign body, severe inflammation and tissue damage.

Iron is an element with very important functions for living organisms. Iron acts as a building block of many proteins, especially hemoglobin (23). One of the most important conditions affecting iron metabolism is severe inflammation (24). Therefore, Fe levels are often examined to determine the severity of the inflammation. It has been reported in studies that Fe levels decrease in severe inflammatory conditions (25-27).

Edema occurs in the intestines as a result of loss of appetite and circulatory disorders due to heart failure. Iron deficiency occurs because this situation causes a decrease in iron intake with food and a decrease in the absorption of iron. Additionally, ferroportin binds and breaks down as inflammation-related cytokine levels increase. This condition impairs the absorption of iron, causing iron to be retained especially in the liver and reticuloendothelial cells and decreasing its level in the blood (28).

One of the most important reasons for this situation is loss of appetite and nutritional deficiency due to severe inflammation, and the other reason is hypoferremia caused by interleukin-6 (IL-6), one of the inflammatory mediators (23,29). It has been stated that total iron binding capacity decreases in inflammatory conditions (30). The organism's defense mechanism aims to retain Fe, which is necessary for the replication of pathogenic agents in inflammatory situations (31). Transferrin saturation is an indicator of how much Fe in serum is bound to transferrin. Transferrin saturation decreases in iron deficiency (32). In the present study, serum Fe, TIBC and TS were found to be significantly lower in the PT and RPT groups compared to the control group, respectively.

Authors think that the main reason for this is the severe course of inflammation due to both trauma and infection in the patient group and the activation of the body's defense system and retention of iron stores in the body to prevent the use of iron by pathogenic agents, as well as the development of iron deficiency as a result of the lack of food intake due to digestive problems and anorexia that occur with the deterioration of the general condition due to severe inflammation.

FINANCIAL SUPPORT

No support was received from any organization in the conduct of this research.

CONFLICTS OF INTEREST

The authors have declared that there are no conflicts of interest associated with this study or its results.

AUTHOR CONTRIBUTIONS

The writing of the study and final checks were carried out with the contributions of all authors

ETHICAL STATEMENT

The study was started after approval was obtained from Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK/2023-003).

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RESEARCH ARTICLE

Prevalence of Parasites Detected in Domestic Dogs from Konya Province: A Retrospective Study

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Abstract

Parasitic infections caused by helminth, protozoa, and ectoparasites pose a threat to animal and human health due to clinical changes and transmission of potentially zoonotic agents. This study's objective was to assess parasitic diseases in dogs admitted to the Selcuk University Veterinary Faculty Animal Hospital (SUVFAH) between 2015 and 2021. Fecal, blood, and skin samples sent to the laboratory of the Department of Parasitology, Faculty of Veterinary Medicine were examined. The majority of samples (n = 846) were collected from domestic dogs, and only 49 were from shelter dogs. During the study period, 33.74% (302/895) of the dogs tested positive for at least one parasite, including single (29.83%), double (4.63%), triple (0.14%) and quadruple (0.14%) internal parasite infections. This study revealed that the prevalences of protozoan and rickettsial parasites, including *Isospora* spp., *Giardia duodenalis, Sarcocystis* spp., *Entamoeba histolytica,* and *Ehrlichia canis* in dogs from Konya province, were 7.01%, 26.79%, 1.26%, 0.7%, and 5.26%, respectively. On the other hand, the prevalences of helminthic parasites *Toxascaris* spp., *Trichuris* spp., *Anyclostoma* spp., *Uncinaria* spp., *Dipylidium caninum*, *Taenia* spp. and *Mesocestoides* spp. were 0.14%, 0.42%, 0.42%, 0.42%, 0.42%, 0.42% and 0.14%, respectively. As ectoparasitic infestations, 11.66% *Demodex canis* infestation and 0.61% myiasis cases were encountered. Although most of the dogs in the study were owned, the rate of internal and external parasite infection/infestation was high. In this case, pet owners have a great responsibility to prevent parasitic infections in pets, which are the source of many parasitic agents with zoonotic properties.

Key Words: Dog, ectoparasites, helminths, protozoa, zoonoses

Konya İlindeki Evcil Köpeklerde Tespit Edilen Parazitlerin Prevalansı: Retrospektif Bir Çalışma

Öz

Helmint, protozoa ve ektoparazitlerin neden olduğu paraziter enfeksiyonlar, klinik değişiklikler ve potansiyel zoonotik ajanların bulaşması nedeniyle hayvan ve insan sağlığı için tehdit oluşturmaktadır. Bu çalışmanın amacı, 2015-2021 yılları arasında Selçuk Üniversitesi Veteriner Fakültesi Hayvan Hastanesine (SUVFAH) başvuran köpeklerdeki paraziter hastalıkları değerlendirmektir. Selçuk Üniversitesi Veteriner Fakültesi Parazitoloji Ana Bilim Dalı Laboratuvarına 2015-2021 yılları arasında gönderilen dışkı, kan ve deri örnekleri incelenmiştir. Örneklerin çoğunluğu (n = 846) evcil köpeklerden ve sadece 49'u barınak köpeklerinden toplanmıştır. Çalışma süresi boyunca, köpeklerin %33.74'ü (302/895) tekli (%29.83), ikili (%4.63), üçlü (%0.14) ve dörtlü (%0.14) iç parazit enfeksiyonları dahil olmak üzere en az bir parazit için pozitif test edilmiştir. Bu çalışma Konya ili köpeklerinde, *Isospora* spp., *Giardia duodenalis*, *Sarcocystis* spp., *Entamoeba histolitica* ve *Ehrlichia canis* gibi protozoon ve riketsiyal parazitlerin prevalanslarının sırasıyla %7.01, %26.79, %1.26, %0.7 ve %5.26 olduğunu ortaya koymuştur. Diğer yandan helmintik parazitlerden *Toxascaris* spp., *Trichuris* spp., *Anyclostoma* spp., *Uncinaria* spp., *Dipylidium caninum*, *Taenia* spp. ve *Mesocestoides* spp. prevalansı da sırasıyla %0.14, %0.42, %0.42, %0.42, %0.42 ve %0.14 olarak tespit edilmiştir. Ektoparaziter enfestasyonlar olarak da %11.66 *Demodex canis* enfestasyonu ve %0.61 miyaz vakasına rastlanmıştır. Çalışmadaki köpeklerin çoğu sahipli olmasına rağmen, iç ve dış parazit enfeksiyonu/enfestasyonu oranı yüksektir. Bu durumda zoonoz özellik gösteren birçok paraziter etkenin kaynağı olan evcil hayvanlarda paraziter hastalıkların önlenmesi için evcil hayvan sahiplerine büyük sorumluluk düşmektedir.

Anahtar Kelimeler: Ektoparazit, helmint, köpek, protozoa, zoonozlar

INTRODUCTION

Domestic dogs (*Canis familiaris*) are the most popular pets among the carnivores (1). Due to their positive effects on adults' and children's social, physical, and psychological health, dogs are kept for many beneficial purposes. Additionally, they are utilized for security, rehabilitative programs, sports, hunting, life-saving, and money production through breeding and sale. There are many factors that can affect a dog's life, and parasitic diseases are one of the most important. The prevalence of internal and external parasites in domestic dogs can vary depending on various factors such as geographical location, climate, living conditions, and preventive measures taken by dog owners. The parasites commonly affecting domestic dogs in many parts of the world include ticks, fleas, intestinal worms (such as hookworms, roundworms, whipworms, and tapeworms), and heartworms. Gastrointestinal parasites are one of the main obstacles to canine health and well-being. They cause direct and indirect losses (2). The majority of intestinal parasites that cause morbidity and mortality in dogs are zoonotic, such as *Ancylostoma* spp., *Toxocara canis*, *Toxascaris leonina*, *Capillaria* spp., *Uncinaria* spp., *Trichuris vulpis*, *Taenia*/*Echinococcus*, *Mesocestoides* spp., and *Sarcocystis* spp. (3). Because of their natural life cycle and the ability of cysts/eggs to remain viable and infective for long periods after being shed in soil, gastrointestinal protozoa and helminths are typically the most prevalent parasites, resulting in environmental contamination and the spread of parasite infections among animal populations and humans (4,5). Dogs and humans share the same environment, which allows gastrointestinal parasites to contaminate human food, drink, and skin. This can lead to diseases with life-threatening consequences (6). Dogs can become infected with gastrointestinal parasites via intrauterine and galactogen transmission (e.g., *Toxocara canis, Ancylostoma caninum,*) or later in life by consuming the infectious stages of protozoa or helminths (6). Whether helminths or protozoa, these parasites typically result in reduced performance, growth retardation, increased susceptibility to other infectious diseases, and, in rare instances, severe clinical symptoms (7). The most severe infections and morbidity rates occur in newborns and puppies, where intestinal parasites can be lethal, especially when paired with other infectious disorders such as parvoviral enteritis (8, 9). Furthermore, some canine intestinal parasites, such as *Taenia* spp. and *Sarcocystis* spp., can spread to farm animals, causing major economic losses (10). However, understanding the epidemiology of canine parasites is necessary to limit the risk of human infections, particularly for pregnant women, children, and immunocompromised individuals (11).

External parasites of dogs include ectoparasites such as ticks, fleas, lice, and mites. They frequently dwell as blood-sucking parasites on the skin, causing pruritis and hypersensitivity reactions, and may serve as vectors for a variety of infections of veterinary or public health concern (12). In Türkiye, dogs are frequently let to roam freely and stray on main city roadways, scavenge for food scraps near garbage dumps. These procedures expose dogs to a wide range of parasitic diseases including external parasites (12).

Rickettsial infections are caused by bacteria from the order Rickettsiales, as well as the genera *Neorickettsia*, *Orientia*, *Anaplasma*, *Neoehrlichia*, *Rickettsia*, and *Ehrlichia* are seen in dogs (13). In Türkiye, ehrlichiosis is one of the most frequent tick-borne diseases of dogs. Clinical infections in dogs are prevalent, but the disease is rarely observed in other hosts (ticks and cats) (14, 15). Canine monocytic ehrlichiosis (CME) is the name of the disease that is primarily linked to *E. canis* infections that are severe in dogs. According to Mylonakis et al. (2019), CME may be one of the leading causes of life-threatening pancytopenia in dogs in *E. canis*-endemic regions as well as Türkiye and South East Asia (16). This viewpoint is supported by the occurrence of *R. sanguineus* across Türkiye (17).

The purpose of this study was to ascertain the prevalence of ectoparasites, hemoparasites, and gastrointestinal parasites in dogs, together with a remark on zoonotic agents, in the province of Konya, Türkiye.

MATERIAL AND METHODS

Feces, blood, and skin samples sent to the Selcuk University Veterinary Faculty Parasitology Department Laboratory, Konya between 2015 and 2021 were included in the present study. Konya is the largest province in the country by area and has a variety of geographical and climatic features that could influence parasite prevalence. To determine gastrointestinal helminth fauna in owned and shelter dogs, fecal samples were collected from 713 dogs and analyzed using Native, Fulleborn flotation, and Benedek sedimentation methods (18). All fecal samples were first macroscopically screened for the presence of nematodes and proglottids of cestodes. 0.9% isotonic saline solution was used for the Native fecal examination method, saturated salt water for the flotation method, and distilled water for the Benedek sedimentation method. According to the literature, oocysts, cysts, and eggs were identified based on morphological characteristics (18-20). Skin samples and maggot larvae of 163 dogs were collected in sterile petri dishes and sent to the laboratory for analysis. The debris is then placed on a microscope slide, coverslipped, and inspected with a 10 × microscope objective. The material was put on a slide, then 10% potassium hydroxide was added in five drops. After placing a cover slip over the sample, it was examined under a microscope to check for the presence of mites, larvae, or ova (21). Under a stereozoom microscope, maggot larvae were visible, however the genus of the larvae could not be identified. Blood samples were taken from 19 dogs. Thin blood smears were made from EDTA-anticoagulated blood, dried in the open air, fixed in absolute methanol for 3-5 minutes, stained with 10% Giemsa solution for 45 minutes to an hour, washed with tap water, and dried. The smears were checked for blood protozoans using a light microscope (100X), and pathogens were investigated by scanning 100 microscopic fields.

RESULTS

As a result of the study ecto- and endoparasites were detected in 302 (33.74%) of 895 dogs. During the study period, 33.74% (302/895) of the dogs tested positive for at least one parasite, including a single (29.83%), double (4.63%), triple (0.14%), and quadruple (0.14%) internal parasite infections. This study revealed that the prevalences of protozoan and

rickettsial parasites, including *Isospora* spp., *Giardia duodenalis, Sarcocystis* spp., *Entamoeba histolytica,* and *Ehrlichia canis* in dogs from Konya province, were 7.01%, 26.79%, 1.26%, 0.7%, and 5.26%, respectively. On the other hand, the prevalences of helminthic parasites *Toxascaris* spp., *Trichuris* spp., *Anyclostoma* spp., *Uncinaria* spp., *Dipylidium caninum*, *Taenia* spp. and *Mesocestoides* spp. were 0.14%, 0.42%, 0.42%, 0.42%, 0.42%, 0.42% and 0.14%, respectively. As ectoparasitic infestations, 11.66% *Demodex canis* infestation and 0.61% myiasis cases were encountered. The parasites detected in the study are shown in Table 1 and Table 2 and the total parasite prevalences are shown in Table 3.

Table 1. Single parasitic infection rates in dogs between 2015-2021

Helminths	(n:713)	Positive	Prevalence(%)
Nematod			
Toxocara spp.		30	4.21
Toxascaris spp.		1	0.14
Cestod			
Dipylidium caninum		3	0.42
Taenia /Echinococcus spp.		3	0.42
TOTAL	713	37	5.19
Protozoans	(n:713)		
Giardia spp.		164	23
Isospora spp.		29	4.07
Sarcocystis spp.		6	0.84
Entamoeba spp.		3	0.42
Chilomastix spp.		1	0.14
TOTAL	713	203	28.47
Blood protozoans	(n:19)		
Ehrlichia spp.		1	5.26
TOTAL	19	1	5.26
Ectoparasites	(n:163)		
Demodex spp.		19	11.66
Sarcoptes spp.		3	1.84
Otodectes cynotis		2	1.23
Trichodectes canis		1	0.61
Anal myiasis/Dipteran larvae		1	0.61
TOTAL	163	26	15.95

Table 2. Mix parasitic infection rates in dogs between 2015-2021

Parasites	n:713	Positive	Prevalence(%)
Toxocara spp.+ *Isospora* spp.		4	0.56
Toxocara spp.+ *Giardia* spp.		9	1.26
Isospora spp. + *Giardia* spp.		13	1.82
Sarcocystis spp.+ *Giardia* spp.		1	0.14
Anyclostama spp.+ *Uncinaria* spp.		1	0.14
Giardia spp.+ *Entamoeba* spp.		1	0.14
Giardia spp. + *Anyclostoma* spp.		1	0.14
Isospora spp.+ *Sarcocyctis* spp.		2	0.28
Isospora spp.+ *Mesocestoides* spp.		1	0.14
Total dual infection	713	33	4.63
Isospora spp.+ *Entamoeba* spp. + *Giardia* spp.		1	0.14
Total triple infection	713	1	0.14
Trichuris spp. + *Toxocara* spp. + *Ancylostoma* spp. + *Giardia* spp.		1	0.14
Total quadruple infection	713	1	0.14

Table 3. Total parasite prevalences

Helminths	Prevalence(%)
Nematod	
Toxocara spp.	6.17
Toxascaris spp.	0.14
Trichuris spp	0.14
Anyclostoma spp.	0.42
Uncinaria spp.	0.14
Cestod	
Dipylidium caninum	0.42
Taenia /Echinococcus spp.	0.42
Mesocestoides spp.	0.14
Protozoans	
Giardia spp.	26.79
Isospora spp.	7.01
Sarcocystis spp.	1.26
Entamoeba spp.	0.7
Chilomastix spp.	0.14
Blood protozoans	
Ehrlichia spp.	5.26
Ectoparasites	
Demodex spp.	11.66
Sarcoptes spp.	1.84
Otodectes cynotis	1.23
Trichodectes canis	0.61
Anal myiasis	0.61

DISCUSSION AND CONCLUSION

Dogs, which play an important role in human life, are associated with many zoonotic microorganisms of parasitic origin. Among these, helminths and gastrointestinal protozoa are the most important enteropathogens causing death in dogs (22). The aim of our study was to determine the prevalence of canine gastrointestinal parasites, hemoparasites and ectoparasites with a focus on zoonotic agents in a large dog population from different districts of Konya. Such studies were necessary due to the lack of large and recent data on the subject. In order to minimize the incidence of parasitic diseases, especially their transmission to humans, the factors influencing their epidemiology should be well understood. Factors such as geographical region, climate, intermediate or final host population, pre-patent or patent period of infection, diagnostic method, and drug use are reflected in the study results and cause differences (22).

Among the protozoa, infections with *Cryptosporidium* spp., *Giardia* spp., *Sarcocystis* spp., and *Isospora* spp. are commonly encountered in dogs (23). In this study, different protozoan species (*Isospora* spp., *Giardia* spp., *Sarcocsyt* spp. and *Entamoeba* spp., *Ehrlichia* spp. and *Chilomastix* spp.) were found in 203 (28.47%) of a total of 713 dogs. The majority of parasites detected in this investigation were protozoa. The prevalence of *G. duodenalis* (26.79%) was higher than the other parasites found in the dogs in this study. However, molecular assays and parasite genotyping are necessary to identify the species and assemblages involved, as well as to assess their zoonotic potential (24). *Giardia* is a common protozoa affecting a wide range of animals, including humans with global significance. They are the most frequent gastrointestinal pathogens for dogs and cats in developed areas, infecting around one billion people

worldwide (25). A review by Ballweber and colleagues notes that the reported prevalence of *Giardia* in feces varies from study to study, and that this variation is partly related to geography, detection method, age of the animal, whether the animal was symptomatic or not, and where the animal was housed (26).

Canine coccidiosis is a disease generally caused by protozoa of the *Isospora* species. The disease causes colitis or enteritis in dogs and has a high mortality rate. The presence of *Isospora* spp. in dogs was found to range from 5.5% to 26.45% in Türkiye (27-30). The prevalence of *Isospora* species was determined to be 7.01% in this study. In a study conducted in Konya province by Uslu et al. (30), the infection rate (26.45%) was found to be higher than in previous studies. This situation is thought to be related to the age of the dogs (2-6 month old puppies) sampled in the study. Canine coccidiosis usually causes clinical signs in young puppies, and infection rates decrease in later periods or the infection progresses asymptomatically (7). The rate of *Isospora* spp. detected in our study is the findings of a very wide age range of dogs.

Sarcosporidiosis is a protozoan infection that rarely causes diarrhoea in the final host dogs. Since sarcosporidiosis is particularly severe in intermediate host ruminants and causes economic losses, most of the studies are focused on ruminants in Türkiye (31, 32). The frequency of *Sarcocystis* spp. in dogs varied between 0.8 and 81.6% in the few studies conducted in dogs in Türkiye (23,27,30,33). In this study, *Sarcocystis* spp. sporocysts were found in 1.26% of the fecal samples examined and the prevalence value obtained was found to be compatible with the results of other studies.

Entamoebiasis (amibiasis), which is common in tropical and subtropical regions and is mostly caused by *Entamoeba histolitica*, which infects humans, is rare in some wild and domestic animals, including cats and dogs. Canine amibiasis has been reported to be of human origin, and it has been suggested that transmission is the result of ingestion of parasite cysts with contaminated water and food, or that *Musca domestica* may act as a mechanical vector (34). In a study conducted by Denizhan and Karakuş (23) in Türkiye, *Entamoeba histolytica* was found at a rate of 11.48%. In this study, *Entamoeba* spp. prevalence was found to be 0.7%. This rate was found to be lower than in the study conducted in Türkiye. This may be due to the fact that there were fewer stray dogs in contact with human remains in our study and most of our material consisted of owned dogs.

The most prevalent canine gastrointestinal (GI) helminths are *Toxocara* sp., *Toxascaris* sp., *Echinococcus* spp., *Taenia* spp., *Ancylostoma* spp., *Dipylidium* spp., *Uncinaria* spp., *Capillaria* spp., and *Trichuris* spp. (35). *Toxocara* spp., *Echinococcus* spp., and *Ancylostoma* spp. are particularly significant in both underdeveloped and emerging countries due to the limited use of antiparasitic medications, low socioeconomic conditions, and a lack of education (36). The genera/species distribution of helminths detected in dogs in Türkiye is mainly based on necropsy or fecal examination of street/owned dogs; the most common species reported are *T. canis*, *T. leonina*, hookworms, *Taenia* spp., and *D. caninum*. In studies on the prevalence of intestinal helminths in dogs in Türkiye, it was found that the prevalence of parasites ranged from 19.4% to 86.96% (30,37-43).

Toxocara canis is a soil-associated nematode that is known as the most frequent intestinal parasite in dogs and wild canids (44). Furthermore, it has been linked to visceral and ocular larval migrans in humans. *T. canis* prevalence has been estimated to be between 4.2% and 51% in Türkiye (41, 45). In the current study, *T. canis* was found in 6.17% of fecal samples. In a study conducted in Konya province by Uslu et al., (30), the infection rate (33.06 %) was found to be higher than in previous studies. It was thought that the reason for this situation was the young age (2-6 months old puppies) of the dogs and the fact that they were stray dogs. Due to transplacental and transmammary transmission, puppies are more susceptible to infection. Additionally, immunity to certain parasites is typically gained with age, most likely as a result of one or more infections (46). The dogs in our study ranged widely in age from puppy to adult.

In the current study, the prevalence rates of *Toxascaris* spp. (0.14%), *Trichuris* spp. (0.14%), *Anyclostoma* spp. (0.42%), *Uncinaria* spp. (0.14%), and cestodes such as *D. caninum* (0.42%), *Taenia* spp. (0.42%), and *Mesocestoides* spp. (0.14%) were very low, which is consistent with the results of some other studies but contradicts others. However, it should be noted that the diagnostic technique used in our study (centrifugal flotation) is more specific for nematodes than for cestodes, which may explain the relatively low prevalence of cestodes in our study (47). In addition, the majority of the dogs studied were on a strict diet with no access to raw meat or carcasses, reducing the likelihood of taeniid tapeworm contamination (48).

Taenia spp. prevalence in dogs is reported to be between 6.1% and 46.0% in Türkiye (38,49,50) and 1.1-33.0% abroad (51-53). In this study, *Taenia* spp. prevalence was determined as 0.42%. This result is lower than the values determined both in Türkiye and abroad. The fact that *Taenia* spp. could not be identified with eggs in the fecal examination, and no ring was observed in the macroscopic examination, suggesting that these eggs may also be *Echinococcus granulosus* eggs.

The prevalence of *Dipylidium caninum*, one of the canine cestodes of zoonotic importance, was found to be between 0.3-52% in Türkiye (30,38,41,45,54-56). In our study, *D. caninum* prevalence was determined as 0.42%. Compared to other studies conducted in this study, *D. caninum* 12.5% in Hatay (54), 3.5% in Van (38), 4.3% in Eskişehir and 2.9% in Afyonkarahisar (45), 2.8% in Kayseri (41) and 2.8% in Diyarbakır (56) was found to be lower than the most of the reported rates, and higher than a study conducted in Konya (55). It is thought that the differences in prevalence values may be due to the differences in the rates of flea or lice infestation, such as *Ctenocephalides canis*, *C. felis*, *Pulex irritans,* and *Trichodectes canis*, which are the vectors of *D. caninum* in dogs, from region to region. According to the results of this study and other studies conducted in Türkiye, *D. caninum* can be considered as a common cestode across Türkiye.

Mites are found throughout the world and have an affinity for a diverse group of mammalian hosts, including humans. With more than 30,000 described species, the most important mites causing dermatopathies found in the Canidae family are *Otodectes cynotis*, *Sarcoptes scabiei*, *Demodex canis,* and *Cheyletiella* spp. (57). Canine demodicosis is a

well-known skin disease seen in veterinary medicine. It is a dermatological disorder caused by mites colonizing the hair follicles and sebaceous glands. Erythema, alopecia, comedones, follicular hyperkeratosis, pustules, crusts, and seborrhea are all dermatological alterations (58). In Türkiye, *Demodex* species have been reported morphologically and molecularly from various companion animals (59-63). In this study, *Demodex canis* was detected at a rate of 11.66 %. Myiasis is defined as the parasitism of some Diptera larvae in human and animal tissues and natural cavities, feeding on dead or living tissues of the host at certain times and causing lesions there. Myiasis is a frequently encountered condition worldwide, including Türkiye (64-70). In previous studies conducted in Konya province, several cases of traumatic myiasis have been reported in dogs, other animals, and humans (70-73). In our study, it was detected at a low rate of 0.61%. The reason for this low rate is thought to be the fact that the majority of the dogs included in the study are domestic dogs and another reason is the problems experienced in the registration of myiasis cases.

Ehrlichiosis is caused by tick-transmitted rickettsial microorganisms of the Anaplasmatacea family. *Ehrlichia canis*, *E. chaffeensis,* and *E. ewingii* are the most important species threatening human and animal health, particularly in dogs. Severe infections in dogs are mainly associated with *E. canis* and the disease caused by this microorganism is called canine monocytic ehrlichiosis (74). CME is mainly characterised by fever, anorexia, generalised lymphadenomegaly, mucosal pallor, lethargy, depression, and splenomegaly. Hypothermia may even occur in severely pancytopenic dogs (74). In this study, the blood sample sent from the veterinary hospital was diagnosed with light microscopy than confirmed with a commercial ELISA kit (Asan Easy Test *E. canis,* Asan Pharm, Korea). Studies conducted in Türkiye have shown that *E. canis* is the only species detected in dogs. CME is common in Türkiye and the prevalence of the disease has been determined by serological and molecular studies (15,75-82). In our study, *E. canis* was determined to be 5.26%.

Besides all this, each dog provided a single fecal sample, and each sample was examined just once. Notwithstanding these drawbacks, the study's data clearly show how environmental contamination endangers the health of farm and companion dogs as well as humans, including pet owners and herders. It is, therefore, advisable for those concerned to seek veterinary advice on how to reduce the incidence of parasitic disease. Public awareness campaigns or creative, informative, and engaging educational programs should be used to inform pet owners about the importance of regular deworming and ectoparasite control.

This study confirmed the prevalence of zoonotic gastrointestinal parasites in dogs in Konya, Türkiye. These parasites constitute a serious public health danger, hence dog deworming programs must be instituted. Other effective preventive strategies include dog management and feces collection, as well as preventing dog feces from contaminating soil and water.

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CONFLICT OF INTEREST

There is no conflict of interest to be declared by the authors.

AUTHOR CONTRIBUTIONS

The initial draft, preparation, conceptualization, technique, and study were conceived and written by CC and MI. The tests were carried out by CC, AE, ŞY, and DSY, who also edited and amended the manuscript. Each author accepted the submitted version of the paper and made contributions to it.

ETHICAL STATEMENT

In our study, we did not examine live animals. We examined faeces, blood and skin samples, which were sent to the laboratory for diagnostic purposes.

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RESEARCH ARTICLE

Characterization of Physicochemical, Colour and Textural Properties of Turkish Type Cheeses

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Abstract

The aim of this study was to evaluate the physicochemical parameters, instrumental colour and texture profile analysis of the most preferred and consumed Turkish cheeses (white cheese, kashar cheese, tulum cheese, and Mihaliç cheese). For this purpose, a total of 200 cheese samples were randomly collected from various markets and bazaars located in İstanbul, Türkiye. The cheese samples were analyzed in terms of the physicochemical parameters (moisture, dry matter, salt, fat, protein, pH and titratable acidity), instrumental colour (CIE *L**, *a**, *b**) and texture profiles (hardness, stringiness, springiness, adhesion, adhesiveness, cohesiveness, gumminess and chewiness). There was a significant difference in proximate composition between cheese types (P<0.001). The differences between the lightness, redness and yellowness values of the cheeses were also significant (P<0.001). The type of cheese showed a strong positive correlation with stringiness, springiness, adhesion and adhesiveness parameters, whereas showed a weak negative correlation with cohesiveness and gumminess, and a weak positive correlation with chewiness. However, there was no significant correlation between hardness and cheese type. All stages in cheese production, such as the type of milk and other ingredients used in cheese production, process conditions, ripening time and conditions, preservation characteristics, which differ according to cheese varieties, directly affect the physicochemical, colour and textural properties of the final product. Correspondingly, the difference between the characteristics of cheese samples was found to be significant.

Key Words: Colour, physicochemical parameters, texture profile, Turkish type cheeses

Türk Tipi Peynirlerin Fizikokimyasal, Renk ve Tekstürel Özelliklerinin Tanımlanması

Öz

Bu çalışmanın amacı, en çok tercih edilen ve tüketilen Türk peynirlerinin (beyaz peynir, kaşar peyniri, tulum peyniri ve Mihaliç peyniri) fizikokimyasal parametrelerini, enstrümantal renk ve tekstür profil analizlerini değerlendirmektir. Bu amaçla, İstanbul'da bulunan çeşitli market ve satış yerlerinden rastgele toplam 200 peynir örneği toplanmıştır. Peynir örnekleri fizikokimyasal parametreler (nem, kuru madde, tuz, yağ, protein, pH ve titre edilebilir asitlik), enstrümantal renk (CIE *L**, *a**, *b**) ve tekstür profilleri (sertlik, liflilik, yaylanma, yapışma, yapışkanlık, bağlayıcılık, sakızımsılık ve çiğnenebilirlik) açısından analiz edilmiştir. Peynir çeşitleri arasında içerik kompozisyonu bakımından anlamlı bir farklılık vardır (P<0,001). Peynirlerin parlaklık, kırmızılık ve sarılık değerleri arasındaki farklar da anlamlıdır (P<0,001). Peynir tipi, liflilik, esneklik, yapışma ve yapışkanlık parametreleri ile güçlü bir pozitif korelasyon gösterirken; yapışkanlık ve sakızımsılık ile zayıf bir negatif korelasyon ve çiğnenebilirlik ile zayıf bir pozitif korelasyon göstermiştir. Bununla birlikte, sertlik ve peynir tipi arasında anlamlı bir korelasyon bulunmamıştır. Peynir üretiminde kullanılan sütün türü ve diğer bileşenler, proses koşulları, olgunlaşma süresi ve koşulları, muhafaza özellikleri gibi peynir çeşitlerine göre farklılık gösteren peynir üretimindeki tüm aşamalar nihai ürünün fizikokimyasal, renk ve tekstürel özelliklerini doğrudan etkilemektedir. Buna bağlı olarak, peynir örneklerinin özellikleri arasındaki fark önemli bulunmuştur.

Anahtar Kelimeler: Fizikokimyasal parametreler, renk, tekstür profili, Türk tipi peynirler

INTRODUCTION

Cheese, which is an important dairy product manufactured in almost every part of the world, is widely consumed by the majority of people due to its high nutritional value, unique taste and flavour (1, 2). In our country, there are many varieties of cheese with different characteristics produced with different regional practices, milk type (sheep, cow or goat) and processing techniques (ripening, brining). Therefore, there are significant variations between the sensorial, chemical and microbiological qualities of cheeses. Among these, the white pickled cheese and kashar cheese are produced in many regions of the country, whereas most of the other types of cheeses (tulum, Mihaliç) are produced in certain regions (3-6). Turkish cuisine boasts a rich diversity of cheeses, each with its own unique flavour, texture, and cultural significance. The most commonly produced cheese is white pickled cheese, followed by kashar cheese, tulum cheese, Mihaliç (Kelle) cheese and herby cheese. In addition to these, other cheese varieties such as dil cheese, örgü cheese, civil cheese, hellim cheese, çerkez cheese, abaza cheese, Urfa cheese, and sıkma cheese have a widespread production and consumption network (7-9).

White cheese, manufactured from cow, sheep, goat's milk or mixtures of these milks, is bright white and non-porous cheese with a high fat content, salty taste and a soft to semi-hard texture. White cheeses manufactured with pasteurized milk are ripened for at least one month, while the best flavour develops in sheep and goat cheeses after one year of ripening (7,10,11).

Kashar cheese (Kashkaval cheese) is a semi-hard, ripened, pasta-filata cheese manufactured primarily from cow's milk, but also from sheep or goat's milk. Although it originates from Türkiye, it is also widely consumed in the Balkans and some parts of the Middle East. Kashar cheese has a mild to slightly sharp flavour, depending on the ripening time and the production techniques used. In addition, it becomes firmer and more elastic as it matures, with a texture that can vary from smooth to slightly grainy (12-14).

Tulum cheese is a traditional Turkish cheese mostly produced in East Anatolian region. Tulum cheese, which differs from other cheeses with its manufacturing method, is traditionally ripened and stored in the skin of animals, especially sheep or goat skins. This aging process gives the cheese a unique flavour and aroma. Tulum cheese has a crumbly texture that can vary from hard to semi-hard. It has a strong and pungent flavour and a highly aromatic taste that develops during the ripening period inside the animal skin. It is traditionally produced from sheep's milk, which contributes to its distinctive flavour and texture. It also has a pale to yellowish colour, depending on the milk used (usually sheep's milk) and the ripening process (4,15,16).

Mihaliç (Kelle) cheese, known for its hard texture and salty flavour profile, is widely produced in Balıkesir, Bursa and Çanakkale region. Mihaliç cheese needs to be ripened for several months (at least 3 months) to develop its flavour and texture. The salty flavour of the cheese has nutty undertones that become more pronounced with age and develop over time to have a characteristic richness and complexity. The cheese has yellowish-cream colour that is influenced by the type of milk used and the ripening process (17,18).

The evaluation of Turkish type cheeses requires a multidimensional approach integrating chemical analysis, colour assessment and detailed texture profiling. This holistic evaluation can help to understand the quality, characteristics and consumer preferences of cheeses. Therefore, the purpose of this study was to evaluate the physicochemical parameters, instrumental colour and texture profile analysis of the most preferred and consumed Turkish cheeses (white cheese, kashar cheese, tulum cheese, Mihaliç cheese) sold in retail.

MATERIAL AND METHODS

Sampling

A total of 200 cheese samples (50 pieces each of white cheese, kashar cheese, tulum cheese and Mihaliç cheese) were randomly obtained from various markets and bazaars located in İstanbul, Türkiye from September 2022 to May 2023. The collected samples (~300 g for each of samples) were immediately transferred to the laboratory under cold chain.

Physicochemical Analysis

The moisture and dry matter contents of cheese samples were determined by drying at 103±2°C to a constant weight using Moisture Analyzer (Sartorious MA45, Germany). Salt (NaCl) content of cheese samples was performed by Mohr method (Method 935.43) and the fat content was determined by the Gerber method (Method 933.05) (19). The total nitrogen (TN) and non-protein nitrogen (NPN) that was soluble in 12% trichloroacetic acid were assayed by the Kjeldahl method (19). The protein content was calculated as 6.38 × (TN - NPN). The pH values were measured at 20±2°C using a pH meter (Hanna HI 1131, Germany) equipped with a combined electrode (HI 9321 Microprocessor pH meter, Hanna Instruments, Germany). The titratable acidity of cheese samples was conducted by the Dornic method (Method 920.124) (19).

Instrumental Colour

The surface colour of cheese samples was determined by measuring Colorflex HunterLab Spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA, USA) in terms of L^* (lightness), a^* (redness) and b^* (yellowness) values using colour difference meter. Colour was evaluated using a diffuse illumination (D65 2° observer) with 8 mm viewing aperture and a 25 mm port size with the specular component excluded. The measurements were performed at five different locations on each cheese and the arithmetic values were calculated (20).

Texture Profile Analysis (TPA)

The textural properties of cheese samples were evaluated using a texture analyzer (Instron Universal Testing Machine, Model 1140, Instron, UK) equipped with a stainless-steel cylindrical probe (diameter 20/36 mm). The texture profile analysis was conducted by a double compression-decompression cycle test with a rest period of 3 s. All measurements were performed at room temperature (20±2°C) by placing 45 × 45 × 25 mm block shape cheese sample (50 g

overweight). The hardness, stringiness, springiness, adhesion, adhesiveness, cohesiveness, gumminess and chewiness characteristics of cheese samples were examined in terms of instrumental texture properties. The arithmetic average of eight measurements was calculated as a mean value for each sample.

Statistical Analysis

The General Linear Model (PROC GLM) in SPSS 21.0 (SPSS Inc., Chicago, IL, USA) was used to determine the least squares means (LSM), standard errors (SE), and the significant differences among means. Duncan's multiple range test was used to evaluate the significance of differences. Pearson's correlation coefficients (r) were used to determine the relation between the textural properties of cheese samples.

RESULTS

Physicochemical Properties

Physicochemical properties of four different Turkish type cheese samples are given in Table 1. The mean moisture value and salt content of cheese samples ranged from 29.16% to 42.33% and 2.71% to 6.15%, respectively. The fat content of the cheese samples varied between 20.39% and 25.62%, while the protein content was between 20.23% and 26.84%. The mean pH and acidity values ranged from 5.19 to 5.63 and 0.69% to 1.29% (LA%) in the analyzed cheese types.

Table 1. Mean values and standard errors of physicochemical properties of cheese samples (n= 200)

Physicochemical properties	Cheese type				
	White cheese (n= 50)	Tulum cheese (n= 50)	Mihaliç cheese (n= 50)	Kashar cheese (n= 50)	P
Moisture (%)	42.33^{a}±0.11	39.09^{b}±0.19	29.16^{d}±0.05	37.25^{c}±0.25	***
Dry matter (%)	57.67^{d}±0.26	60.91^{c}±0.33	70.84^{a}±0.19	62.75^{b}±0.25	***
NaCl (%)	3.89^{c}±0.14	5.04^{b}±0.05	6.15^{a}±0.05	2.71^{d}±0.07	***
Fat (%)	22.48^{b}±0.16	20.39^{c}±0.13	25.62^{a}±0.32	24.55^{a}±0.22	***
Protein (%)	20.23^{c}±0.08	21.73^{b}±0.07	22.05^{b}±0.16	26.84^{a}±0.18	***
pH	5.34±0.12	5.19±0.11	5.46±0.09	5.63±0.10	NS
Acidity (%)	1.16^{b}±0.03	1.29^{a}±0.07	0.94^{c}±0.02	0.69^{d}±0.02	***

n: number of analyzed samples

a-d: Means within a row with different letters are significantly different at P<0.001(***).

There was a significant difference in proximate composition between cheese types (P<0.001). The moisture contents of the cheese samples were significantly different from each other with the lowest in Mihaliç cheese and the highest in white cheese. In addition, kashar cheese was the least salty cheese and Mihaliç cheese had the highest salt content (P<0.001). In terms of fat content, tulum cheese had the lowest content, while Mihaliç and kashar cheeses had the highest contents (P<0.001). Kashar cheese had the highest protein content, whereas white cheese had the lowest protein content (P<0.001). However, tulum and Mihaliç cheeses were found to have similar protein contents (P>0.05). The acidity value, which is an important criterion in the formation of flavour in cheese, showed significant differences among cheese varieties due to differences in production processes. Tulum cheese had the highest acidity value, while kashar cheese had the lowest acidity.

Colour Evaluation

The instrumental colour parameters of different Turkish type cheeses are shown in Table 2. The mean lightness (*L**) values of cheese types ranged from 52.33 to 64.18, the mean redness (*a**) values ranged from -0.78 to 0.39 and the mean yellowness (*b**) values varied between 9.28 and 12.03.

Table 2. Mean values and standard errors of the instrumental colour (CIE *L**, *a**, *b**) parameters of cheese samples (n=200)

Colour parameters	Cheese type				
	White cheese (n=50)	Tulum cheese (n=50)	Mihaliç cheese (n=50)	Kashar cheese (n=50)	P
*L**	60.57^{b}±0.17	64.18^{a}±0.07	59.78^{c}±0.11	52.33^{d}±0.12	***
*a**	-0.25^{b}±0.02	-0.78^{d}±0.03	-0.49^{c}±0.03	0.39^{a}±0.02	***
*b**	9.28^{c}±0.09	10.88^{b}±0.09	11.85^{a}±0.15	12.03^{a}±0.04	***

n: number of analyzed samples

a-c: Means within a row with different letters are significantly different at P<0.001(***).

The colour characteristics of the cheeses varied depending on the content of the raw materials and the production processes of the cheese types. Thus, the differences between the lightness, redness and yellowness values of the cheeses were significant (P<0.001). Tulum cheese had the highest *L** value (the brightest), while kashar cheese had the lowest *L** value (the dullest) (P<0.001). The yellowness values of the cheeses were higher for kashar cheese, while white cheese had the lowest *b** value (P<0.001). However, the differences in the yellowness values of Mihaliç and

kashar cheeses were not significant (P>0.05). In addition, kashar cheese had the highest redness value among the cheese types (P<0.001). The *a** values of white, tulum and Mihaliç cheeses showed a tendency towards a green shade on the axis, whereas kashar cheese was on the red coordinates of the scale.

Texture Profile Analysis

The mean values of TPA parameters (hardness, stringiness, springiness, adhesion, adhesiveness, gumminess, chewiness) obtained from different cheese types are presented in Table 3. The difference between the hardness values of the four different cheese types was significant (P<0.001), whereas white cheese differed from the other three cheese types which were the same with each other in terms of stringiness and adhesiveness values. A significant difference was observed between cheese types in cohesiveness values, while the gumminess of Mihaliç and kashar cheeses were similar to each other. In terms of chewiness values, the difference between tulum and kashar cheeses was not significant, whereas a significant difference was found between other cheese types.

Table 3. Mean values and standard errors (SE) of texture profile analysis of cheese samples

Cheese type	n	Hardness		Stringiness		Springiness		Adhesion		Adhesiveness		Cohesiveness		Gumminess		Chewiness	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
White cheese	50	2.124^{c}	0.164	4.179^{a}	0.645	4.822^{a}	0.239	0.373^{a}	0.041	0.938^{a}	0.085	0.244^{d}	0.008	0.518^{b}	0.034	2.498^{a}	0.150
Tulum cheese	50	1.489^{cd}	0.046	0.886^{b}	0.114	0.937^{b}	0.184	0.059^{c}	0.008	0.052^{b}	0.008	0.491^{b}	0.008	0.731^{b}	0.033	0.685^{c}	0.174
Mihaliç cheese	50	12.039^{a}	0.440	0.233^{b}	0.066	0.267^{c}	0.040	0.104^{b}	0.039	0.012^{b}	0.009	0.382^{c}	0.035	4.599^{a}	0.281	1.228^{b}	0.185
Kashar cheese	50	6.394^{b}	0.268	0.267^{b}	0.067	0.233^{c}	0.066	0.084^{bc}	0.011	0.020^{b}	0.008	0.650^{a}	0.006	4.156^{a}	0.206	0.968^{c}	0.258
P	200		***		***		***		***		***		***		***		***

n: number of analyzed samples. a-d: Means within a column with different letters are significantly different at P<0.001(***).

Mihaliç cheese had the highest hardness value among the four cheese types analyzed (P<0.001). There was a significant difference in adhesiveness value between white cheese and other three cheese types (P<0.001). A similar difference was observed in stringiness and springiness properties of white cheeses. The firm structure of Mihaliç and kashar cheeses resulted in high gumminess values of these two cheese types (P<0.001).

The Pearson correlation coefficients (r) of textural properties of cheese samples are shown in Table 4. Hardness showed strong correlation with gumminess (r= 0.888, P<0.01) and weak correlation with chewiness (r= 0.400, P<0.05), whereas this value showed a negative correlation with springiness (r= -0.400, P<0.05) and adhesiveness (r= -0.372, P<0.05). Gumminess positively correlated with hardness (r= 0.888, P<0.01), while negatively strong correlated with springiness (r= -0.485, P<0.01) and adhesiveness (r= -0.483, P<0.01), and weakly correlated with stringiness (r= -0.409, P<0.05). Chewiness values showed strong positive correlation with gumminess (r= 0.536, P<0.01) and positive correlation with hardness (r= 0.400, P<0.05). There was a strong positive correlation between cheese types and stringiness, springiness, adhesiveness and adhesion properties, whereas no significant correlation was observed for hardness. There was a weak positive correlation between chewiness and cheese type, while a weak negative correlation was observed for cohesiveness and gumminess.

Table 4. Pearson correlation coefficients (r) among texture profile analysis (TPA) of cheese samples

Textural properties	Hardness	Stringiness	Springiness	Adhesion	Adhesiveness	Cohesiveness	Gumminess	Chewiness	Cheese type
Hardness	1	-0.338	-0.400(*)	-0.210	-0.372(*)	-0.060	0.888(**)	0.400(*)	-0.321
Stringiness		1	0.896(**)	0.723(**)	0.958(**)	-0.660(**)	-0.409(*)	0.295	0.647(**)
Springiness			1	0.882(**)	0.947(**)	-0.702(**)	-0.485(**)	0.276	0.673(**)
Adhesion				1	0.841(**)	-0.792(**)	-0.407(*)	0.093	0.462(**)
Adhesiveness					1	-0.711(**)	-0.483(**)	0.181	0.605(**)
Cohesiveness						1	0.270	-0.095	-0.427(*)
Gumminess							1	0.536(**)	-0.346(*)
Chewiness								1	0.380(*)
Cheese type	-0.321	0.647(**)	0.673(**)	0.462(**)	0.605(**)	-0.427(*)	-0.346(*)	0.380(*)	1

* Correlation is significant at P<0.05. ** Correlation is significant at P<0.01 .

DISCUSSION AND CONCLUSION

According to the Turkish regulations, the maximum limit of moisture content in white, kashar and tulum cheese should be 60-65%, 40-45% and 45% respectively, and salt in dry matter should be maximum 6.5% for white cheese, 3-4% for kashar cheese and 5% for tulum cheese (21). The average moisture contents of the cheese types examined in present study were below the maximum limits specified in the Turkish Food Codex and the cheese samples were within the obligated limits in terms of nutritional components. Salt content, which is one of the determining criteria especially in the formation of product flavour, was found to be 3.89% for white cheese, 2.71% for kashar cheese and 5.04% for tulum cheese and these values were determined to be within the regulations. The salt content of Mihaliç cheese, whose brining and ripening conditions were similar to white cheese, was recorded as 6.15%.

In addition, cheeses are divided into 4 different groups according to fat in dry matter (FDM). These are full-fat cheese (FDM% ≥ 45), half-fat cheese (25 ≤ FDM% < 45), low-fat cheese (10 ≤ FDM% < 25) and non-fat cheese (FDM% < 10) (21). In the present study, it was determined that the cheese samples were in the categories of half-fat and low-

fat cheese according to their fat content. Among cheese types, Mihaliç cheese was found to be more fatty with an average value of 25.62%.

Bilgin et al. (22) stated that the titratable acidity (LA%) of 26 full-fat white cheese samples was between 0.60-3.96% and the average acidity value of white cheeses was 1.19% in accordance with legal regulations. In addition, they reported the average moisture content as 53.70%, the average salt content as 7.71% and the average fat content as 49.03%.

The main differences in the properties of cheeses varied depending on the raw milk composition and physicochemical characteristics (1). Based on this, the acidity of cheeses produced with raw milk is higher than cheeses produced with pasteurized milk (23). The acidity value of cheeses was also influenced by a series of factors such as moisture, lactose and salt contents, ripening process, glycolytic and lipolytic effects (13). During the ripening process of the cheese, the breakdown of the available lactose to lactic acid results in a decrease in pH value, which is followed by changes in pH depending on the type of cheese (5, 24). The salt content of cheese was a delicate physicochemical property that directly affected the water activity and moisture value, ripening process and fermentation attributes of cheese. In addition, a change in salt content could also influence the protein content and pH value (6).

Significant differences were observed between the *L**, *a** and *b** values of the cheese types evaluated in the present study. This difference in colour values between types of cheese may be due to the ingredients, especially the milk used in manufacturing, and the variation in production, ripening and storage processes. There is a positive correlation between the lightness of cheese and moisture content, indicating that cheeses with higher moisture content are lighter and brighter. Furthermore, it was stated that the high lightness value in the cheeses was mainly provided by the yellow component (*b**) and that the white-yellowish hue was responsible for the final colour of the cheese (25).

The increase in salt concentration due to the ripening process causes colour changes in cheeses and thus decreases the lightness value. Additionally, cheese colour varies because of different milk sources and heat treatments during cheese production. The yellow colour of cheeses produced from cow's milk is associated with the transfer of carotenoids in feed to milk resulted the increase in *b** value (18, 24). Also, the decrease in fat content of cheese leads to an increase in lightness value of the product (26).

The texture of cheese was among the most important factors defining the product, determined by the combination of its physical and chemical properties (27). Textural profile of cheeses was also influenced by the processing factors such as water content, processing time and temperature. The changes in texture values such as hardness, springiness, cohesiveness, gumminess and chewiness may be associated with the decrease in moisture values as well as the diffusion of salt into the cheese, decrease in pH and changes in casein matrix. Increasing the water content from 30% to 50%, which caused the most significant change in textural characteristic of cheese, led to a significant decrease in TPA parameters especially hardness, chewiness and gumminess. Besides water content, high pH value, dry matter and protein content lead to a harder texture in cheeses (2,11,28).

Furthermore, the type of milk used in cheese production also affected the hardness of cheeses. Studies have shown that white cheeses produced with goat milk were harder than those produced with sheep milk. This difference in the textural properties of cheeses produced with different milk types was explained by different casein structures or casein concentration in milk. The hardness of cheese depends on the water-binding of casein and the presence of fat (1, 29).

In the present study, Mihaliç cheese with the lowest moisture content had the highest hardness value. On the other hand, the lowest hardness values were recorded in white and tulum cheeses that had higher moisture contents. Moreover, high hardness and springiness values of Mihaliç and kashar cheeses resulted in higher gumminess values. However, the highest chewiness value was recorded in white cheese based on high stringiness and springiness values. Due to these properties, white cheese had to be chewed for a longer time to be swallowed, whereas tulum cheese with the lowest hardness was the easiest cheese to chew.

Among the texture profile parameters, hardness and chewiness were more susceptible to the variation of processing conditions and these two parameters showed similar trends with each other (30). In agreement with our study, Jia et al. (30) and Zheng et al. (31) reported a positive correlation between chewiness and hardness. On the other hand, Boukria et al. (32) reported that the difference in the milk (cow, sheep, goat or camel) used in cheese production, or the length of the storage period did not affect the texture parameters such as adhesiveness, cohesiveness or springiness. Higher moisture content in cheese resulted in a decrease in its hardness, while higher unsaturation of fatty acids was associated with a softer texture. Moreover, casein gels were responsible for fracture and stretch properties of cheeses (32). The coagulation and protein degradation during the ripening of cheese led to an increase in springiness, resulting in a weakening of the protein chains or a low molecular weight peptide network (33). In addition, Tarakçı and Yolaşan (34) highlighted that cohesiveness, adhesiveness and springiness parameters of the texture profile did not differ in terms of cheese varieties.

All stages in cheese production, such as the type of milk and other ingredients used in cheese production, process conditions, ripening time and conditions, preservation characteristics, which differ according to cheese varieties, directly affect the physicochemical, colour and textural properties of the final product. Thus, the difference between the characteristics of cheese samples was found to be significant. Textural properties of cheese types are widely influenced by moisture, fat in dry matter, pH value, ripening of the cheese, type and amount of emulsifying salts, and also by processing conditions such as processing (melting) temperature and rate of cooling of the molten mass. These differences are based on the region and manufacturing process. Consequently, the variety of cheeses obtained through the different applications of the production stages provides the diversity of the product manufactured in a geographical area and responds to the tastes of consumers. In this way, consumers with different palates have the opportunity to consume cheeses with different flavour and textural characteristics.

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CONFLICT OF INTEREST

There is no conflict of interest to be declared by the author.

AUTHOR CONTRIBUTION

Planning, sample collection, analysis and writing of the study were carried out by EA.

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RESEARCH ARTICLE

Investigation of Protective and Therapeutic Efficacy of Lactoferrin on Neonatal Calf Diarrhea

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Abstract

The aim of this study was to investigate the prevalence of rotavirus, coronavirus, *Cryptosporidium*, *E. coli* F5 (K99), *Cl. perfringens* and *Salmonella spp*. and clinical, haematological and biochemical changes in calves with neonatal diarrhoea and the efficacy of lactoferrin supplementation in standard treatment in Van and Diyarbakır provinces. The calves with diarrhea were investigated by immunochromatographic test kit and conventional bacteriological methods. Rotavirus was detected in 51%, *Cryptosporidium* in 35%, *Cl. perfringens* in 23%, coronavirus in 16%, *E. coli* F5 (K99) in 4%, *Salmonella spp*. in 2% of the calves with diarrhea. *Giardia spp*. was not detected in any calf, while 65% of the calves had a single agent, 33% had more than one agent. In vitro bactericidal and bacteriostatic effects of lactoferrin on *E. coli* O157, *E. coli* F5 and *Salmonella enteriditis* were investigated. Lactoferrin was found to be effective on bacteria at concentrations of 100 mg/ml and higher, decreased the mortality rate and showed a positive effect on clinical parameters. It was concluded that lactoferrin can be used for preventive and therapeutic purposes at doses of 100 mg/ml and higher and will be more effective in treatment.

Key Words: Calf, diarrhea, lactoferrin, neonatal

Neonatal Buzağılarda Lactoferrinin İshalde Koruyucu ve Terapotik Etkisinin Araştırılması

Öz

Bu çalışma Van ve Diyarbakır illerinde neonatal ishalli buzağılarda rotavirus, coronavirus, *Cryptosporidium*, *E. coli* F5 (K99), *Cl. perfringens* ve *Salmonella spp*.'nin prevalansı ile klinik, hematolojik, biyokimyasal değişiklikler ve standart tedavide laktoferrin ilavesinin etkinliğini araştırmak amacıyla yapıldı. İshalli buzağılarda immunokromatografik test kiti ve konvansiyonel bakteriyolojik yöntemlerle etken araştırması yapıldı. İshali buzağıların %51'inde rotavirus, %35'inde *Cryptosporidium*, %23'ünde *Cl. perfringens*, %16'sında coronavirus, %4'ünde *E. coli* F5 (K99), %2'sinde *Salmonella spp*. tespit edilirken. *Giardia spp*. hiç bir buzağıda tespit edilmemiştir. Buzağıların %65'inde tek bir etken bulunurken, %33'ünde birden fazla etken tespit edilmiştir. Sağaltımda laktoferrinin *E. coli* O157, *E. coli F5* ve *Salmonella enteriditis* üzerindeki in vitro bakterisidal ve bakteriyostatik etkisine bakıldı. Laktoferrinin 100 mg/ml ve daha yüksek konsantrasyonlarda bakteriler üzerinde etkili olduğu ve ölüm oranını düşürdüğü, ayrıca klinik parametreler üzerinde olumlu bir etki gösterdiği tespit edilmiştir. Laktoferrinin 100 mg/ml ve daha yüksek dozlarda koruyucu ve tedavi amaçlı kullanılabileceği ve sağaltımda daha etkili olacağı kanaatine varılmıştır.

Anahtar Kelimeler: Buzağı, ishal, laktoferrin, neonatal

INTRODUCTION

While more than one enteric pathogen (viruses, bacteria and protozoa) usually play a role in the etiology of neonatal calf diarrhea, sometimes one primary pathogen may play a role alone (1-6).

Lactoferrin (Lf), a bioactive peptide that is highly abundant in humans, cattle, mice and pigs, is a multifunctional iron-binding glycoprotein belonging to the transferrin family that contains approximately 690 amino acids. Lf plays an important role in the regulation of defense and immune mechanisms against bacteria, viruses and fungi (7,8). Lf prevents bacterial adhesion to abiotic surfaces through ionic binding to biomaterials or specific binding to bacterial structures or both (9,10). The iron-independent bactericidal effect of Lf has also been reported. It interacts with the lipoteicoic acid layer of gram-positive bacteria and the lipopolysaccharide layer of gram-negative bacteria which eventually leads to a lethal effect on bacteria (11,12).

It has been shown in many in vitro and animal studies that Lf reduces the ability of enteric pathogens to adhere to and invade mammalian cells by disrupting the function of surface virulence factors and that Lf has a protective effect on infections with enteric microorganisms, including rotavirus, *Giardia spp*. and *Shigella spp.* (13).

In order to maintain vital functions in neonatal calves with diarrhea, fluid-electrolyte therapy, regulation of acid-base balance and parenteral administration of nonsteroidal and antimicrobial agents are required (14). Alternative treatments may also be necessary in order to reduce the use of antimicrobials, especially since excessive use of antimicrobial agents will create further bacterial resistance in animals, and Lf is known to prevent septicemia progressing with enteritis in calves at high risk (15). In addition, it has been reported that the administration of Lf in the early stages of diarrhea significantly reduces mortality in calves (16).

The aim of this study is to determine the pathogens detected in diarrheic calves, to reveal the clinical, hematological and biochemical alterations caused by diarrhea, to investigate the protective and therapeutic effect of Lf against the pathogens in enteritis.

MATERIAL AND METHODS

Animals

The material of the study consisted of 100 neonatal calves with diarrhea aged 3-20 days of different breeds and sexes from 42 enterprises around Van and Diyarbakir provinces and 20 healthy neonatal calves as control group. For clinical data, the patient's history including address, sex, gender, age, recovery period and prognosis were recorded.

Physical examination of each patient was performed and physical activity was categorised as lively, quiescent, depressed and comatose. Alive was defined as a normal response to stimuli and a sucking reflex; stagnant was defined as a state of stagnation in which the animal exhibited relative indifference to normal stimuli and a weak sucking reflex; depressed was defined as a state of marked indifference in which the animal did not respond at all to external stimuli, had no sucking reflex but was able to stand and move; coma was defined as a state of complete apathy in which the animal was unconscious and could not be aroused.

Dehydration scores were classified according to the degree of eyeball retraction estimated by the distance between the eyeball and the palpebral conjunctiva.

Dehydration was evaluated as follows; dehydration less than 6% when eyeball regression was 3 mm, dehydration 8% when it was 4.5 mm, dehydration 10% when it was 6 mm, dehydration 12% when it was 7 mm, and dehydration over 12% when it was over 7 mm. The values of fever, pulse, respiration, skin elasticity and diarrhoea scores of the calves were recorded. Dehydration was calculated according to haematocrit value, Na+, TP and urea results obtained from clinical findings and Edan I15 blood gas device.

In order to determine the diarrhoea scores of the sick animals, normal stools were scored as 1, soft pasty 2, loose 3, watery 4, and the scores before and after treatment were recorded.

Aetiological Agents Analysis

Salmonella spp. stool samples were examined with conventional bacteriological methods and identified with the VITEK II (Biomerieux, France) device and the results were serologically confirmed.

VETİMA 313/5 lateral flow immunochromatographic rapid diagnosis kit(BioX diagnostic, Belgium) was used for the detection of rotavirus, coronavirus, *cryptosporidium, E. coli* K99 (F5) and *Cl. perfringens* colonies suspected of *E. coli* and *E. coli* K99 (F5) by using immunochromatographic test kit were further analysed by conventional bacteriological methods and identified in VITEK II identification device.

The stool samples were diluted one to one with physiological saline and zinc sulphate flotation method and examined by direct microscopic examination with x10, x20 magnification for *Giardia spp.*.

Blood Samples and Analysis

For haematological study, blood was collected from the jugular vein into K3 EDTA tubes, and WBC (x10*9/L), Hct %, RBC (x10*12/L), Hb (g/dl), MCV (fL), MCH (pg), MCHC (g/dl), PLT (x10*9/L) before and after treatment were analysed by Genius KT6200 automatic haemogram device. Serum samples were analyzed in terms of TP, GGT, albumin, BUN and glucose on Mindray BS 120 biochemistry device.

1 ml blood sample were taken from the jugular vein using a 23 G needle with heparin from 44 animals with diarrhea for blood gas analyses. Blood samples were placed on 15VET Veterinary Test Card BG 10 (pH, pCO2, pO2, Na+, K+, Cl-, Ca++, Hct, Glu, Lac) and analysed on EDAN I15 blood gas analyser device.

Microbiological Analyses

To determine the invitro activity of Lf on bacteria, *E. coli* O157 (ATCC 43895), *Salmonella enteriditis* (ATCC 14028), *E. coli* F5 (K99) field strains as 0.5 MFU ($1.5x10^8$ cells/ml) were added to the dilutions prepared in tryptose broth containing 400 mg/ml, 200 mg/ml, 100 mg/ml, 10 µl/ml Lf according to Cowan and Steel's Manual for the Identification of Medical Bacteria method. After the tubes were incubated at 37°C for 24 hours, the number of bacteria in the petri dishes was calculated as cfu/ml and the activity of Lf on bacteria in vitro

was calculated. In order to determine the MIC activity of Lf on bacteria using a different technique simultaneously, 4 wells with a diameter of 0.5 mm were opened in Müller Hinton Agar and 50 µl of 400 mg/ml, 200 mg/ml, 100 mg/ml, 10 mg/ml Lf dissolved in sterile distilled water was added into the wells and the bacteriostatic activity zone diameter was determined.

Neonatal calves wit diarrhea were divided into 2 equal groups (n= 50); as Lf Supported Treatment Group (LSTG) and as Standart Treatment Group (STG).

In both groups were treated as follows parenteral enrofloxacin (2.5 mg/kg/day, im), ceftiofur (1 mg/kg/day im), and/or the oral antibiotic neomycin (500 mg/kg/day) was given. In calves infected with Cl. perfringens, metronidazole (7.5 mg/kg/day) was administered, and those infected with *Cryptosporidium spp*. halofuginone (0.1 mg/kg/day) was administered. In addition to calves in the first group was given120 mg/kg/day of Lf (Tatura-BIO™ LF Powder) orally for 3 days,

Serum total protein (STP) and GGT levels were analyzed in Mindray BS 120 biochemistry device for analysing passive transfer failure in blood taken from calves before treatment.

In this study, descriptive statistics for continuous variables were expressed as mean and standard error. Two-tailed independent t-test was used to compare group means in terms of continuous variables. The statistical significance level was taken as 5% and IBM SPSS Statistics 22 statistical package programme was used for calculations.

RESULTS

Etiological Findings

According to the results of the immunochromatographic test kit in the diarrheal calves, rotavirus was detected in 51, coronavirus in 16, *Cryptosporidium* in 35, *Cl. perfringens* in 23, *E. coli* F5 (K99) in 4, and *Salmonella spp*. was detected in 2 animals in the study performed by classical bacteriological methods. *Giardia spp*. was not detected in any of the calves included in the study. However none of the agents investigated were found in the two animals included in the study (Table 1).

Table 1. Agents detected in diarrheic calves.

Agents	n= 100	%
Rotavirus	51	51%
Coronavirus	16	16%
Cryptosporidium	35	35%
Cl. perfringens	23	23%
E.coli K99 (F5)	4	4%
Salmonella spp.	2	2%
Giardia	-	0%
None of any agents were detected	2	2%

In this study, 65% of the calves with diarrhoea had a single agent associated with diarrhea and 33% had multiple agents. Rotavirus-*Cryptosporidium* in 18 animals, Rotavirus-*Cl. perfringens* in 11 animals, Rotavirus-Coronavirus in 1 animal, Rotavirus-Coronavirus-*Cryptosporidium* in 1 animal, Coronavirus-*Cl. perfringens* in 1 animal, Rotavirus-*Cryptosporidium-Cl. perfringens* in 1 animal were determined as mixed infection (Table 2).

Table 2. Mix Agents detected in diarrheic calves.

Agents	n= 100	%
Rotavirus –Cryptosporidium	18	%18
Rotavirus- *Cl. perfringens*	11	%11
Rotavirus –Coronavirus	1	%1
Rotavirus-Coronavirus –Cryptosporidium	1	%1
Rotavirus-Cryptosporidium- *Cl. perfringens*	1	%1
Coronavirus-*Cl.perfringens*	1	%1

Clinical Findings

During treatment period, 6 out of 50 animals died in LSTG and 9 out of 50 animals in STG.

The mean body temperature of the calves with diarrhea in LSTG was 38.04±1.89°C before treatment, 38.38±0.3°C on the 3rd day of treatment and 38.34±0.2°C on the 5th day.

The mean body temperature of the calves in the STG was 37.85±1.76°C before treatment, 38.25±0.33°C on the 3rd day and 38.18±1.01°C on the 5th day of treatment.

Body temperature in the control group was 38.6°C and the statistical difference on the 1st day was $p<0.05$ and there was no difference between the body temperature values on the 3rd and 5th days ($p>0.05$). There was no difference between LSTG and STG in the statistical analysis ($p>0.05$) as shown in Table 3.

Table 3. Clinical findings in diarrheic calves

	Lf Supported Treatment Group (LSTG) ($\bar{x} \pm S\bar{x}$) (Min-Max)	Classical Treatment Group (CTG) ($\bar{x} \pm S\bar{x}$) (Min-Max)	p value
Body Temperature °C			
0th day	38.04±1.89(32.0-39.9)	37.85±1.76(33.0-39.8)	
3rd day	38.38±0.3(37.4-38.9)	38.25±0.33(37.4-38.6)	>0.05
5th day	38.34±0.2(38.3-38.7)	38.18±1.01(38.2-38.6)	>0.05
Heart rate/min			
0th day	109.9±25.05(24-144)	115.2±20.54(60-136)	
3rd day	111.5±9.8(94-128)	113.81±6.72(96-124)	>0.05
5th day	108.4±7.21(96-116)	112.7±5.93(92-122)	>0.05
Respiratory rate/min			
0th day	34.48±10.25(20-52)	34.76±8.7(18-56)	
3rd day	33.6±296(28-36)	32.9±2.8(30-36)	>0.05
5th day	32.89±2.44(30-36)	32.4±2.08(28-36)	>0.05
Enophtalmus/mm			
0th day	2.44±1,19(0-4)	2.2±1.05(0-4)	
3rd day	0.28±0.50(0-2)	0.26±0.49(0-2)	>0.05
5th day	0.04±0.20(0-1)	0.12±0.33(0-1)	>0.05
Skin folding/sec			
0th day	2.65±1,4(1-5)	2.44±0.09(1-6)	
3rd day	1.06±0.25(1-2)	1.14±0.42(1-2)	>0.05
5th day	1±0.01(1-1)	1±0.01(1-1)	>0.05
Diarrhea score before and after treatment			
0th day	3.18±0.75(1-4)	3.02±0.68(1-4)	
3rd day	1.51±0.59(1-3)	1.88±0.71(1-3)	<0.05
5th day	1.16±0.37(1-2)	1.3±0.56(1-3)	<0.05

*p value was obtained by independent t-test

Hematologic and Biochemical Findings

As shown in the tables (Table 4, 5, 6). WBC values in LSTG and STG groups were statistically significant before and after treatment ($p<0.001$). In comparison with the control group and treatment groups, the statistical difference on the 1st day was $p<0.05$ and no difference was found on the 3rd day ($p>0.05$).

Table 4. Hematological findings of LSTG ($\bar{x} \pm S\bar{x}$)

	Before Treatment	After Treatment	Control Group	p value
WBC (×109 /L)	23.79±17.2	7.59±3.6	8.45±1.32	<0.001
Hct (%)	38.17±14.9	31.43±7.71	27.45±2.06	<0.01
RBC(×1012 /L)	10.59±2.06	9.06±1.02	9.12±0.83	<0.001
Hb (g/dL)	11.01±3,62	9.45±1,87	9.83±0.62	<0.01
MCV (fL)	35.55±9.55	35.12±7.74	33.37±3.5	>0.05
MCH (pg)	9.90±2.44	10.52±2.27	10.92±1.11	>0.05
MCHC (g/dL)	29.82±6,09	31.20±9.67	36.02±3.6	>0.05
PLT (×109/L)	455.9±9.86	517.96±225.2	585.5±105.3	>0.05

Table 5. Hematological findings of CTG ($\bar{x} \pm S\bar{x}$)

	Before	After	Control	p value
WBC (×109 /L)	21.77±12.32	6.86±3.42	8.45±1.32	<0.001
Hct (%)	37.34±8.30	30.52±6.52	27.45±2.06	<0.001
RBC(×1012 /L)	11.68±2.03	10.01±2.52	9.12±0.83	<0.001
Hb (g/dL)	11.40±2.03	9.82±2.52	9.83±0.62	<0.001
MCV (fL)	33.191±5.84	31.26±5.51	33.38±3.5	>0.05
MCH (pg)	10.02±1.59	10.22±2.67	10.92±1.11	>0.05
MCHC (g/dL)	30.13±4.32	30.01±4.86	36.02±3.6	>0.05
PLT (×109/L)	497.90±213.8	546.54±305.4	585.5±105.3	>0.05

Table 6. Compare of biochemical findings in diarrheic calf and control group

	$\bar{x} \pm S\bar{x}$ (Min-Max) (n=100)	Control Group Mean ± SD (n=20)	p value
STP g/L	46.4±13.45 (10.5-70)	57.9±2.93	<0.001
GGT U/L	172.41±253 (2.2-2233.6)	486±256	<0.001
Alb g/L	37.16±7.15 (17-46.2)	32.25±2.14	<0.01
BUN mmol/L	8.86±7.76 (2.16-28.36)	3.76±1.3	<0.001
GLU mmol/L	3.15±3.77 (<1-32.5)	2.92±0.58	>0.05

In the LSTG and STG groups, Hct results before and after treatment were statistically significant as shown in the table (p<0.01). There was no difference between LSTG and STG (p>0.05).

In the Control Group, there was a statistical difference on day 1 (p<0.001) and no difference was found on day 3 after treatment (p<0.05). In the LSTG and STG groups, the results before and after treatment were statistically significant (p<0.01) as shown in the table (p<0.001). There was no statistical difference between LSTG and STG (p>0.05). Hb (g/dl) in LSTG was statistically different before and after treatment (p<0.05).

In STG, the difference in Hb (g/dl) before and after treatment was statistically significant (p<0.05).

In the control group was found to be statistically significant on day 1 (p<0.05) and no difference was found on day 3 (p<0.05). There was no difference between LSTG and STG (p>0.05).

DISCUSSION AND CONCLUSION

In newborn calves, mostly rotavirus, coronavirus, enteropathogenic *E. coli, Salmonella spp*., *Cl. perfringens* and *Cryptosporidium* spp. caused by diarrhea has a high mortality rate. Other important causes of calf deaths include immunodeficiency, seasonal effects, difficult birth, faulty herd management and insufficient colostrum intake (3).

Brunauer et al. (5), meta-analysed from 1293 studies (94 sub-studies) in 21 different countries, and reported the highest combined mean prevalence of worldwide diarrhea agents in calves. According to his study 12 208 animals in approximately 2110 herds; mean prevalence was reported as 6.69% for rotavirus-*Cryptosporidiosis*, 2.84% for rotavirus-coronavirus, and 1.64% for rotavirus-*E. coli* K99 (ETEC).

In this study, according to the immunochromatographic test kit results, rotavirus was found in 51 (51%) of the diarrheic calves, *Cryptosporidium* in 35 (35%), *Cl. perfringens* in (23%), coronavirus (16%) in 16, *E. coli* F5 (K99) in 4 (4%), and *Salmonella spp.* was determined in 2 (2%) calves. *Giardia spp.* could not be determined in any of the calves included to the study, and none of any relevant agents were detected in two calves.

In our study, a single factor was determined in 65% of the diarrheic calves, and multiple factors were determined in 33%. As mixed infections, rotavirus-*Cryptosporidium* was found in 18 animals, rotavirus-coronavirus in 1 animal, rotavirus-*Cl. perfringens* in 11 animals, rotavirus-coronavirus-*Cryptosporidium* in 1 animal, coronavirus-*Cl. perfringens* in 1

animal and rotavirus-*Cryptosporidium-Cl. perfringens* were found in 1 animal.

When our findings are compared with the previous studies, the rate of rotavirus-*Cryptospordium* is also in the highest percentile in this study, but the rate of rotavirus-*E.coli* F5, rotavirus-coronavirus was found to be lower than in previous studies. We think that this is probably due to the geographical conditions, newer diagnostic methods, herd management systems, vaccination, colostrum management, lateral flow and higher sensitivity of immunochromatographic test kits than acid-fast staining methods.

We were examined enophthalmia, skin wrinkling time and diarrhea scores were examined in two different treatment groups. When these parameters were compared with control groups the body temperature, heart and respiratory rate, dehydration levels were high and there was an increased enopthalmia and skin folding time was prolonged. The results were consistent with previous studies on neonatal diarrheal calves (17-19).

In this study, the clinical findings improved rapidly in two different groups, and the efficacy before and after the treatment was found to be statistically significant between the groups ($p< 0.05$). In the LSTG, a statistically significant difference was found in the diarrhea score compared to the classically treated group ($p< 0.01$).

The blood gas evaluation of 44 calves, it was observed that pH, Na^+, Cl^-, HCO_3^-, Ca^{++} were at low concentrations, and CO_2 and K^+ concentrations were high, and the findings are similar to the findings of many researchers (20-23). According to the literature searches, it has been stated that it Ca^{++} increases the reabsorption of ions and fluids by decreasing the intestinal calcium-sensing receptor (CaSR) and leading to a reduction in intestinal secretion and motility (24).

In our study, according to the analyzes performed with the blood gas kit; Ca^{++} was found to be low in 33 (75%) of 44 animals and at normal levels in 11 (25%). The reason for the low Ca^{++} level is thought to occur probably by the factors that cause diarrhea caused the destruction of CaSR in the intestine. In the literature review, adequate studies on serum Ca^{++} levels in calves with diarrhea have not been determined.

Mean serum total protein level in diarrheic calves was determined as 46.4 mmol/L, in the study. When animals with diarrhea were compared with the control group (57.9 ± 2.93), 69 animals (69%) had low, 15 (15%) had normal, 16 (16%) had high serum total protein levels. Serum GGT levels were 172.41 U/L in diarrheic calves that were 1-13 days old and 486 U/L in the control group. In this study, when the diarrhea group and the control group are compared in the direction of passive transfer failure (PTF), a highly significant ($p<0.001$) difference is obtained. Considering the data obtained, it is seen that the low serum GGT level, especially in calves with diarrhea, indicates PTF and is consistent with the literature data of the researchers (25). At the same time, a positive correlation was found between low GGT level and low STP level. Therefore, it is seen that GGT can be used safely in the detection of PTF and in colostrum management.

The mean blood glucose level was determined as 3.18 mmol/L in calves with diarrhea and 3.92 mmol/L in the control group, and the statistical difference between the groups was found to be significant ($p<0.05$) in our the study. When the data we obtained are compared with the literature data, it is seen that the restriction of food intake due to diarrhea in calves, the pathological changes in the intestines, the food cannot be digested and as a result hypoglycemia is formed. The current findings of our study are compatible with previous studies (23,26).

In our study, statistically significant differences were found in WBC, Hct, Hb, RBC levels, before and after treatment. Panousis et al. (26) stated in their study that calves with diarrhea had higher RBC, Hb and Hct values compared to healthy calves. Again, various researchers reported that Hct, Hb, and RBC concentrations in calves with diarrhea were high due to dehydration (27-29). It was determined that our present findings were consistent with the studies, and the hydration status was effective on Hct, Hb and RBC. WBC levels were high due to infectious agents.

Lf has two main effects on enteric pathogens: it inhibits proliferation and impairs the function of surface virulence factors, reducing their ability to adhere and invade mammalian cells (13). Paredes et al. (30) investigated the anticryptosporidial activity of Lf on different stages of *Cryptosporidium* and determined that physiological concentrations of Lf killed *C. parvum* sporozoites and had no significant effect on the viability of oocysts or the intracellular development of the parasite. Since sporozoites in the digestive tract are essential for the infection process, Lf in breast milk inhibits intracellular migration of sporozoites during lactation, pointing to the potential of lactoferrin as a new therapeutic agent for *Cryptosporidiosis*.

In this study, microbiological examinations were carried out to determine both the MIC value and in vitro effectiveness of Lf on *Salmonella spp.*, *E.coli* F5 (K99*), E.coli* O157 under laboratory conditions. In our study, it was determined that Lf was effective at the lowest concentrations of 100 mg/ml and above to show bacteriostatic effect and inhibited the growth of bacteria, while its effectiveness was weak at lower concentrations.

Biernbaum et al. (31) studied various concentrations of lactoferrin against common dairy pathogens such as *S. enterica* and *E. coli* O157:H7. They report that the growth of *E. coli* O157:H7 was significantly reduced at levels higher than 14.05 mg/ml lactoferrin, while for *S. enterica*, lactoferrin concentrations of 112.5 mg/ml or above suppressed the growth on in milk.

The findings obtained in our study was consistent with the findings of Biernbaum et al. (30). For this reason, it is clear that researchers should apply at the lowest concentration of 100 mg/ml in the future studies.

Mosquito et al. (32) reported that *Salmonella enterica subsp. enterica serovar typhimurium* causes systemic infection and acute diarrhea in humans, especially in children younger than 2 years of age. In an in vivo study they performed on rats, using cattle Lf on this infection reduced the severity, mortality rate and inflammatory rate of the infection. Laboratory findings obtained in this study support the findings of Mosquito et al. (31) and clearly show that Lf has a bacteriostatic effect on *Salmonella spp.* In our study to determine the in vitro efficacy of Lf on *Salmonella spp*., *E.coli* F5 (K99), *E.coli* O157 under laboratory conditions, it was observed that after 24 hours of incubation, it changed from bacillus morph to coccobacillus morph in bacterial morphology. Ostan et al. (33) reported that Lf can damage the outer membrane in gram-negative bacteria and change the bacterial outer

membrane permeability, which showed that Lf also had an effect on cell morphology.

In this study, to determine the effect of Lf application on total bacterial release in the stool of in animals in the control group; it was determined that the average of fecal bacterial load before Lf administration was 3880 cfu/g and after Lf administration, the bacterial load was 1736 cfu/g, and the difference between the 1st and 2nd day was statistically significant ($p<0.001$). In the control group that did not received Lf, the average fecal bacterial load was 3950 cfu/g on the 1st day and 3870 cfu/ml on the 2nd day. There was no statistical difference between the mean bacterial load on the 1st and 2nd day ($p>0.05$). This supports the view that Lf may be significantly effective against pathogens, especially in the gastrointestinal tract.

In this study, an infectious agent associated with diarrhea was determined in 98% of neonatal calves with diarrhea. With field and laboratory studies, it has been determined that lactoferrin contributes to clinical improvement on the factors determined in diarrhea and the minimal dose to be used in treatment is 100 mg/ml.

The study fully demonstrated that lactoferrin may have bioprotective potential and its efficacy as an antimicrobial additive. It is thought that the application of adequate and appropriate doses, especially on animals at risk, will give successful results in both prevention and treatment, and it is found that more field and experimental studies are needed to limit the use of antibiotics in this regard and to create an alternative in protection against pathogens in sensitive periods.

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CONFLICT OF INTEREST

There is no conflict of interest between the authors.

AUTHOR CONTRIBUTIONS

NI, took part in the study planning and sample collection and AK, HI in the study planning and control. Laboratory and field studies were carried out by NI. The writing of the study and final checks were carried out with the contributions of all authors.

ETHICAL STATEMENT

Final report of the research Project detailed above was approved by Van Yuzuncu Yil University Animal Researches Local Ethic Committee in the sesion held on 26/05/2022 and decision number 2022/05-17

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ARAŞTIRMA MAKALESİ

Elazığ ve Diyarbakır Yöresi Koyun Ayak Hastalıklarının Değerlendirilmesi

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Öz

Bu çalışma ile Elazığ ve Diyarbakır yörelerindeki koyunculuk işletmelerinde 2018 yılı içerisindeki mera öncesi ve mera sonrası dönemde karşılaşılan ayak hastalıklarının görülme sıklıklarının araştırılması amaçlandı. Farklı yaş, ırk ve cinsiyetlerdeki 10000 koyun/koç üzerinde yapılan incelemelerde hayvanların %5.3'ünde ayak lezyonlarına rastlandığı belirlendi. Piyetenin hem mera öncesi (%38) hem mera sonrası (%46.43) dönemlerde en sık rastlanılan ayak lezyonu olduğu tespit edildi. Mera öncesi dönemde ayak lezyonlarına çoğunlukla ön ayaklarda (%64) rastlanırken, mera sonrası dönemde arka ayaklarda (%60.71) rastlandı. Çalışma boyunca değerlendirilen erkek hayvanların sayılarının az olması ve herhangi tırnak hastalığına rastlanmaması dolayısıyla cinsiyete bağlı bir değerlendirme yapılmadı. Sonuç olarak bakım ve besleme şartlarındaki yetersizlikler gibi yetiştirici kaynaklı olumsuzluklar ile çevresel şartların etkisinin ayak lezyonlarının oluşmasında oldukça etkili olduğu kanısına varıldı.

Anahtar Kelimeler: Ayak hastalıkları, Elazığ, Diyarbakır, görülme sıklığı, koyun, piyeten

Evaluation of Foot Diseases Encountered in Sheep Farms in Elazig and Diyarbakir Regions

Abstract

This study aimed to investigate the frequency of foot diseases encountered in the pre-pasture and post-pasture periods in sheep farming enterprises in Elazig and Diyarbakir regions in 2018. In the examinations carried out on 10000 sheep/rams of different ages, breeds and genders, it was determined that foot lesions were found in 5.3% of the animals. It was determined that footrot were the most common foot lesion in both the pre-pasture (38%) and post-pasture periods (46.43%). In the pre-pasture period, foot lesions were mostly found on the forelimbs (64%), while in the post-pasture period they were found on the hind legs (60.71%). Since the number of male animals (n=15) evaluated throughout the study was low and no foot diseases were observed, no gender-related evaluation was made. As a result, it was concluded that breeder-related negativities such as inadequate care and feeding conditions and the effect of environmental conditions are very effective in the formation of foot lesions.

Key Words: Elazig, Diyarbakir, foot diseases, footrot, incidence, sheep

GİRİŞ

Ayak hastalıklarına rastlanan çiftlik hayvanları sağaltım masrafları, üretimden erken çıkarılmaları, kilo kaybı, süt verimindeki azalmalar ve döl veriminin düşmesi sebebiyle çok büyük ekonomik kayıplara sebep olmaktadır (1-5).

Koyun ve keçilerde ayak hastalıkları, etiyolojisinde birçok hazırlayıcı ve yapıcı sebeplerin olduğu multifaktöriyel problemlerdir. Barınak şartlarındaki problemler, mevsimsel değişiklikler, beslemeye bağlı problemler, ırk ve genetik yatkınlık durumu, gebelik, tırnağı doğrudan etkileyen travmalar, sürüdeki sistemik hastalıklar ve çeşitli enfeksiyon etkenleri ayak hastalıklarının oluşumunda rol oynamaktadır (6).

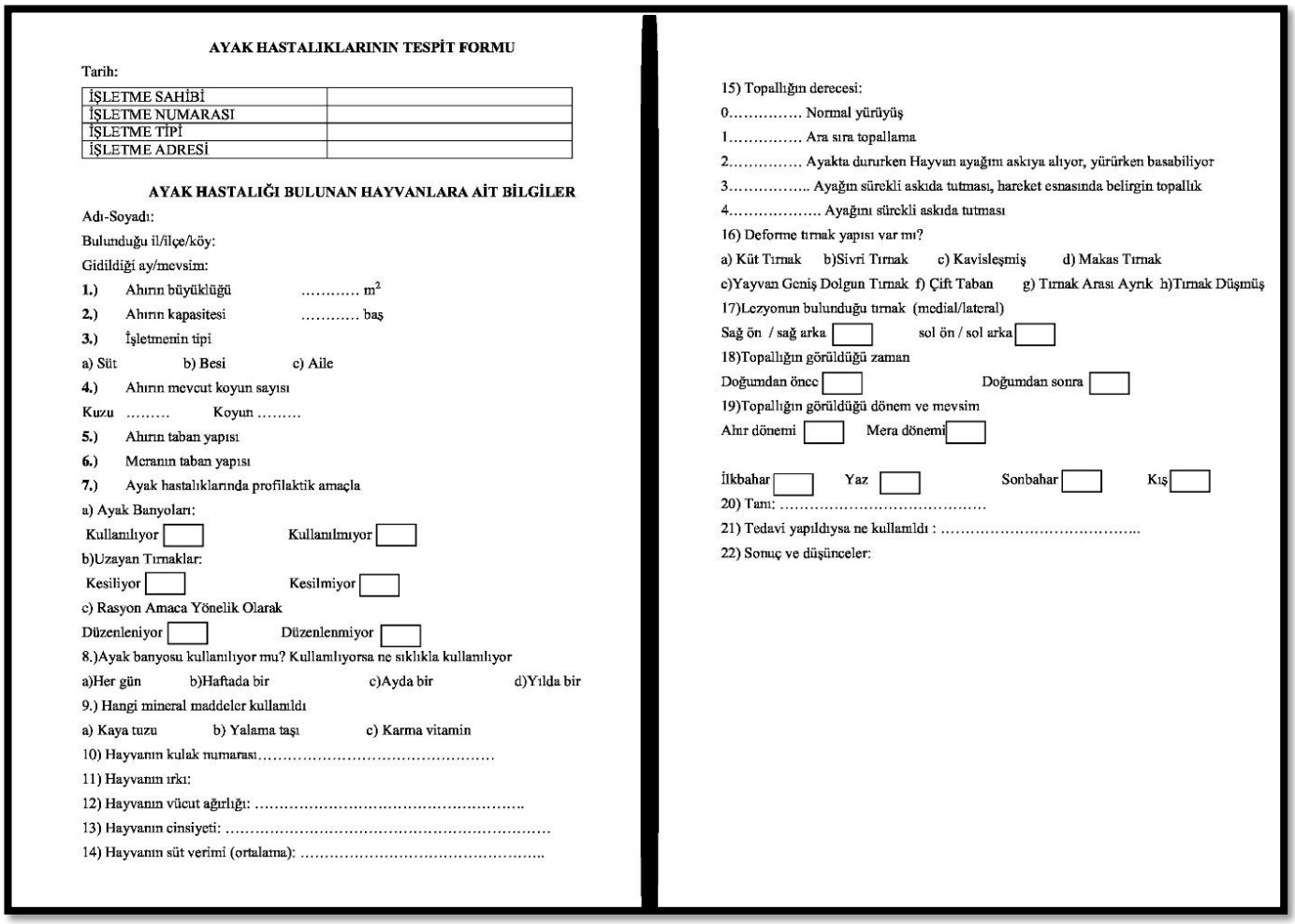
Koyunlarda başta piyeten olmak üzere birçok ayak hastalığı ile karşılaşılabilmektedir. Piyeten, koyun yetiştiriciliğinin yoğun olduğu İngiltere, Avusturalya, Yeni Zelanda gibi dünyanın pek çok ülkesi ile Türkiye'de görülen ve en çok ekonomik kayba sebep olan ayak hastalığıdır (7). Barınma ve bakım koşullarının kötü olduğu sürülerde hızlı bir yayılım göstermesi, süt ve döl veriminde azalma olması sebebiyle oldukça ciddi bir ayak hastalığıdır (8). Koyunlarda sık rastlanılan ve ekonomik kayıplara sebep olan diğer bir ayak hastalığı ise tüylüce ya da sinüzitis interdigitalis olarak adlandırılan her iki tırnak arasında bulunan içerisinde yağ ve apokrin ter bezlerini bulunduran sinüs bifleksin yangısıdır (9-11). Ülkemizde koyun yetiştiriciliği yapılan işletmelerde tırnak bakımı konusundaki yetersizliklerin en fazla sebep olduğu diğer bir problem ise tırnak deformasyonları olduğu yapılan diğer çalışmalarda tespit edilmiştir (6,12).

Bu çalışma ile Elazığ ve Diyarbakır yörelerindeki küçük ve büyük ölçekli işletmelerde bulunan koyunlarda karşılaşılan ayak hastalıklarının prevalansı ile ayak hastalıkları üzerine bölgenin toprak ve iklim şartlarının etkisinin araştırılması amaçlandı.

MATERYAL VE METOT

Çalışmanın Dizaynı ve Ayak Hastalıklarının Tespiti

Araştırmanın materyalini, Elazığ ve Diyarbakır yöresinde yetiştirilen değişik ırk, cinsiyet ve yaştaki 10000 koyun oluşturdu. Çalışmaya dâhil edilen hayvanların 5000 tanesi mera öncesi dönemde, 5000 tanesi ise mera sonrası dönemde değerlendirildi. Çalışmaya dâhil edilen sürülerin işletme sahiplerinden hayvan sayısı, ırk, cinsiyet ve yaş dağılımı ile barınak, bakım ve beslenme koşulları gibi sürü geneline ait bilgiler ayrı ayrı toplanıp değerlendirildi. Daha sonra sürüdeki koyunların inspeksiyonla muayeneleri yapılarak topallık gözlenen veya ayak bölgesinde patolojik bir görüntü tespit edilen hayvanlar ayrı bir bölüme alınıp sistematik ayak muayenesi yapılarak hastalığın teşhisi yapıldı. Sürü geneline ait tüm bilgiler aşağıda belirtilen ayak hastalıkları tespit formu (Şekil 1) ile kayıt altına alındı. İşletmelerdeki eksiklikler ve hatalar işletme sahiplerine bildirildi.



AYAK HASTALIKLARININ TESPİT FORMU

Tarih:

İŞLETME SAHİBİ	
İŞLETME NUMARASI	
İŞLETME TİPİ	
İŞLETME ADRESİ	

AYAK HASTALIĞI BULUNAN HAYVANLARA AİT BİLGİLER

Adı-Soyadı:
Bulunduğu il/ilçe/köy:
Gidildiği ay/mevsim:
1.) Ahırın büyüklüğü ..... m^2
2.) Ahırın kapasitesi ..... baş
3.) İşletmenin tipi
a) Süt b) Besi c) Aile
4.) Ahırın mevcut koyun sayısı
Kuzu ..... Koyun .....
5.) Ahırın taban yapısı
6.) Meranın taban yapısı
7.) Ayak hastalıklarında profilaktik amaçla
a) Ayak Banyoları:
Kullanılıyor ☐ Kullanılmıyor ☐
b)Uzayan Tırnaklar:
Kesiliyor ☐ Kesilmiyor ☐
c) Rasyon Amaca Yönelik Olarak
Düzenleniyor ☐ Düzenlenmiyor ☐
8.)Ayak banyosu kullanılıyor mu? Kullanılıyorsa ne sıklıkla kullanılıyor
a)Her gün b)Haftada bir c)Ayda bir d)Yılda bir
9.) Hangi mineral maddeler kullanıldı
a) Kaya tuzu b) Yalama taşı c) Karma vitamin
10) Hayvanın kulak numarası.....
11) Hayvanın ırkı:
12) Hayvanın vücut ağırlığı: .....
13) Hayvanın cinsiyeti: .....
14) Hayvanın süt verimi (ortalama): .....

15) Topallığın derecesi:
0..... Normal yürüyüş
1..... Ara sıra topallama
2..... Ayakta dururken Hayvan ayağını askıya alıyor, yürürken basabiliyor
3..... Ayağın sürekli askıda tutması, hareket esnasında belirgin topallık
4..... Ayağını sürekli askıda tutması
16) Deforme tırnak yapısı var mı?
a) Küt Tırnak b)Sivri Tırnak c) Kavisleşmiş d) Makas Tırnak
c)Yayvan Geniş Dolgun Tırnak f) Çift Taban g) Tırnak Arası Ayrık h)Tırnak Düşmüş
17)Lezyonun bulunduğu tırnak (medial/lateral)
Sağ ön / sağ arka ☐ sol ön / sol arka ☐
18)Topallığın görüldüğü zaman
Doğumdan önce ☐ Doğumdan sonra ☐
19)Topallığın görüldüğü dönem ve mevsim
Ahır dönemi ☐ Mera dönemi ☐
İlkbahar ☐ Yaz ☐ Sonbahar ☐ Kış ☐
20) Tanı: .....
21) Tedavi yapıldıysa ne kullanıldı : .....
22) Sonuç ve düşünceler:

Şekil 1. Ayak hastalıkları tespit formu

Toprak Analizinin Yapılması

Çalışmaya dahil edilen koyun sürülerinin mera döneminde otlatıldıkları çayır veya meralardan alınan toprak numuneleri, çalışmanın toprak materyalini oluşturdu. Toprak numunesi meranın büyüklüğüne göre 8 ya da 10 farklı noktadan alınarak mineral analizi ve pH tayini yapıldı. Toprak örneklerinin analizleri Dicle Üniversitesi Ziraat Fakültesi Toprak Analiz Laboratuvarı'nda gerçekleştirildi.

Aylık Ortalama Nem Oranlarının Belirlenmesi

Koyunların ayak hastalıkları yönünden takip edildikleri proje süresi boyunca çalışma alanı olarak belirlenen bölgelerin aylık ortalama nem oranları Elazığ ve Diyarbakır Meteoroloji istasyonlarının ölçümleri esas alınarak Devlet Meteoroloji İşleri Genel Müdürlüğü'nden temin edildi.

BULGULAR

Yapılan çalışmada 5000 tanesi mera öncesi dönemde, 5000 tanesi de mera sonrası dönemde olmak üzere toplam 10000 hayvan ayak hastalıkları yönünden değerlendirildi. Mera öncesi dönemde değerlendirilen hayvanların %5'inin mera sonrası dönemde değerlendirilen hayvanların ise %5.6'sının ayaklarında lezyonlara rastlandı. Mera öncesi dönemde ayak hastalıklarına rastlanan koyunların %64'ünün ön ayaklarında, %36'sının ise arka ayaklarında lezyonlara rastlandı. Mera sonrası dönemde ayak hastalıklarına rastlanan koyunların ise %39.29'unda ön ayaklarda, %60.71'inde ise arka ayaklarda lezyonlara rastlandı. Çalışmada değerlendirilen erkek hayvan sayısının çok az olması (n=15) ve bu hayvanların hiçbirinde tırnak hastalığına rastlanmaması sebebiyle cinsiyete göre değerlendirme yapmanın doğru olmayacağını düşündürdü.

Çalışma sırasında ağılların çoğunluğunun bilinçsiz ve imarsız olduğu, hijyenik koşullarının uygun olmadığı ve hayvanların alanlarının oldukça dar olduğu tespit edildi. Çalışmada değerlendirilen işletmelerin zeminlerinin çoğunlukla toprak olduğu, dışkı kanallarının yetersiz hatta bazı işletmelerde hiç olmadığı belirlendi. Yine barınakların birçoğunda havalandırmanın olmadığı tespit edildi. İşletme sahiplerinden alınan bilgiler doğrultusunda tırnak kesimi ve ayak banyoları başta olmak üzere tırnak bakımına gereken önemin verilmediği belirlendi. Hayvanların yetiştirilme amacına uygun rasyonlarla beslenmediği, yalama taşı dışında tırnak sağlığında önemli yeri olan ek minerallerin kullanılmadığı belirlendi.

Çalışmada değerlendirilen koyunlarda tespit edilen ayak hastalıklarının mera öncesi ve sonrası dönemlere göre dağılımı Tablo 1'de sunuldu. Çalışma boyunca karşılaşılan lezyonlar ise Şekil 2'de sunuldu. Çalışmaya dâhil edilen koyunların yaşadıkları bölgelerden alınan toprak numunelerinin makro ve mikro analiz verilerinin ortalama değerleri ile Ocak-Ekim 2018 tarihleri arasındaki aylık ortalama nispi nem oranları ise Tablo 2 ve Tablo 3'de sunuldu.

Tablo 1. Ayak hastalıklarının mera öncesi/mera sonrası dönemlere göre dağılımı (%)

Ayak Hastalığı	Mera öncesi (%)	Mera sonrası (%)
Tırnak deformasyonu	30	25.00
Piyeten	38	46.43
Sinüzitis interdigitalis	32	28.57
Toplam	100	100.00

Şekil 2. Piyeten tespit edilmiş bir koyun (a), müdahale edilmiş bir piyeten olgusu (b), tırnak deformasyonu (c,e), arka ayaklarında topallık semptomu gösteren bir koyun (d), çalışma sırasında ağıl ve merada karşılaşılan zemin tipleri (f, g).

Tablo 2. Çalışmada alınan toprakların mikro ve makro analizleri sonucu elde edilen ortalama veriler

Toprak analizleri	Değerler
Su ile doymuşluğu (%)	66.55
Bünye	Killi ve tınlı
Tuz oranı (%)	0.14 (Tuzsuz toprak)
pH	8.28 (Alkali toprak)
Kireç CaCO3 oranı (%)	4.33 (Az kireçli toprak)
Fosfor kg/da	37.38 (Çok fazla)
Organik madde (%)	3.86 (İyi)
Se	3.42
Ni (mg/kg)	37.46
Ca (mg/kg)	26.647
K (mg/kg)	6.376
Mg (mg/kg)	9.829
Na (ppm)	272.8
Cd (mg/kg)	0.1032
Co (mg/kg)	19.04
Cu (mg/kg)	35.72
Fe (mg/kg)	27.078
Mn (mg/kg)	826.4
Zn (mg/kg)	99.95
Pb (mg/kg)	11.74

Tablo 3. Çalışmanın yapıldığı dönemde (Ocak-Ekim 2018) yılı Elazığ ve Diyarbakır illerinin aylık ortalama nispi nem oranları (%)

	1	2	3	4	5	6	7	8	9	10
Elazığ	72.8	67.4	54.0	39.6	62.1	43.2	24.3	23.6	29.0	51.8
Diyarbakır	77.3	74.5	63.2	53.0	67.5	37.9	24.4	24.5	29.2	51.8

TARTIŞMA VE SONUÇ

Ayak hastalıkları koyun yetiştiriciliği işletmelerinde önemli verim kayıplarına sebep olan genetik, besleme durumu, mevsimsel dönem, barınak ve mera şartlarının hazırlayıcı etkisinin olduğu multifaktöriyel hastalıklardır (4,13). Yapılan birçok çalışma (4-6,12,13), bilinçsiz yetiştiricilik ve çevresel şartların ayak hastalıklarının oluşumu üzerindeki etkisini ortaya koymuştur. Bu çalışma ile Elazığ ve Diyarbakır yörelerinde toplam 10000 koyunda ayak hastalıklarının prevalansı, oluştuğu dönem, mevsimsel şartlar, mera ve barınak şartlarının ayak hastalıkları üzerine olan etkisi incelendi.

Koyun ve keçi ayak hastalıklarının oluşmasında mevsimsel değişikliklerin oldukça önemli olduğu yapılan çalışmalarla ortaya konulmuştur. Birçok çalışmada koyun ve keçilerde karşılaşılan ayak hastalıklarının prevalans incelemelerinde mera öncesi ve mera sonrası dönemler oldukça detaylı bir şekilde değerlendirilmiştir (6,12). Polat (6), Elazığ yöresindeki koyun ve keçilerde ayak hastalıklarını değerlendirdiği çalışmasında mera öncesi dönemde hayvanların %6.57'sinde, mera sonrası dönemde ise hayvanların %7.08'inde ayak lezyonlarına rastlandığını bildirmiştir. Yurdakul'un (12), Sivas yöresindeki koyunlarda karşılaşılan ayak hastalıklarını değerlendirdiği çalışmasında mera öncesi dönemde değerlendirilen 4070 hayvanın 857 tanesinde (%21.06), mera sonrası değerlendirilen 2257 hayvanın ise 234 tanesinde (%10.37) ayak lezyonlarına rastlandığı bildirilmiştir. Yapılan bu çalışmada ise mera öncesi dönemde hayvanların %5'inde, mera sonrası dönemde ise %5.6'sında ayak hastalıklarına rastlandığı belirlendi. Bu yönüyle diğer çalışmalarla karşılaşıldığında ayak lezyonlarına hem mera öncesi dönemde hem de mera sonrası dönemde daha az rastlandığı belirlendi. Yurdakul'un (12) Sivas bölgesindeki koyunlarda ayak hastalıklarını değerlendirdiği çalışmada hem mera öncesi dönemde (%60.68) hem de mera sonrası dönemde (%70.94) ayak lezyonlarına çoğunlukla ön tırnakta rastlandığı bildirilmesine rağmen bu çalışmada mera öncesi dönemde ön tırnaklarda (%64) mera sonrası dönemde ise arka tırnaklarda (%60.71) ayak lezyonlarına daha sık rastlandığı tespit edildi.

Küçük ruminant işletmelerinde en sık karşılaşılan ayak hastalıklarını non-infeksiyöz tırnak deformasyonları olduğu birçok çalışmada bildirilmiştir (6,12,14,15). Polat (6) Elazığ bölgesindeki koyun ve keçilerde karşılaşılan ayak hastalıklarının %77.47'sinin non-enfeksiyöz tırnak deformasyonları olduğunu ve bunların mera öncesi dönemde %92.86 seviyelerine ulaştığını mera sonrası dönemde ise %65.87 seviyelerine indiğini bildirmiştir. Yurdakul (12) ise Sivas bölgesindeki koyunlarda karşılaşılan ayak hastalıklarının %67.74'ünün non-enfeksiyöz tırnak deformasyonları olduğunu ve bunların mera sonrası dönemde %86.75 seviyelerine ulaştığını mera öncesi dönemde ise %62.54 seviyelerine indiğini bildirmiştir. Yapılan bu çalışmada ise koyunlarda karşılaşılan non-enfeksiyöz tırnak deformasyonlarının oranının mera öncesi dönemde %30, mera sonrası dönemde ise %25 seviyelerinde olduğu belirlendi.

Koyun yetiştiriciliğinin yoğun olarak yapıldığı ülkelerdeki işletmelerde en sık karşılaşılan ayak hastalıklarından birisi de piyetendir. Ülkemizdeki küçük ruminant işletmelerinde yapılan prevalans çalışmalarında ayak hastalıklarının %6.49-30.99'unun piyeten olduğu tespit edilmiştir (6,12,13,16,17). Polat'ın (6) Elazığ bölgesindeki küçük ruminant işletmelerinde yaptığı çalışmada piyetenin diğer ayak lezyonlarına oranının %6.49 olduğu belirlenmiştir. Yapılan bu çalışmada ise ayak lezyonu tespit edilen hayvanların mera öncesi dönemde %38'inde; mera sonrası dönemde ise %46.43'ünde piyetene rastlandığı belirlendi.

Sonuç olarak 2018 yılı içerisinde Elazığ ve Diyarbakır illerindeki koyunculuk işletmelerinde mera öncesi ve mera sonrası dönemde karşılaşılan ayak hastalıklarının prevalansının incelendiği bu çalışmada %5.3 oranında ayak lezyonlarına rastlandığı belirlendi. Mera öncesi ve sonrası dönemde birçok çalışmanın aksine en çok rastlanılan ayak lezyonunun tırnak deformasyonları değil piyeten olduğu tespit edildi. Çalışmada karşılaşılan ayak lezyonlarının en büyük sebebi olarak yetiştirici kaynaklı bakım ve besleme şartlarının yetersizliği olarak belirlendi. Tüm bu verilerin ışığında koyunculuk işletmelerinde düzenli tırnak bakımlarını yapılması, ağıl ve mera şartlarının uygun duruma getirilmesi ve tırnağın mekanik temizliği başta olmak üzere antiseptikli solüsyonlarla temizlenmesi oldukça önemlidir.

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Bu çalışma Hasip Okay'ın yüksek lisans tezinden üretilmiştir.

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ARAŞTIRMA MAKALESİ

Soğutma ve Dondurma Tekniklerinin Etlik Piliç Göğüs Eti Kalite Özellikleri Üzerine Etkisi

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Öz

Bu çalışma soğutma ve dondurma tekniklerinin etlik piliç göğüs eti kalite özellikleri üzerine olan etkisini araştırmak amacı ile yapılmıştır. Çalışmada 10'ar adet, soğutulmuş ve dondurulmuş, hızlı gelişen etlik piliç göğüs eti örnekleri kullanılmıştır. Dondurulmuş göğüs eti örnekleri standart koşullarda kesilmiş ve -18 ºC sıcaklıkta 11 ay süre ile dondurularak depolanmış etlik piliç karkaslarından, soğutulmuş kas örnekleri ise standart koşullarda kesilmiş ve +4 ºC sıcaklıkta 24 saat soğutulmuş karkaslardan alınmıştır. Her iki gruptaki göğüs eti örneklerinde pH, renk özellikleri, pişirme kaybı, damlama kaybı ve rutubet analizleri yapılmıştır. Renk özellikleri derili ve derisiz göğüs kasında, diğer özellikler derisiz örneklerde incelenmiştir. Etlik piliç etlerinin soğutulmuş ya da dondurulmuş olarak muhafaza edilmesi; parlaklık (L*) hariç incelenen göğüs eti renk özelliklerinin tamamını önemli düzeyde etkilemiştir. Göğüs etinin derili ya da derisiz olmasının; parlaklık (L*), kırmızı renk koordinatı (a*), chrome ve E renk özellikleri üzerine önemli bir etkiye sahip olduğu bulunmuştur (P<0.001). Göğüs eti rutubet düzeyi soğutulmuş göğüs etinde (P<0.004), damlama kaybı (P<0.022) dondurulmuş göğüs etinde önemli düzeyde daha yüksek bulunmuştur. Sonuç olarak; incelenen göğüs eti kalite özellikleri etin muhafaza şeklinden önemli düzeyde etkilenmiş, göğüs etinin derili ya da derisiz olması bazı et kalitesi renk özelliklerini önemli düzeyde etkilemiştir. Dondurulmuş piliç eti kalitesi ile ilgili olarak dondurma şekli, dondurma ve depolama süresi ile donmuş eti çözdürme yöntemleri vb. dikkate alan yeni çalışmaların planlanmasının faydalı olacağı düşünülmektedir.

Anahtar Kelimeler: Dondurma, et kalitesi, piliç eti, soğutma

Effects of Chilling and Freezing Techniques on Broiler Breast Meat Quality Characteristics

Abstract

The aim of this study was to determine the some physico-chemical quality of chicken breast meat stored at chilled (2-4 °C) and frozen (-18 °C) conditions. In the study, 10 chilled and frozen fast-growing broiler chicken breast meat samples were used. Frozen breast meat samples were taken from chicken carcasses that were slaughtered under standard conditions and stored frozen at -18 °C for 11 months, whereas chilled muscle samples were taken from carcasses that were slaughtered under standard conditions and cooled at +4 °C for 24 hours. Color (L*, a*, and b*), pH, humidity, cooking losses, and drip losses were measured. Color characteristics were measured with skin and skinless sample while other parameters were performed only on skinless sample. Color properties, pH, cooking loss, dripping loss and moisture analyzes were performed on meat samples taken from both groups. Color characteristics were examined in skinned and skinless chest muscles, and other characteristics were examined in skinless samples. Storage condition as cooling or freezing significantly affected all breast meat color characteristics examined, except L* value. It was determined that breast meat with and without skin had a significant effect on L*, a*, chrome and E color characteristics (P<0.001). Breast meat moisture level was significantly higher in chilled carcasses (P<0.004), while drip loss (P<0.022) was significantly higher in frozen carcasses. In conclusion; some breast meat quality characteristics were significantly affected by the preserving method or meat condition as with or without skin. Planning new research would be useful taking some issues as freezing method, freezing and storage time, thawing methods etc. into consideration.

Key Words: Broiler, breast meat quality, chilling, freezing

GİRİŞ

En önemli hayvansal protein kaynaklarından birisi olan piliç eti tüketilinceye kadar genelde soğutulmuş olarak kısa süreli ya da dondurularak daha uzun süreli muhafaza edilmektedir. Dondurarak muhafaza özellikle arz-talep dengesinin değiştiği durumlarda ya da etin uzak mesafelere nakledilmesi gerektiği koşullarda tercih edilmektedir. Dondurarak depolama piliç etinde olduğu gibi günümüzde pek çok gıdanın muhafazasında kullanılan başlıca tekniklerden birisidir (1). Etin soğutularak ya da dondurularak muhafaza edilmesi ve depolama süresi etin fiziki, kimyasal, duyusal ve mikrobiyolojik kalitesini etkileyebilmektedir (2,3). Dondurulmuş etlerin kalitesi; etin orijini, dondurma yöntemi ve etkinliği, muhafaza koşulları, depolama süresi ve sıcaklığı ile donmuş etin çözdürme metodu gibi çok sayıda faktörden etkilenmektedir (4,5).

Kısa sürede tüketime sunulacak etler tekniğine uygun bir şekilde soğutulur ve soğukta muhafaza edilirse, başta besleyici değerleri olmak üzere görünüş, lezzet, renk ve tekstür gibi özelliklerinde genelde bir değişiklik olmamaktadır. Uygulama koşullarına bağlı olarak donma-depolama ve donma-çözünme işlemi sırasında, etin kalitesi ve ticari değerinde düşme olabilir. Özellikle etin rutubet içeriği dondurma ve çözdürme tekniklerinden önemli düzeyde etkilenir. Kas lifleri arasında bol miktarda bulunan su donduğunda protein, karbonhidrat, lipid, vitamin ve mineral vb. çözünen maddelerin konsantrasyonu artar ve etteki biyokimyasal sistemin dengesi değişir. Kas liflerindeki bu değişiklikler, hücre zarlarının performansını etkileyerek et kalitesini de etkiler. Donma ve çözünme sırasında, miyoglobinin oksitlenmesi daha kolaydır ve et rengi daha koyu olur. Son yıllarda etin ticari değerindeki düşmeyi önlemek ya da azaltmak için hızlandırılmış donma ve çözünme gibi değişik teknikler uygulanmaktadır. Bu çalışma soğutulmuş ve dondurulmuş etlik piliç göğüs etlerinde bazı et kalitesi özelliklerini araştırmak amacı ile yapılmıştır.

MATERYAL VE METOT

Bu çalışmada Bursa Uludağ Üniversitesi Veteriner Fakültesi Araştırma ve Uygulama Merkezi Tavuk Yetiştirme Ünitesi araştırma kümesinde yetiştirilerek standart koşullarda kesilen ROSS PM_3 genotipi etlik piliç karkasları kullanılmıştır. Soğutulmuş ve dondurulmuş karkasların elde edildiği hayvanların beslenmesinde; deneme başından 10 günlük yaşa kadar etlik piliç başlangıç yemi (%22 protein ve 3000 ME, kcal/kg), 11-35. günler etlik piliç büyütme (%20 protein ve 3050 ME, kcal/kg), 35. günden kesime kadar kesim öncesi yem (%18 protein ve 3100 ME, kcal/kg) kullanılmıştır. Kesim sonrası ön soğutma işleminden sonra ev tipi buzdolabında +4 °C sıcaklıkta 1 gün süre ile soğutulan 10 adet etlik piliç karkasından alınan göğüs kası örnekleri ile kesim sonrası soğutma işleminden sonra paketlenerek -18 °C sıcaklıkta dondurularak 11 ay süre ile bu sıcaklıkta depolanmış 10 adet etlik piliç karkasından alınan göğüs kası örnekleri bu çalışmanın materyalini oluşturmuştur (3,6).

Dondurulmuş piliç karkasları analiz öncesi buzdolabında +4 °C sıcaklıkta 24 saat süre ile çözdürme işlemine tabi tutulmuş ve analizlerde kullanılmıştır. Çalışmada kullanılan örnekler analizler esnasında buzdolabında muhafaza edilmiştir. Et kalitesi analizleri BUÜ Veteriner Fakültesi Zootekni Anabilim Dalı Karkas Değerlendirme ve Et Kalitesi Analiz laboratuvarında yapılmıştır. Hayvan Deneyleri Etik Kurulları Çalışma Usul ve Esaslarına Dair Yönetmelik (7), Madde 8 19-k gereği bu çalışma için etik kurul onayı almaya gerek yoktur.

Veri Toplama

Soğutulmuş ve dondurulmuş etlerde kalite analizleri musculus pectoralis major göğüs kasından yapılmıştır. Renk analizleri derili ve derisiz kas örneklerinde, pH, rutubet, damlama (drip loss) ve pişirme kaybı analizleri ise derisiz örneklerde gerçekleştirilmiştir. Zootekni Anabilim Dalında mevcut pH metre (ExStik PH100 pH-meter, Extech Instruments) ve renk ölçüm cihazı (PCE-XXM 20, PCE Instruments LTD) karkas göğüs eti kas örneklerinde pH ve renk özelliklerinin ölçümü amacıyla kullanılmıştır.

Göğüs kası örneklerinde pH ölçümü yapmadan önce pH metrenin ucu distile su ile temizlenip sonrasında ölçümler gerçekleşmiştir. Ölçüm esnasında cihazın göstergesinde sabit değer okunduğunda pH değeri belirlenmiştir. Renk ölçümleri; önce derili örneklerde yapılmış, sonrasında aynı noktadan deri kaldırılarak derisiz olarak doğrudan kas üzerinden ölçüm yapılmıştır. Renk özellikleri için parlaklık (*L**), kırmızı renk koordinat *(a*)* ve sarı renk koordinat *(b*)* değerleri üç ölçümün ortalamasını esas alan Uluslararası Aydınlatma Komisyonu (Commission Internationale de l'Eclairage, CIE, CIELAB) tarafından verilen standartlara göre yapılmıştır (8). Bu standartlara göre; parlaklık (*L**; 0-100) koyu/siyah' tan yaygın beyaza kadar değişen renk tonunu, kırmızı renk koordinatı (*a**); 60' a kadar negatif değerler yeşil/mavi renk yoğunluğunu, 60' da kadar pozitif değerler kırmızının değişik tonlarını ifade etmektedir. Sarı renk koordinatı (*b**); mavi/sarı; 60' a kadar negatif değerler maviyi, pozitif değerler sarının değişik tonlarını göstermektedir (8-12). Kas örneklerinden ölçümle elde edilen bu değerler kullanılarak kas örneklerinin renk açısı (Hue *h**, arctan), $h^\circ = \tan^{-1}(b^*/a^*).180/\pi$) ve renk doygunluk/canlılık (Chrome *C**), $C^* = (a^{*2}+b^{*2})^{1/2}$ değerleri ile *ΔE* değerleri ($\Delta E = (L^2 + a^2 + b^2)1/2$) hesaplanmıştır (13,14). Göğüs kaslarında pH ve renk ölçümleri yapıldıktan sonra, her bir göğüsten 30 gram ağırlığında örnek alınarak su geçirmez plastik torbalara konulmuştur. Torbalara konulan et parçaları 80 derece sıcaklıktaki su banyosunda 20 dakika ısıl işleme tabi tutulmuş, sonrasında torbalardan çıkarılarak tekrar tartılmıştır. Elde edilen değerler; *Pişirme Kaybı*, %=(çiğ/pişmemiş örnek ağırlığı - pişmiş örnek ağırlığı) / çiğ/pişmemiş örnek ağırlığı) formülünde yerlerine yazarak pişirme kaybı analizi tamamlanmıştır (15). *Rutubet* analizi amacıyla; her iki gruptaki göğüs etlerinden 30'ar gram ağırlığında parçalar kesilerek ayrı ayrı petri kaplarına konulup 105 derece sıcaklıktaki etüv cihazında 1 saat kurutulmuştur. Petri kaplarının darası alınmış (G1), et örnekleri tartılarak ağırlıkları ölçülmüştür (G2). Örnekler tekrardan petri kaplarına konularak etüv cihazından 4 saat işleme tabi tutulmuş ve işlem sonrası tekrardan ağırlıkları ölçülmüştür. Elde edilen değerler % Rutubet ((G1 – G2)) / G1*100 formülünde yerlerine yazılarak kuru madde değeri hesaplanmış ve elde edilen değerlerin ortalaması alınmıştır. *Damlama Kaybı (Drip Loss) analizi* amacıyla et örneklerinden 6 gram ağırlığında (W1) parçalar kesilerek plastik torbaların içine konulmuş, et paçalarının uç kısmı plastik torbaya kürdan ile sabitlenerek +4 derecedeki buzdolabı ızgarasında dik şekilde sabit durması sağlanmıştır. Bu

şekilde etten akacak suyun örnek parçaya temas etmeden torbanın altında toplanması sağlanmıştır. Buzdolabında 24 saat bekletilen örnek parça paketlerden çıkartılarak tartılmıştır. Elde edilen değerler % (W1– W2)/W1*100 formülünde yerlerine yazılarak damlama kaybı hesaplanmıştır (15,16).

İstatistiki Analizler

Rutubet, damlama kaybı, pişirme kaybı ve pH bakımından gruplar arası farklılıklar t testi (paired sample test) ile renk kalite özellikleri için gruplar arası farklılıklar çok yönlü varyans analizi (general linear model) ile analiz edilmiş, gruplar arası farklılıkların önemli bulunması halinde Duncan testi kullanılmıştır (17). İstatistiki analizler SPSS (Version 28.0) bilgisayar programında yapılmıştır (18).

BULGULAR

Soğutulmuş ve dondurulmuş piliç göğüs etlerinde tespit edilen renk kalite özellikleri Tablo 1' de sunulmuştur. Renk kalite özelliklerinden a* (P<0.002), b* (P<0.001), hue, h (P<0.001), chrome C* (P<0.003) ve *ΔE* (P<0.025) değerleri etin muhafaza koşullarından önemli düzeyde etkilenmiş, b*, C* ve *ΔE* değerleri dondurulmuş etlerde daha yüksek bulunmuştur. Piliç etinin derili ya da derisiz olması L* (P<0.001), a* (P<0.001), C* (P<0.001) ve *ΔE* (P<0.001) renk kalite özelliklerini önemli düzeyde etkilemiş, derili göğüs etinde bu değerler daha yüksek bulunmuştur.

Tablo 1. Soğutulmuş ve dondurulmuş piliç etlerinde renk kalite özellikleri *(x̄±Sx̄).*

Grup/Özellikler	L*	a*	b*	h*	C*	*ΔE*
Muhafaza Koşulları						
Soğutulmuş	60.77±1.04	-10.26±2.68	-2.61±1.40	0.741±0.28	14.36±2.32	2030±106
Dondurulmuş	61.04±1.03	-23.02±2.67	5.59±1.41	-0.896±0.27	25.73±2.11	2376±104
Fiziksel Yapı						
Derili	68.00±1.02	-26.71±2.66	-0.01±1.39	0.135±0.29	27.47±2.49	2842±103
Derisiz	53.80±1.03	-6.56±2.67	3.06±1.40	-0.290±0.30	12.63±2.50	1564±105
Muhafaza Koşulları x Fiziksel Yapı						
Soğutulmuş x Derili	65.37±1.46[b]	-16.74±1.79	-2.76±1.97	0.875±0.39	17.67±3.60	2385±146
Soğutulmuş x Derisiz	56.17±1.44[a]	-3.77±3.81	-2.46±1.99	0.607±0.41	11.06±3.59	1675±148
Dondurulmuş x Derili	70.65±1.44[b]	-36.68±3.81	2.57±1.98	-0.606±0.41	37.26±3.54	3299±148
Dondurulmuş x Derisiz	51.44±1.43[a]	-.9.36±3.80	8.60±1.99	-1.186±0.40	14.20±3.56	1453±147
ANOVA						
Muhafaza Koşulları	0.855	0.002	0.001	0.001	0.003	0.025
Fiziksel Yapı	0.001	0.001	0.121	0.293	0.001	0.001
Muhafaza Koşulları x Fiziksel Yapı	0.002	0.067	0.158	0.697	0.027	0.001

L*:parlaklık, **a***; kırmızı renk koordinatı, **b***;sarı renk koordinatı, **h***; renk açı değeri, **C***; chrome

Soğutulmuş ve dondurulmuş piliç etlerinde rutubet, damlama kaybı, pişirme kaybı ve pH değerleri Tablo 2' de gösterilmiştir. Soğutulmuş piliç etlerinde rutubet (P<0.004) ve pH (P<0.05) değerleri, dondurulmuş piliç etlerinde ise damlama kaybı (P<0.022) değeri önemli düzeyde daha yüksek bulunmuştur. Pişirme kaybı bakımından soğutulmuş ve dondurulmuş piliç etlerinde önemli düzeyde bir farklılık tespit edilmemiştir.

Tablo 2. Soğutulmuş ve dondurulmuş piliç etlerinde bazı kimyasal ve fiziksel et kalitesi özellikler *(x̄±Sx̄).*

Grup/Özellikler	Dondurulmuş Et	Soğutulmuş et et	*P*
Rutubet, %	27.80±5.39	36.66±2.91	0.004
Damlama kaybı (Drip loss), %	3.94±1.73	2.20±0.68	0.022
Pişirme kaybı, %	11.70±4.10	13.62±4.73	0.127
pH	5.49±0.64	6.03±0.20	0.050

TARTIŞMA VE SONUÇ

Piliç eti kalitesi genotip, hayvan besleme, barındırma sistemi, kas miyopatileri, etin muhafaza yöntemi gibi çok sayıda faktörden etkilenmektedir (3,19-21). Üretim sonrası, etin tüketilmesine kadar olan süreçte kalitesinde herhangi bir değişim olmadan muhafazası oldukça önemli bir konu olup, uzun süreli muhafazada dondurma yöntemi yaygın olarak kullanılmaktadır. Soğutma işlemi ile genelde etin kalitesinde bir değişme neden olmazken, usulüne uygun yapılmadığı takdirde dondurma işlemi ile etin renginde değişme ve tekstürün bozulması gibi bazı istenmeyen değişiklikler meydana gelebilir. Tüketici beğenisi açısından et rengi en önemli kalite parametrelerinden birisidir. Bu çalışmada; dondurarak muhafaza yöntemi piliç göğüs etinin L* değeri hariç incelenen bütün et kalitesi renk özelliklerini, etin derili ya da derisiz olması L*, a*, C* ve *ΔE* değerlerini önemli düzeyde etkilemiştir. Genelde renk kalite özelliklerinden parlaklık ve kırmızı renk koordinatı değerlerinin çok düşük, sarı renk koordinatı değerinin de yüksek olması istenmez (22). Bu çalışmada ölçülen renk yoğunlukları dikkate alındığında soğutulmuş ve dondurulmuş etlerin her ikisin de de kırmızılık renk koordinatının kırmızıdan yeşile doğru yoğunlaştığı söylenebilir. Normalde tavuk etlerinde renk dondurma yöntemi ile değişmese de etlik piliçler gibi genç kanatlılarda kemiğe yakın bölümler dondurulup çözdürüldüğünde etler koyu renkli olabilmektedir

(23). Bu çalışmada b* bakımından soğutulmuş etlerin maviye, dondurulmuş etlerin sarıya doğru yoğunlaşmış olduğu bulunmuştur. Derili etlerde a* yönünden yeşile doğru kayma daha yüksek bulunmuştur. Etlerde renk özellikleri birçok faktörden etkilenmektedir. Dondurulmuş etlerde; a* değerindeki düşme ette kırmızı rengin oluşmasından sorumlu olan miyoglobin kaybından dolayıdır. Karkas ağırlığı arttıkça et rengi parametrelerinden kırmızı renk koordinatı ve parlaklık değerleri yükselmektedir (24). Piliç eti renk kalite özelliklerinden L* ve a* değerleri farklı sıcaklık koşullarından önemli düzeyde etkilenmektedir (25). Dondurulmuş et yüzeyinde zamanla ortaya çıkan metmyoglobin birikimi diskolorasyona yol açmakta ve buna bağlı olarak parlaklık azalırken, kırmızılık düzeyi yükselmektedir. Artan lipit oksidasyonu ve MetMB formasyonu dondurulmuş etlerde sarı renk koordinatı değerinde ortaya çıkan farklılıkların başlıca nedenidir. Donma ve çözülme süresinin uzaması ile ette kırmızı renk koordinatı, parlaklık ve sarı renk koordinatı değerleri azalmaktadır. Et renginin oluşumu miyoglobin gibi kas pigmentlerine bağlıdır. Etlik piliçlerde deri rengi beyazdan sarıya kadar değişebilir ve ırk ile beslemeden önemli düzeyde etkilenir. Kas kontraksiyonlarına bağlı olarak da et rengi değişkenlik gösterebilir. Tavukların daha hareketli olan bacak kasları daha koyu renklidir. Etlik piliç göğüs eti, düşük kas kontraksiyonundan dolayı az miktarda miyoglobine gereksinim duyduğundan göğüs eti, but etine göre daha açık renklidir. Yaş ilerledikçe miyoglobin miktarı arttığından genç hayvanlara göre yaşlı hayvanların etleri daha koyu renklidir. Etlerde E renk değeri insanın renkleri ayırt etme yeteneği ile, renk doygunluk ve açı değerleri insanlardaki görsel renk algıları ile yakından ilgilidir. Etin doygunluk değeri, et renginin saflığını göstermekte olup, dondurulmuş etlerde daha yüksek bulunmuştur. Et renginin canlılığını ifade eden h* değerinin yüksek olması canlı renkleri, düşük olması renklerin donuk olduğunu göstermektedir (26). Bu çalışmada; h* değeri soğutulmuş ve derili etlerde daha yüksek bulunmuş olup, soğutulmuş ve derili etlerin daha canlı renkli olduğu söylenebilir. Ölçülen bütün renk değerlerinin tek bir rakam ile ifade edildiği *ΔE* değeri (26) bu çalışmada dondurulmuş ve derili et örneklerinde daha yüksek bulunmuştur.

Pişirme kaybı, rutubet ve damlama kaybı; etin su tutma kapasitesi ile ilişkili önemli parametrelerdir. Etten sızan su miktarı veya etteki suyun uzaklaştırılması olarak tanımlanan damlama kaybı (drip loss) üzerine; kesim sonrası karkas sıcaklığı ve pH düşme hızı, etin işlenme özellikleri (parçalama, kesme vb.), depolama sıcaklığı, donma hızı ve donma sıcaklığı gibi faktörlerin önemli bir etkisi vardır. Parçalanmamış bütün karkasta ette damlama kaybı daha düşük olup, et ne kadar çok parçalanırsa damlama kaybı o kadar artmaktadır. Kesim sonrası et ne kadar kısa zamanda soğutulursa su tutma kapasitesi de o kadar fazla olur. Et pH'sının hızlı düşmesi su tutma kapasitesinde azalmasına ve damlama kaybının artmasına neden olmaktadır. Bu çalışmada soğutma yöntemi ile karşılaştırıldığında, dondurulmuş piliç göğüs etlerinde önemli düzeyde daha düşük pH ve önemli düzeyde daha yüksek damlama kaybı saptanmıştır. Damlama kaybı, kas dokusu içindeki suyun miyofibrillerden hücre dışı boşluğa transferini içeren bir süreç olup (27) kasın ete dönüşümü sırasında su, demir ve protein kaybı ile kas liflerinden ortaya çıkan sızıntı olarak tanımlanabilir (28). Damlama kaybı ile pişirme kaybı arasında doğrusal bir ilişki olup, genelde daha yüksek damlama kaybı daha yüksek pişirme kaybına neden olmaktadır (29). Damlama kaybı; işleme teknolojisi ve tüketici kabulü yönünden en önemli parametre olan su tutma kapasitesinin de en önemli göstergesidir (30). Karkas ağırlığı fazla olan etlerde pişirme kaybı da daha yüksektir (31). Pişirme ve damlama kaybı düşük pH' ı olan etlerde daha yüksektir (19). Hızlı gelişen etlik piliçlerden üretilen etlerde pH, pişirme kaybı ve damlama kaybı değerleri yavaş gelişen genotiplerden genelde daha yüksektir (32,33).

Etin pH'sı raf ömrü bakımından önemli bir parametre olup, çok düşük pH et rengi ve su tutma kapasitesini etkilemektedir. Çetin ve ark. (34) farklı zeminlerde yetiştirilen yavaş ve hızlı gelişen etlik piliçlerde soğutulmuş göğüs eti pH'sının sırası ile 5.75 ve 5.73 olduğunu bildirmişlerdir. Göğüs eti pH değeri ile parlaklık ve kırmızı renk koordinatı değeri arasında doğrusal bir ilişki olup, et rengi koyulaştıkça pH değeri yükselmektedir (35). Kaslarda ortaya çıkan miyopatilerde et pH'sını etkilemekte, solgun ve eksüdatlı kaslarda pH değeri daha düşük olmaktadır (36). Bianchi ve ark. (24) ile Yalçın ve ark. (31) daha yüksek karkas ağırlığına sahip etlik piliç etlerinde kas pH değerinin daha düşük olduğunu bildirmişlerdir. Etin dondurulma işlemi esnasında peptit ve amino asit üretimine yol açan protein denatürasyonu etin pH değerini ve metabolitlerin birikimini etkilemektedir (4). Bu çalışmada beklenildiği gibi (1) soğutulmuş piliç etinde pH değeri dondurulmuş ete göre daha yüksek bulunmuştur ($P<0.05$). Kesim sonrası laktik asitlerin oluşumu ile pH seviyesinin düşmesi etteki proteinlerin denatüre olmasına neden olmaktadır. Ette su proteinlere bağlı olarak bulunduğundan proteinlerin denatüre olması su tutma kapasitesini azaltmaktadır. Bu nedenle ette pH seviyesinin kesim sonrası hızlı düşüşünü önlemek için kesim işlemlerinden sonra mümkün olan en hızlı sürede soğutma işleminin yapılması gerekir. Ette su, proteinlere bağlı olarak bulunmakta, yüksek pH ortamında proteinlere daha fazla su bağlanmaktadır. Su tutma kapasitesi de depolama esnasında etin ağırlık kaybını doğrudan etkilemektedir. Laktik asit oluşumu ette pH seviyesinin düşmesine neden olmakta ve protein denatürasyonunu etkilemektedir. Bu çalışmada pişirme kaybı bakımından soğutulmuş ve dondurulmuş etlerde bir farklılık bulunmamıştır. Özbek ve ark. (37) farklı zeminlerde yetiştirilen hızlı gelişen etlik piliçlerde göğüs etinde pişirme kaybının %27.88 olduğunu ve yavaş gelişenlere göre daha yüksek olduğunu bildirmişlerdir. Dondurulmuş piliç göğüs etinde depolama süresi arttıkça pişirme kaybının arttığı bildirilmiştir (1). Bu çalışmada beklenildiği gibi rutubet değeri soğutulmuş etlerde, damlama kaybı dondurulmuş etlerde önemli düzeyde daha yüksek bulunmuştur (33).

Bu çalışmada elde edilen veriler bütünü ile değerlendirildiğinde; piliç etinin soğutularak ya da dondurularak muhafaza yöntemi parlaklık hariç bütün renk özelliklerini önemli düzeyde etkilemiş, piliç göğüs etinin derili ya da derisiz olması parlaklık ve kırmızılık renk değeri üzerine önemli bir etki göstermiştir. Renk özelliklerinden chrome ve *ΔE* değerleri de göğüs etinin derili ya da derisiz olmasından önemli düzeyde etkilenmiştir. Dondurma işlemi etin damlama kaybını artırırken, pH değerini önemli düzeyde düşürmüştür. Uzun süre depolanması gereken etlerde; etin özelliğine en uygun dondurma şekli, depolama süresi, çözdürme yöntemi vb. uygulanması ile et kalitesinde ortaya çıkabilecek kayıplar en aza indirilebilir. Farklı dondurma teknikleri, dondurma süresi, depolama süresi, çözdürme yöntemleri vb. konusunda yeni çalışmaların planlanmasının faydalı olacağı düşünülmektedir.

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RESEARCH ARTICLE

Lipid Peroxidation and Thiol/Disulfide Homeostasis in Cattle with Trichophytosis

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Abstract

The aim of this study was to determine thiol/disulfide homeostasis in cattle with trichophytosis and to determine the changes in malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px=GPx) levels due to the disease. 15 cattle with trichophytosis and 15 healthy cattle in the control group constituted the material of the study. Blood samples were taken from the jugular vein of the animals in tubes with and without anticoagulant. MDA, CAT, SOD, total thiol, native thiol analyses were performed in serum and GSH-Px analyses were performed in plasma. It was determined that total thiol, native thiol and native thiol/total thiol levels among biochemical parameters in cattle with trichophytosis decreased statistically significantly. It was determined that disulfide/native thiol and disulfide/total thiol levels increased statistically compared to the control group. Although the disulfide level increased compared to the control group, it was not statistically significant. In addition, it was determined that MDA level increased compared to the control group, CAT, SOD and GSH-Px levels decreased statistically significantly compared to the control group. In conclusion, the findings obtained from the study showed that trichophytosis causes oxidative stress in cattle, and the use of oxidative stress markers, especially thiol/disulfide homeostasis markers, would contribute to the pathogenesis of trichophytosis.

Key Words: Cattle, oxidative stress, thiol/disulfide homeostasis, trichophytosis

Trikofitozisli Sığırlarda Lipid Peroksidasyonu ve Tiyol/Disülfid Homeostazı

Öz

Amacımız trikofitozisli sığırlarda tiyol/disülfid homeostazisini ortaya koymak ve hastalığa bağlı olarak oluşan malondialdehit (MDA), katalaz (CAT), süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px= GPx) seviyesindeki değişimleri belirlemektir. Trikofitozisli 15 ve kontrol grubunu oluşturan 15 adet sağlıklı sığır çalışmanın materyalini oluşturdu. Hayvanların Vena jugularis'inden antikoagulanlı ve antikoagulansız tüplere kan örnekleri alındı. Serumda MDA, CAT, SOD, total tiyol, natif tiyol, plazmada ise GSH-Px analizleri yapıldı. Trikofitozisli sığırlarda total tiyol, natif tiyol ve natif tiyol/total tiyol düzeylerinin istatistiksel olarak anlamlı düzeyde azaldığı belirlendi. Disülfid/natif tiyol ve disülfid/total tiyol düzeylerinin ise kontrol grubuna göre istatistiksel olarak arttığı tespit edildi. Disülfid düzeyi kontrol grubuna göre artmakla beraber istatistiksel olarak anlamsızdı. Bunun yanı sıra MDA düzeyinin kontrol grubuna göre arttığı, CAT, SOD ve GSH-Px düzeylerinin ise kontrol grubuna göre istatistiksel olarak anlamlı düzeyde azaldığı belirlendi. Sonuç olarak çalışmadan elde edilen bulgular trikofitozisin sığırlarda oksidatif strese neden olduğu, oksidatif stres belirteçlerinden özellikle de tiyol/disülfid homeostazis belirteçlerinin kullanımının trikofitozisin patogenezine katkı sağlayacağı kanısına varıldı.

Anahtar Kelimeler: Oksidatif stres, sığır, tiyol/disülfid homeostazı, trikofitozis

INTRODUCTION

Trichophytosis is a skin disease that is characterized by dandruff and keratinized crusting of the skin caused by fungi, causing losses such as growth slowdown, live weight loss, and deterioration of skin quality (1). These factors, which are found all over the world, cause infections in humans and animals, and generally epidermophyton species are effective in humans, microsporum species in carnivores, and trichophyton species in horses, pigs and ruminants (2). The disease is transmitted to healthy animals through direct contact (3). Clinical symptoms include hair loss, skin crusting, erythema and itching (4). The lesions, which are commonly found on the head and neck, are grayish in appearance, slightly raised, and round in shape. Diagnosis is made by seeing round, grayish lesions on the skin. Definitive diagnosis is made by microscopic examination of samples taken from the lesions (3).

Antioxidants are classified according to their structures as enzymes (catalase 'CAT', superoxide dismutase 'SOD', glutathione peroxidase 'GSH-Px = GPx') and non-enzymes (reduced glutathione 'GSH') or according to their cell localization (5). Oxidative stress is formed as a result of insufficiency of antioxidant mechanisms and increase of reactive oxygen species (6). Thiol is a very important antioxidant in preventing damage caused by oxidative stress and protects the cell against oxidative stress. It appears that the thiol status changes in various diseases and that thiol/disulfide homeostasis is very important in the pathogenesis of diseases. Therefore, determination of thiol/disulfide homeostasis can provide very important information about various physiological or pathological processes (7,8). For these reasons, the aim of this study is to reveal the thiol/disulfide homeostasis in cattle with trichophytosis and to determine the changes in MDA, CAT, SOD, and GSH-Px levels that occur due to the disease.

MATERIAL AND METHODS

The animal material of the study was obtained from livestock farms in Digor district of Kars province, of different breeds (8 Montofon crossbreeds and 22 Simmental crossbreeds), of both genders, aged between 5-18 months, 15 with trichophytosis and 15 control animals. Blood samples taken from the jugular vein of the animals into tubes with and without anticoagulant, ethylene diamine tetraacetic acid (EDTA), were centrifuged at 3000 rpm for 15 minutes to obtain serum and plasma. The disease was diagnosed according to clinical symptoms (grayish, round, raised, chalk dust-like lesions on the head and neck), and the final diagnosis was made microscopically. Skin scrapings were taken from the lesioned areas of the cattle with a sterile scalpel and processed with 10% KOH. Preparations were examined under the microscope and the observation of typical spores was evaluated as positive for trichophytosis.

MDA measurement was performed by the method reported by Yoshoiko et al. (9), CAT, SOD, GSH-Px (Cayman Chemical Co., USA), total thiol and native thiol (Rel Assay Diagnostics, Turkey) were measured colorimetrically (Epoch, Biotek, USA) using a commercial test kit. Disulfide = (Total thiol-Native thiol)/2, Disulfide/Total Thiol (%) = (Disulfide x 100)/Total thiol, Disulfide/Native Thiol (%) = (Disulfide x 100)/Native thiol, and Native Thiol/Total Thiol (%) = (Native thiol x 100)/Total thiol was calculated with the formulas (10).

Statistical Analysis

SPSS 20.0 package program was used to evaluate the study data. Independent Sample T-test was used to compare the groups.

RESULTS

In animals infected with trichophytosis that underwent clinical examination, chalk-like, round lesions were detected on various parts of the animal's body, especially on the head and neck. Hyphae were found in direct microscopic examination of the lesional skin scrapings of the animals used in the study.

It was determined that MDA levels (P<0.001) increased statistically significantly in cattle with trichophytosis. In addition CAT, SOD, (P<0.001), and GSH-Px (P<0.01) levels decreased statistically significantly compared to the control group (Table 1). It was determined that the biochemical parameters total thiol, native thiol (P<0.001) and native thiol/total thiol (P<0.01) levels in cattle with trichophytosis decreased statistically significantly compared to the control group. It was determined that disulfide/native thiol and disulfide/total thiol (P<0.01) levels increased statistically compared to the control group. Although disulfide level increased, it was statistically insignificant (P>0.05) (Table 2).

Table 1. Means and standard errors of lipid peroxidation (MDA) and some antioxidant (CAT, SOD, and GSH-Px) parameters in clinically healthy and trichophytosis cattle

Parameters	Control	Infected	P
Malondialdehyde (µmol/L)	2.93±0.11	7.31±0.29	P<0.001
Catalase (nmol/min/mL)	31.50±1.55	15.73±0.90	P<0.001
Superoxide Dismutase (U/mL)	226.78±6.14	107.59±3.71	P<0.001
Glutathione Peroxidase (nmol/min/mL)	0.40±0.04	0.24±0.02	P<0.01

Table 2. Means and standard errors of thiol/disulfide homeostasis parameters in clinically healthy and trichophytosis cattle

Parameters	Control	Infected	P
Total Thiol (µmol/L)	476.15±7.01	418.45±6.65	P<0.001
Natif Thiol (µmol/L)	378.67±6.65	297.94±4.81	P<0.001
Disulfide (µmol/L)	48.74±4.88	60.26±4.17	NS
Disulfide/Native Thiol (%)	13.17±1.50	20.57±1.78	P<0.01
Disulfide/Total Thiol (%)	10.12±0.93	14.28±0.85	P<0.01
Native Thiol/Total Thiol (%)	79.77±1.86	71.44±1.69	P<0.01

NS: Non Significant

DISCUSSION AND CONCLUSION

In the study, in animals with trichophytosis that underwent clinical examination, round lesions with a chalk appearance were detected in various parts of the body, especially in the head and neck region (11-13).

Tissue damage and inflammation in the organism activate many cells. Among the activated cells, phagocytic cells, which play an important role in body defense, cause oxidative stress due to excessive oxygen consumption while performing this task (14). In case of oxidative stress, excessively produced free radicals damage cell compounds (15). Studies have shown that bacterial/viral diseases such as brucellosis (16), sheeppox in sheep (17), hypodermosis (18,19), and pneumonia (20), it has been reported that the oxidant-antioxidant balance is disrupted and oxidative stress occurs. In study on cattle with trichophytosis, found a significant increase in MDA levels (15). Additionally, Bayyit and Merhan (21) reported in another study they conducted in cattle with dystocia that the MDA level was higher in cattle with dystocia. In this study, in parallel with the above studies, it was determined that the oxidant-antioxidant balance was disrupted and as a result, the MDA concentration increased when the control group and the trichophytosis group were compared. The reason for this is; It is thought that it may occur due to lipid peroxidation caused by stress in animals with trichophytosis and/or free radicals formed by phagocytes, which have an important role in host defense.

Antioxidants are classified according to their structures as enzymes/non-enzymes or according to their cell localization. The most important enzymatic defense systems against oxygen radicals are CAT, SOD, and GSH-Px (22). Important antioxidant enzymes such as CAT, SOD and GSH-Px neutralize free radicals (23). Kataria et al. (24) reported in a study conducted on cattle with brucellosis that serum CAT, SOD, glutathione reductase, monoamine oxidase, and peroxidase activities increased significantly in cattle with brucellosis. In another study, a significant increase in CAT, SOD, and GSH-Px activities was reported in cattle with trichophytosis (15). In a study conducted in dogs with trichophytosis, low SOD, and CAT activities were reported (25). In another study conducted in sheep and goats infected with *Haemonchus contortus*, it was reported that MDA increased and SOD which one of the antioxidant markers, decreased (26). Additionally, two different studies conducted in calves with trichophytosis reported that antioxidant activity decreased (27,28). Similarly, in this study, decreased antioxidant levels (SOD, CAT and GSH-Px) in cattle with trichophytosis may be due to the defense mechanism against lipid peroxidation.

Thiol/disulfide homeostasis has functions, antioxidant defense, apoptosis, regulation of enzyme functions, etc. Alteration of thiol/disulfide homeostasis plays a role in the pathogenesis of many diseases, especially chronic diseases (29). Thiols, which regulate intracellular redox status, are the first antioxidants consumed in the oxidative environment. In addition, plasma values are a good indicator of tissue redox potentials (30). Thiols, which are reported to be formed by dendritic cells in the skin, have an important role in oxidative stress and disease pathogenesis (29). In human medicine, changes in thiol-disulfide concentrations have been reported in many inflammatory diseases (31,32). In veterinary medicine, it has been reported that there is a decrease in total thiol and native thiol levels during dehorning of calves with hot cautery, and there is no significant difference in disulfide level (33). Also conducted in sheep with toxoplasmosis, it was reported that total thiol and native thiol levels were significantly lower, and disulfide, disulfide/native thiol and disulfide/total thiol were higher (34). In the study, it was determined that total thiol, native thiol, and native thiol/total thiol levels decreased statistically significantly compared to the control group. It was determined that disulfide/native thiol and disulfide/total thiol levels increased statistically compared to the control group. Although the disulfide level increased, it was statistically insignificant. The levels of thiol/disulfide homeostasis parameters in the study are compatible with the above studies, and we believe that this may be due to the formation of oxidative stress and the organism's consumption of thiols to combat oxidative stress against the increasing oxidative stress level.

As a result, the findings obtained from the study indicate that trichophytosis causes oxidative stress in cattle, and it is thought that the use of oxidative stress markers, especially thiol/disulfide homeostasis markers, will contribute to the pathogenesis of trichophytosis and more detailed studies should be conducted on this subject.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

OM took part in the study planning and LB sample collection. The writing of the study and final checks were carried out with the contributions of all authors.

ETHICAL STATEMENT

This study was started after receiving the ethics committee approval of Kafkas University Animal Experiments Local Ethics Committee dated 24.03.2023 and coded 2023/029.

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RESEARCH ARTICLE

Can Serum Amyloid A Levels be Used in the Diagnosis of SIRS in Cats with Pyometra?

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Abstract

Serum amyloid A (SAA) level increases in conditions such as infection, tissue damage and trauma and is not specific to a disease but provides information about the presence and severity of inflammation. As a life-threating conditions pyometra usually causes systemic inflammatory response syndrome (SIRS) and therefore may lead an increase in SAA levels. The present study was designed to determine SAA levels in cats with pyometra that developed SIRS, and to demonstrate the diagnostic value of SAA by comparing it with hematological and biochemical parameters as well as SIRS criteria. For this purpose, data were used from cats with open-cervix pyometra (OP, n=6) and closed-cervix pyometra (CP, n=6), which were identified as having developed SIRS and were admitted to hospital as well as from healthy cats brought in for routine neutering, which were identified to be in the diestrus phase of the sexual cycle (DE, n=6). Cats with pyometra had higher SAA levels and leukocytosis compared to cats in the DE group (P= 0.002 and P= 0.000, respectively). The highest SAA level was detected in the CP group (168.6 µg/ml) and this level is statistically significant compared to the other groups (P= 0.028). While there was no correlation between SAA levels and SIRS criteria, SAA levels were negatively correlated with both AST (P= 0.045, rs=-0.478) and GGT (P= 0.019, rs= -0.548). In the study, it was determined that sepsis and SIRS progressed with different symptoms in cats and SIRS criteria were less specific in cats. It was concluded that SAA levels may be an important marker in closed-cervix pyometra cases. We think that the study data are promising but further and comprehensive studies are needed considering the number of patients included in the study.

Key Words: Cat, pyometra, serum amyloid A, systemic inflammatory response syndrome

Serum amiloid A düzeyleri piyometralı kedilerde SİRS tanısında kullanılabilir mi?

Öz

Serum amiloid A (SAA) düzeyi enfeksiyon, doku hasarı ve travma gibi durumlarda artar ve bir hastalığa özgü olmamakla birlikte inflamasyonun varlığı ve şiddeti hakkında bilgi verir. Hayatı tehdit eden bir durum olan pyometra genellikle sistemik inflamatuar yanıt sendromuna (SIRS) neden olur ve bu nedenle SAA seviyelerinde artışa yol açabilir. Bu çalışma, SIRS gelişen piyometralı kedilerde SAA düzeylerini belirlemek ve SAA'nın tanısal değerini hematolojik ve biyokimyasal parametrelerin yanı sıra SIRS kriterleriyle karşılaştırarak göstermek amacıyla tasarlanmıştır. Bu amaçla SIRS geliştirdiği tespit edilen ve hastanemize getirilen açık serviks pyometralı (OP, n=6) ve kapalı serviks pyometralı (CP, n=6) kedilerin yanı sıra rutin kısırlaştırma için getirilen ve seksüel siklusun diöstrus döneminde olduğu tespit edilen sağlıklı kedilerden (DE, n=6) elde edilen veriler kullanılmıştır. Piyometralı kediler, DE grubundaki kedilere kıyasla daha yüksek SAA seviyelerine ve lökositoza sahipti (sırasıyla P= 0.002 ve P= 0.000). En yüksek SAA seviyesi CP grubunda tespit edilmiştir (168.6 µg/ml) ve bu seviye diğer gruplardakine göre istatistiksel olarak anlamlı bulunmuştur (P= 0.028). SAA düzeyleri ile SIRS kriterleri arasında korelasyon saptanmazken, SAA düzeyleri hem AST (P= 0.045, rs= -0.478) hem de GGT (P= 0.019 rs= -0.548) ile negatif korelasyon göstermiştir. Çalışmada sepsis ve SIRS'in kedilerde farklı semptomlarla ilerlediği, SIRS kriterlerinin kedilerde daha az spesifik olduğu tespit edilmiştir. Serum amilod A'nın CP olgularında önemli bir belirteç olabileceği sonucuna varılmıştır. Çalışma verilerinin umut verici olduğunu ancak çalışmaya dahil edilen hasta sayısı göz önünde bulundurulduğunda daha ileri ve kapsamlı çalışmalara ihtiyaç olduğunu düşünmekteyiz.

Anahtar Kelimeler: Kedi, piyometra, serum amiloid A, sistemik yangısal cevap sendromu

INTRODUCTION

Pyometra, which implies the acute or chronic suppurative infection of the uterus, occurs in the luteal period and is life-threatening in cats and dogs due to the general deterioration and systemic infection it causes (1). The repeated exposure of the reproductive system to hormonal stimulation has an important place in the etiology of the disease. Estrogen (E2) exposure during the follicular period prepares the uterus for the effect of progesterone (P4). Progesterone stimulates endometrial development and glandular secretion and suppresses myometrial contractions and uterine immunity. Eventually, this process prevents the uterus from eliminating bacterial contamination and creates favorable conditions for bacterial growth and the development of pyometra (2). This process may either occur as a result of repeated exposure to endogenous hormones or be triggered by exogenously administered E2 and P4 (3,4).

The clinical course of the disease varies, depending on the duration of its development, the presence of an irregular cycle, the effects of exogenous and endogenous hormones, the severity of liver and kidney dysfunction, the presence of bacterial infection, the patency of the cervix, and the patient's immune response (5,6). Usually, closed-cervix pyometra (CP) cases are more severe than open-cervix pyometra (OP) cases (7). However, the general clinical findings are similar in both types of pyometra. Symptoms such as purulent vaginal discharge (if the cervix is open), expansion of the uterine volume, polyuria, polydipsia, lethargy, anorexia, vomiting - which are actually not specific to pyometra - are observed (3).

Diagnosis can be made based on anamnesis, physical and gynecological examinations, laboratory findings, radiography, ultrasonography (USG), and the type of the genital discharge in cases, where the cervix is open (8). Recently, acute-phase proteins (APP) have also started to be evaluated in cats and dogs for diagnostic purposes (9,10).

Acute-phase proteins are produced by the liver against trauma, viral and bacterial infections, and inflammatory conditions. During the inflammatory process, species specific increases (positive APP) and decreases (negative APP) occur in some APP levels. For example, the APP, which increases most rapidly and at the highest level is the C-reactive protein (CRP) in the dog, and serum amyloid A (SAA) in the cat. This means that SAA is a positive acute-phase protein in the cat. Since SAA increases in various feline diseases, it can be used as a marker to determine the presence and severity of systemic inflammation and its prognosis in cats (11). Studies show that there is a correlation between APP production and the clinical signs of systemic inflammatory response (9,10).

Systemic inflammatory response syndrome (SIRS) is the body's clinical, hematological and immunological response to infectious and non-infectious factors. Toxins produced by bacteria involved in pyometra, after entering the systemic circulation, cause the development of sepsis and SIRS, which result in multiple organ failure and increased mortality rates (12). In veterinary medicine, clinical and laboratory findings adapted from human medicine, such as hyperthermia/hypothermia, tachycardia/bradycardia, tachypnea/bradypnea, leukocytosis/leukopenia and band neutrophils, are used as SIRS diagnostic criteria. These criteria show a significant correlation with the prognosis of the disease (13). Therefore, patients with sepsis or SIRS should be regularly assessed for these parameters (14). Furthermore, Yazlık et al. (15) suggested that the results of hematological and biochemical analyses could also be used as markers in dogs with pyometra that develop SIRS, especially in cases of CP.

Today, the diagnostic criteria established for dogs continue to be used for the diagnosis of non-species-specific diseases in cats. However, there are very important metabolic, enzymatic and endocrinological differences between cats and dogs. For example, as mentioned above, there are various species-specific APPs, and evaluating the CRP instead of SAA in cats would yield irrelevant results and mislead the physician (9). Furthermore, while pyometra occurs after the age of six in dogs not administered with any exogenous hormone, it may occur at any age in cats (1). Therefore, there is a need to detect the differences of cats. In this context, the present study was designed to determine SAA levels in cats with pyometra that developed SIRS, and to demonstrate the diagnostic value of SAA measurement by comparing it with hematological and biochemical parameters as well as SIRS criteria.

MATERIAL AND METHODS

In the present study, data were used from cats with open- and closed-cervix pyometra, which were identified as having developed SIRS and were admitted to our clinic, as well as from healthy cats brought in for routine neutering, which were identified to be in the diestrus phase of the sexual cycle.

Cats, which were admitted to the clinic with signs of vaginal discharge, anorexia, polyuria, polydipsia, and lethargy, diagnosed with pyometra by USG, vaginal cytology, and blood analysis, and determined to have developed SIRS, were included in the open-cervix pyometra group (OP, n=6). Cats showing the same clinical signs, except for vaginal discharge, were included in the closed-cervix pyometra group (CP, n=6). Cats, which were brought in for neutering, found to be healthy upon clinical examination, and identified to be in the diestrus phase of the sexual cycle based on gynecological examination and operational findings, were included in the diestrus group (DE, n=6).

In this study, animals with SIRS were identified according to the diagnostic criteria reported (16). Accordingly, the following reference intervals were used: <6, >20 $\times 10^9$/L for WBC,<37.2°C,>39.4°C for body temperature, >40 breaths/min for respiratory rate, and <140, >220 bpm for pulse. Cats, which met at least three of these four criteria, were considered to be SIRS-positive (16).

Animals with no history of exogenous hormone administration at any time, no treatment before being brought to our clinic, and no acute or chronic systemic and/or metabolic disorders other than pyometra, and without any organ dysfunction caused by pyometra were included in the study.

Routine procedures were performed on the cats. Accordingly, after anamnesis was performed, physical examinations were conducted, and the findings were recorded. The report of Thomovsky et al. (17) was used as a reference for these examinations, which were part of the routine evaluation.

Ultrasonographic examinations were performed transabdominally with an 5-7.5 MHz probe (Mindray VETUS 9,

Hasvet). During USG, a preliminary diagnosis of suspected pyometra was made by observing an anechoic content with a snowstorm appearance in the distended lumen, which expanded cranially and dorsally from the bladder. Cats diagnosed with pyometra were assigned to the OP group if they had vaginal discharge, the CP group if they had no vaginal discharge. Cats with no pathology in the uterus were assigned to the DE group. In all groups, uterine diameter measurements were performed with USG, as described by Gatel et al. (18), and the results were recorded.

The definitive classification of the animals into the study groups was based on the macroscopic and microbiological evaluation of the reproductive tissue after surgery. Accordingly, cats with suspected pyometra were definitively classified into the pyometra group if bacterial growth was found in the microbiological evaluation of the uterus. Cats with no pathology in the uterus and with corpus luteum identified in the ovary during the macroscopic evaluation were classified into the DE group.

For hemogram, serum biochemistry, and SAA measurement, blood samples were taken from the antebrachial cephalic vein into 10-ml serum tubes and EDTA-coated tubes before surgery (Mindray BC-60, Hasvet). In this study, only WBC (reference interval: 6-20 x10^9/L) were counted as the WBC count is included among the SIRS criteria. After the samples were centrifuged at 5000 rpm for 10 minutes, blood urea nitrogen (BUN; reference interval: 19-34 mg/dl), creatinine (CREA; reference interval: 0.8-2.1 mg/dl), alanine aminotransferase (ALT; reference interval: 28-109 U/L), alkaline phosphatase (ALP; reference interval: 11-49 U/L), aspartate amino transferase (AST; reference interval: 17-46 U/L) and gamma glutamyl transferase (GGT; reference interval: 0-2 U/L) levels were measured in the serum samples (Mindray BS-120Vet, Hasvet). The SAA levels were determined in the sera with the aid of an automatic veterinary hormone analysis and immunity testing device (Vcheck V200, Hasvet) using Feline SAA 3.0 test kits, which were 97% compatible with ELISA. Evaluations were performed according to the prospectus data, such that <5 µg/ml was accepted normal; 5-10 µg/ml raised a suspicion of systemic inflammation; and >10 µg/ml indicated the presence of systemic inflammation.

Statistical analysis

Statistical analyses were performed with the IBM SPSS 25.0 package program. The normality distribution of the study data was determined by the Shapiro-Wilk test. When the normal distribution of the data was verified, a one-way ANOVA test was used for the comparison of the groups, and when the data did not show a normal distribution, the Kruskal-Wallis and Mann-Whitney U tests were employed. The correlations between SAA and the other parameters were determined by Spearman's correlation coefficient.

RESULTS

No statistical differences were found between the mean ages and body weights of the study groups (Table 1). The clinical symptoms detected in the cats with pyometra and the severity of these symptoms are presented in (Table 2).

Table 1. Values of mean age and body weight of the groups

Group	Age (year; X±SE)	Body weight (kg; X±SE)
OP	2.8 ± 2.7	3.4 ± 0.2
CP	2.0 ± 1.3	3.3 ± 0.1
DE	1.3 ± 0.4	3.2 ± 0.1
P value	0.501	0.815

Data are presented as X ± SE.OP; Open-cervix pyometra, CP; Closed-cervix pyometra, DE; Diestrus.

Table 2. Clinical symptoms and its rates identified in cats with pyometra

Clinical Symptoms	OP (%; n)	OP (%; n)	Total (%; n)
Depression/Lethargy	0.0 (0/6)	100 (6/6)	50.0 (6/12)
Anorexia	66.7 (4/6)	100 (6/6)	83.3 (10/12)
Vomiting	0.0 (0/6)	16.7 (1/6)	8.3 (1/12)
Color change in mucous membranes	33.3 (2/6)	66.7 (4/6)	33.3 (6/12)
Prolongation of CRT	33.3 (2/6)	66.7 (4/6)	50.0 (6/12)
Hyperthermia	50.0 (3/6)	66.7 (4/6)	58.3 (7/12)
Dehydration	33.3 (2/6)	50.0 (3/6)	41.7 (5/12)
Abdominal pain	16.7 (1/6)	50.0 (3/6)	33.3 (4/12)
Polydipsia	0.0 (0/6)	50.0 (3/6)	25.0 (3/12)
Polyuria	0.0 (0/6)	50.0 (3/6)	25.0 (3/12)

Data were presented as % and "n" show the number of cats. OP; Open-cervix pyometra, CP; Closed-cervix pyometra, DE; Diestrus, CRT; Capillary refill time.

Data on the hematological and serum biochemical parameters, SAA levels and SIRS criteria are presented in (Table 3). The cats with pyometra had higher SAA levels and leukocytosis, when compared to cats in the DE group (P= 0.002 and P= 0.000, respectively). Also, bradycardia was more common than tachycardia in the cats with pyometra.

Table 3. Rates of hematological, serum biochemical and SAA findings of the groups

Parameters	Reference interval	OP (%; n)	Groups CP (%; n)	DE (%; n)	P value
High SAA levels	>5 µg[a]	50 (3/6)	100 (6/6)	0 (0/6)	0.002*
Leukocytosis	>20×10^9/L[b]	100 (6/6)	100 (6/6)	0 (0/6)	0.000**
Hyperthermia	>39.4°C[b]	50 (3/6)	66.7 (4/6)	0 (0/6)	0.207
Tachypnea	>40 bpm[b]	100 (6/6)	83.3 (5/6)	50 (3/6)	0.301
Bradycardia	<140 bpm[b]	66.7 (4/6)	83.3 (5/6)	0 (0/6)	0.04*
Tachycardia	>220 bpm[b]	0 (0/6)	16.7 (1/6)	0 (0/6)	-
Higher BUN levels	>34 mg/dl[c]	50 (3/6)	66.7 (4/6)	33.3 (2/6)	0.513
High GGT activity	> 2 U/L[c]	33.3 (2/6)	50 (3/6)	0 (0/6)	0.059
High ALT activity	> 109 U/L[c]	33.3 (2/6)	0 (0/6)	0 (0/6)	0.073
Low ALT activity	< 28 U/L[c]	0 (0/6)	33.3 (2/6)	0 (0/6)	-
High AST activity	> 48 U/L[c]	33.3 (2/6)	0 (0/6)	0 (0/6)	0.073
Low AST activity	< 17 U/L[c]	0 (0/6)	33.3 (2/6)	0 (0/6)	-
High ALP activity	> 49 U/L[c]	16.7 (1/6)	16.7 (1/6)	33.3 (2/6)	0.575
Low ALP activity	< 11 U/L[c]	16.7 (1/6)	0 (0/6)	0 (0/6)	-

Data were presented as % and "n" show the number of cats.OP; Open-cervix pyometra, CP; Closed-cervix pyometra, DE; Diestrus, SAA; Serum Amiloid A, BUN; Blood urea nitrogen, GGT; Gamma glutamyl transferase, ALT; Alanine aminotransferase, AST; aspartate amino transferase, ALP; alkaline phosphatase.[a] : Prospectus of Feline SAA 3.0 test kits, [b]: (16), [c]: Vet Cornell Refence Intervals, *; p<0.05, **p<0.001 .

The mean data for the SIRS criteria and SAA levels are presented in Table 4. Statistical significance was determined only between WBC, pulse and SAA levels among the parameters examined. The mean body temperature of the cats with CP was higher than the reference interval values, but this difference was statistically insignificant. The mean respiratory rates were above the reference values in all groups. Although the respiratory rates were higher in the pyometra groups than in the control group, no statistically significant difference was detected. The highest SAA level was determined in the CP group (168.6 µg/ml), and this level was statistically significant compared to the levels of both the OP and DE groups (P= 0.028). On the other hand, no significant difference was found between the SAA levels of the OP and DE groups (Table 4).

Table 4. Mean values obtained for SIRS criteria and SAA levels in the study

Parameters	Reference interval	Groups			P value
		OP	CP	DE	
WBC	<6 ; >20 x10^9/L	36.0 ± 5.3[a]	39.1 ± 6.4[a]	10.0 ± 1.4[b]	0.001**
Body temperature	<37.2; >39.4 °C	39.1 ± 0.2	39.4 ± 0.5	38.6 ± 0.2	0.216
Respiratory rates	>40 breaths/min	55.0 ± 3.9	58.7 ± 9.5	49.3 ± 9.4	0.714
Pulse	<140; >220 bpm	130.0 ± 7.7[a]	129.2 ± 18.5[a]	166.3 ± 8.3[b]	0.028*
SAA	0-5 µg/ml	18.2 ± 8.7[a]	168.6 ± 17.9[b]	5.0 ± 0.0[a]	0.028*

Data are presented as X ± SE. Different letters[(a, b)] in the same row indicates statistically significant. OP; Open-cervix pyometra, CP; Closed-cervix pyometra, DE; Diestrus, WBC; white blood cell, SAA; Serum Amiloid A, *; p<0.05, **p<0.001.

No statistically significant difference was detected in the serum biochemical parameters, except for AST (P= 0.040). Although the GGT results were higher than the reference interval values, no statistical significance was determined in the CP group (Table 5).

Table 5. Mean results of serum biochemical test

Parameters	Reference interval	Groups			P value
		OP	CP	DE	
BUN	19-34 mg/dl	38.7 ± 4.3	36.2 ± 5.2	31.1 ± 3.8	0.487
CREA	0.8 - 2.1 mg/dl	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.189
ALT	28 -109 U/L	85.7 ± 18.8	37.9 ± 9.5	67.5 ± 9.6	0.066
AST	17- 48 U/L	38.2 ± 5.1[a]	20.6 ± 5.7[b]	26.9 ± 0.9	0.040
ALP	11- 49 U/L	36.7 ± 9.5	30.9 ± 4.9	43.5 ± 3.6	0.414
GGT	0- 2 U/L	2.8 ± 1.4	3.2 ± 1.3	1.0 ± 0.0	0.353

Data are presented as X ± SE. Different letters[(a, b)] in the same row indicates statistically significant. OP; Open-cervix pyometra, CP; Closed-cervix pyometra, DE; Diestrus, BUN; Blood urea nitrogen, CREA; Creatinine, GGT; Gamma glutamyl transferase, ALT; Alanine aminotransferase, AST; aspartate amino transferase, ALP; alkaline phosphatase.

While the CP group had the largest uterine diameter, the DE group had the smallest diameter (1.6 ± 0.2 cm vs. 0.4 ± 0.1 cm). It was found that the uterine diameters were significantly larger in the CP group than in the OP (1.2 ± 0.3 cm) and DE (P= 0.023 and P= 0.001, respectively) groups, and were also higher in the OP group than in the DE group (P=0.01).

While no correlation was determined between the SAA levels and SIRS criteria (Table 6), the SAA levels were negatively correlated with both AST (P= 0.045, $r_{s=}$-0.478) and GGT (P= 0.019, $r_{s=}$ -0.548) (Table 7).

Table 6. Correlation between SAA level and SIRS criteria. r_s; Spearman correlation coefficient

		WBC	Body temperature	Respiratory rate	Pulse
SAA	P value	0.057	0.138	0.281	0.065
	rs	0.456	0.364	0.269	-0.444

SAA; Serum Amiloid A, WBC; White blood cells.

Table 7. Correlation between SAA level and serum biochemical results. r_s; Spearman correlation coefficient

		BUN	CREA	ALT	AST	ALP	GGT
SAA	P value	0.451	0.523	0.656	0.045	0.141	0.019
	rs	0.190	-0.161	0.113	-0.478	-0.361	-0.548

SAA; Serum Amiloid A, BUN; Blood urea nitrogen, CREA; Creatinine, GGT; Gamma glutamyl transferase, ALT; Alanine aminotransferase, AST; aspartate amino transferase, ALP; alkaline phosphatase.

DISCUSSION AND CONCLUSION

Despite being a life-threatening disease, the number of studies on the prevalence, characteristics and prognosis of pyometra in cats is much less than that in dogs, and therefore, pyometra in cats is assessed based on the data available for dogs (1). The incidence of pyometra in dogs is much higher than that in cats, such that it is >20% in ten-year-old dogs and 2% in 13-year-old cats. However, contrary to incidence, death from pyometra is higher in cats than in dogs (6% vs 4%), which means that pyometra is more fatal in cats than in dogs. Although clinical findings are similar to those observed in dogs, they are often nonspecific to cats, and therefore, pyometra is difficult to diagnose in cats (1,19). For that reason, it is necessary to determine the species-specific changes caused by pyometra in the cat, and this issue is open to study.

In the present study, none of the patients died and all survived after being treated. Therefore, an evaluation for the correlation between the SAA levels and survival rate or time could not be performed. On the other hand, the physical examination findings demonstrated that 50% to 55% of the cases presented with symptoms of depression/lethargy, prolongation of the CRT, loss of appetite, and color change in the mucous membranes, which can be observed in various diseases. It has been reported that the clinical findings of pyometra in cats are non-specific (1,19). However, the significant advantages offered by USG in the diagnosis of pyometra eliminated the disadvantage of the non-specific clinical findings.

As is the case with many bacterial infections, as pyometra progresses, it may lead to sepsis, endotoxic shock or SIRS, which can result in death (20). Although the definitions of sepsis and SIRS are different, they are closely related to each other: SIRS is found in cases with sepsis, but sepsis does not occur in every case with SIRS (21). The mortality rate of sepsis varies between 29% and 79% in cats (22-24). Therefore, diagnosing sepsis or severe sepsis in feline pyometra cases, based on clinical findings, is important for determining the extent of systemic disorder and managing the clinical course (25). However, although proven to be sensitive in both dogs (26) and cats (27), SIRS diagnostic criteria are not specific to these species and lack a consensus (25). This is because hyperthermia, tachycardia and tachypnea, which are among the SIRS criteria, have many causes other than systemic inflammation, such as anxiety and pain, especially in cats (28). As a matter of fact, in the present study, no statistically significant difference was determined between the body temperatures and respiratory rates of the groups. According to our results, these parameters increased only numerically in the pyometra groups.

In the hyperdynamic phase of sepsis, symptoms such as tachycardia, hyperthermia, and hyperemic mucosa develop due to peripheral vasodilation. As sepsis progresses, it evolves into the hypodynamic phase and presents with symptoms such as vasoconstriction, tachycardia, pale mucous membranes, prolonged CRT, and weakening of the pulse (25). However, in cats, this process progresses differently, and the hyperdynamic phase does not develop as it does in other species (25,29). In affected cats, although many of the classic clinical signs of sepsis in other animal species are observed, additional signs such as bradycardia, hypothermia, and abdominal pain also develop (22,24,25).

The results obtained for hyperthermia (58.3%; 7/12) and tachypnea (91.7%; 11/12) in the present study confirm with the changes observed during the hyperdynamic period of sepsis. These results agree with previous reports by DeClue et al. (25) and Brady et al. (30) indicating 42% and 59% of hypothermia, respectively. Brady et al. (25) also reported to have detected hyperthermia in 35% of cats with sepsis. These differences could be due to variances in the reference intervals used. In the study of Brady et al. (25), the mean body temperature was reported as 37.2 ± 2.5°C, and this value corresponds to the lower reference limit (<37.2 and >39.4°C) used in this study.

In addition, a total of 75% (9/12) of the cats were bradycardic and incompatible with the hyperdynamic phase (29). While similar data have been previously reported, indicating bradycardia in 66% of cats (25), the pathophysiology of this species-specific change in cats is unknown (30). Hypothermia may be a cause of bradycardia, but we cannot establish such a relationship since hypothermia did not occur in any of the animals in this study (31). On the other hand, much older studies have suggested that cats may not develop tachycardia in response to hypotension due to the simultaneous baroreceptor stimulation of the vagal and sympathetic fibers (25,32).

In cases of sepsis and SIRS, an increase occurs in inflammatory mediators such as pro-inflammatory cytokines, T cells and macrophages, and the activation of WBC during this increase plays a key role in the pathogenesis of SIRS (29). The most characteristic changes observed in the hemogram of cats and dogs with pyometra are a marked increase in WBC accompanied by an inflammatory leukogram and a regenerative left shift in WBC (1,13). Therefore, the leukocytosis observed in 100% of the pyometra cases in this study is an expected finding. The mean WBC values of the cats with pyometra in this study were higher than those reported in the studies of (25,30). These researchers reported mean WBC counts of $15x10^9$/L and $12.3x10^9$/L, respectively. However, they evaluated many different SIRS or septicemia cases such as pyothorax and septic peritonitis in their studies. Our results were obtained only from pyometra cases, and this could explain our higher results. On the other hand, it has been determined that WBC levels are higher in CP cases compared to OP cases in cats. It has been suggested that this is due to the drainage of the purulent content from the uterus through the open cervix, causing a milder level of septicemia compared to the closed cervix (7). According to these reports, there is a correlation between the uterine lumen being filled with pus and WBC levels, and this is expected to be more intense in CP in dogs (33,34). In our study, uterine diameter measurements how similarity to these data, but indicate no correlation. This may be because the uterine lumen of cats is smaller than that of dogs, although there are very large differences in size and uterine size between dog breeds, cases are detected and intervened faster in cats, and the cat's immune system has a different working mechanism than that of dogs. An example of this difference is the rapid increase in CRP levels in dogs and SAA levels in cats during the acute phase response (11).

There are differences between the evaluation of SIRS in cats and dogs. Since cats have their own pathophysiology, while two criteria are sufficient for the diagnosis of SIRS in dogs, three criteria are required in cats (29). Therefore, in this study, three of the four positive criteria were investigated. However, no specific findings were obtained, which seems to be in agreement with previous reports suggesting that SIRS criteria are not specific to cats and dogs (25-28). Besides, the fact that there was no correlation between SAA and body temperature, respiration rate and pulse in this study can be considered as proof of SIRS criteria not being specific to cats.

Acute-phase proteins are more sensitive markers than WBC in the early diagnosis of inflammation. During the inflammatory process, changes occur in both APP and WBC levels (35). Proteins that increase/decrease in the acute-phase response have been previously investigated in mares, cows and dogs with pyometra (36,37). It has been stated that SAA levels increase during inflammatory processes in many animal species, especially in reproductive tissues, and therefore can be used as a biomarker of inflammatory processes in reproductive tissues (11,38).

In the present study, it was determined that SAA levels had increased in the pyometra groups, but not in the control group, and these results are in agreement with previous reports (39,40). Similar results have been reported in previous studies in dogs (12,41), and it has been concluded that SAA could be used as a marker in dogs with pyometra (41). However, in the aforementioned studies, pyometra was evaluated without being classified under open- and closed-cervix cases. Given that clinical findings are more severe in CP (7), it is important to make the distinction between OP and CP cases. The statistical difference (P= 0.028) determined between the SAA levels of the two pyometra groups in this study confirm the importance of this distinction. Vilhena et al. (40) determined a mean SAA level of 63.6 µg/ml in their study, which included 96% of cats with OP. This level is much higher than the results we obtained in the OP group (18.2 µg/ml). The difference between the results of the two studies could be related to the number of animals used (6 vs 23) or the age of the animals. While this study included young animals, in the study of Vilhena et al. (40), most of the animals were old. It has been determined that the age-related increased incidence of subclinical disease may lead to increased SAA levels in older cats (42). Similar data were reported in dogs by Jitpean et al. (41). These researchers also determined that SAA levels were significantly higher in septic cases. On the other hand, in a pyometra study conducted without evaluating cervical patency, Yuki et al. (43) determined the mean SAA level as 154.8 µg/ml, which is similar to the mean SAA level of 168.6 µg/ml we determined in the CP group. Considering that all the cats with pyometra in this study were SIRS-positive, despite clinically exhibiting three out of the four positive criteria of SIRS, the difference between the SAA levels of the groups suggests that assessing SAA levels together with SIRS criteria would be beneficial in understanding the severity of the condition.

In human medicine, WBC and the CRP are important in detecting the presence of serious infection and determining the type of treatment required (44). On the other hand, although the use of APPs alongside WBC is beneficial for evaluating the inflammatory state in both humans and animals, APPs are reported to be more sensitive than leukocyte counts in detecting infection and inflammation (39,45). Our results agree with the aforementioned studies. In the present study, while no statistical difference was determined between the WBC levels of the pyometra groups, a difference was found between their SAA levels. We consider this to be an important finding in evaluating the clinical significance of OP or CP. Additionally, upon evaluating the relationship between SAA and WBC, although we thought there might be a tendency towards a positive correlation, it was determined that there was no correlation between these parameters (P= 0.057).

In cases of pyometra, sepsis or SIRS, the kidney and liver are the two most rapidly affected organs. There was no difference between the groups for the BUN and CREA values we measured in this study. The fact that these values were normal in the kidneys, which are the first organs affected by pyometra, indicates that kidney function changes had not started yet. The low rates of polyuria and polydipsia (25%) we detected confirm this data. This is because, in pyometra, the antigen-antibody complex affects the glomeruli and causes glomerulonephritis, the first clinical findings of which are polyuria and polydipsia (46,47). However, it has been reported that azotemia was detected in 77.8% of dogs with pyometra and high CREA levels were detected in a percentage of 42.2% (48). Jitpean et al. (41) reported that BUN and CREA levels in septic and nonseptic dogs with pyometra remained within the reference limits and there was no statistical difference between the two groups. DeClue et al. (30) showed that BUN levels increased in 26.3% and CREA levels increased in 10.5% of septic cats.

High AST and ALT activities may be detected in cats and dogs with sepsis (13,30). DeClue et al. (30) found an increase in ALT activity in 36.8% and ALP activity in 10.5% of cats with sepsis. Besides, they also reported that ALT activity was significantly increased in septic cats compared to healthy cats (30). The reason for this increase may be stress and damage to tissues such as the heart, liver and bone marrow due to sepsis (49). In addition, there are also reports suggesting that increased ALP activity in pyometra may result from intrahepatic cholestasis (50). However, as liver function parameters may not always increase in cases of sepsis or pyometra. In this study, GGT activity increased in 41.7% (5/12), AST and ALT activity increased in 33.3% (2/6), and ALP activity increased in 33.3% (2/6) of the cats in the pyometra groups. However, despite these increases and decreases, the mean values of the liver function parameters remained within the reference ranges. This may be due to liver enzyme activity in cats being faster than that of the dog. The half-lives of ALT, AST and ALP are 3.5 hours, 1.5 hours and 6 hours, respectively, in cats, and 60 hours, 12 hours and 66 hours, respectively, in dogs. The difference between these half-lives indicates that hepatic enzymes in cats will not increase as much as in dogs in cholestasis (51). In other words, ALT and AST levels, which increase in the serum following acute injury, will decrease very rapidly within hours after a significant increase due to differences in serum and the cellular localizations of the enzymes (52). Thus, we consider that the negative correlation detected in this study between SAA and AST or SAA and GGT has no clinical significance, as we are suspicious of the AST and GGT levels measured in the cats due to the half-lives of these enzymes.

When evaluating the results of the present study, we observed that there was 100% leukocytosis in all cases in the pyometra groups. Furthermore, tachypnea was detected at a level of 100% in the OP group and 83.3% in the CP group. Moreover, bradycardia was detected at a level of 66.7% in the OP group and 83.3% in the CP group, and finally, hyperthermia was determined at a level of 50% in the OP group and 66.7% in the CP group. Statistical significance was detected only for the WBC and pulse parameters, when compared to the control group. On the other hand, while SAA levels increased in only 50% of the animals in the OP group, they increased in all the cats in the CP group.

The serum biochemical test results demonstrated that the percentages of animals with increased/decreased levels of BUN, CREA, GGT, ALT, AST, and ALP did not differ significantly between the groups. The results of this study, we think that SAA levels could be used alongside SIRS criteria in cats, and can be use of as a marker especially in cases of CP, and even be evaluated before other SIRS criteria. We consider the study data to be promising but considering the number of patients included in the study, further and more comprehensive studies are needed.

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CONFLICT OF INTEREST

There is no conflict of interest to be declared by the authors.

ETHICAL STATEMENT

The procedures followed in the present study presented are part of routine veterinary practice and constitute a routine treatment/intervention to treat cats with pyometra. Therefore, since the presented study falls within the scope of "Clinical applications for diagnosis and treatment purposes", which is described in the first article of the "Regulation on the working procedures and principles of animal experiments ethics committees" said regulation, the HADYEK permission of the Local Ethics Committee for Animal Experiments (HADYEK) is was not required (Article 8, paragraph (k) of the "Regulation on the working procedures and principles of animal experiments ethics committees, February 15, 2014 / 28914).

AUTHOR CONTRIBUTIONS

Idea/Concept: SSA, AGA, Eİ
Design: SSA, MF, FB
Supervision/Consultancy: AGA, VF, FB, İİ
Data Collection and/or Processing: SSA, MF, AGA, Eİ
Analysis and/or Interpretation: AGA, VF, FB, İİ
Source Search: AGA, VF, FB, İİ, Eİ
Manuscript Writing: SSA, MF, AGA, VF, FB, İİ, Eİ
Critical Review: SSA, MF

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RESEARCH ARTICLE

Determination of Oxidative Stress and Sialic Acid Levels in Cattle Infected with Hydatid Cyst

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Abstract

The aim of the study was to determine oxidative stress and sialic acid levels in cattle with hydatid cysts. In the study, 15 hydatid cyst infected and 15 healthy cattle were used. Total sialic acid (TSA), lipid-bound sialic acid (LBSA), protein-bound sialic acid (PBSA), nitric oxide (NO) and malondialdehyde (MDA) levels were determined colorimetrically in the animals. It was determined that TSA, LBSA, NO, and MDA levels were increased in the cattle with hydatid cysts compared to the control group, while PBSA level increased but was statistically insignificant. In conclusion, it was concluded that oxidative stress occurs in the cattle infected with hydatid cyst and TSA can be used as an indicator in the diagnosis of the disease.

Key Words: Cattle, hydatid cyst, oxidative stress, total sialic acid

Hidatik Kist ile Enfekte Sığırlarda Oksidatif Stres ve Siyalik Asit Düzeylerinin Belirlenmesi

Öz

Çalışmanın amacı hidatik kistli sığırlarda oksidatif stres ve siyalik asit seviyelerinin belirlenmesidir. Çalışmada, 15 adet kist hidatik ile enfekte ve 15 adet sağlıklı sığır kullanıldı. Hayvanlarda total siyalik asit (TSA), lipid bağlı siyalik asit (LBSA), proteine bağlı siyalik asit (PBSA), nitrik oksit (NO) ve malondialdehit (MDA) düzeyi kolorimetrik olarak tespit edildi. Hidatik kistli sığırlarda TSA, LBSA, NO ve MDA düzeylerinin kontrol grubuna göre yükseldiği, PBSA düzeyinin ise artmakla beraber istatistiksel olarak anlamsız olduğu belirlendi. Sonuç olarak, hidatik kist ile enfekte sığırlarda oksidatif stresin oluştuğu ve hastalığın teşhisinde TSA'nın bir indikatör olarak kullanılabileceği kanısına varıldı.

Anahtar Kelimeler: Kist hidatik, oksidatif stres, sığır, total siyalik asit

INTRODUCTION

Echinococcus granulosus is a parasitic zoonosis that is widespread worldwide and important in terms of public health, causing hydatid cysts in many mammalian species, especially ruminants (1). In farm animals, it causes significant economic losses due to decreased meat and milk yield, decreased wool quality, infertility, and destruction of cystic organs such as the liver and lungs. Infective eggs excreted with the feces of the final host are ingested orally or rarely through the respiratory tract, resulting in infection in intermediate hosts (2,3). Since the disease is chronic, the development of cysts in intermediate hosts takes a long time, and symptoms vary depending on the size of the cyst, the organ it is located in, its number, and the stage of development of the cyst. Cysts located in the liver cause persistent diarrhea, jaundice and liver enlargement in animals, while cysts in the heart cause heart failure and cysts in the lungs cause symptoms such as cough, dyspnea, wheezing and rapid breathing (2,4).

In the organs and tissues where the cyst is located, an acute phase response (APR) occurs depending on the cellular and humoral response, and changes occur in the synthesis of acute phase protein (APP) in the liver (5,6). Sialic acid, which is found in the structure of many APPs, consists of pyruvate and mannosamine. Sialic acid, which is found in large amounts in animal tissues and bacteria, is an important component of the cell membrane, which has important functions such as acting as a receptor in membranes and playing a role in the adjustment of cellular stimuli, and recognizing each other in pathogen and host interactions (7). Total sialic acid (TSA), whose levels increase in inflammation and infection, is a very important biomarker in the diagnosis of inflammatory diseases (8).

Tissue damage, inflammation and infections occurring in the organism activate many mononuclear cells such as monocytes and macrophages. As a result of this activation, mononuclear cells consume excessive oxygen and ultimately cause the formation of free radicals such as hydrogen peroxide and superoxide anion (9). Oxidative stress occurs due to the excessive formation of free radicals. As a result of oxidative stress, end products of lipid peroxidation such as MDA accumulate, causing damage to tissues and organs (10). It has been reported that parasitic infections in particular cause damage to cells and tissues due to the increase in free radicals in host cells (11). NO is a free radical produced as a result of the reaction catalyzed by nitric oxide synthase and mediates many physiological or pathological events such as regulating cellular transmission in the organism and acting as a receptor in the structure of membranes (12,13). It has been reported that NO, produced by macrophages, neutrophils and mast cells, has anti-inflammatory, antimicrobial and antitumoral functions and its concentration increases in many bacterial, viral and parasitic infections (14-16). For these reasons, our aim in this study was to determine oxidative stress and sialic acid levels in cattle with hydatid cysts.

MATERIAL AND METHODS

In the study, a total of 30 cattle (3-4 years old, Brown Swiss cattle) were used as the hydatid cyst infected group (n=15) and the control group (n=15). After routine clinical examination of the animals brought to the Department of Internal Medicine of the Faculty of Veterinary Medicine of KAU with complaints such as dyspnea, neck extension, cough, cyanosis in the mucous membranes etc., the disease was diagnosed radiographically. The diagnosis was confirmed after slaughter. The animals constituting the control group were composed of clinically healthy animals with the same care and feeding conditions.

Blood samples were taken from the *jugular veins* of the animals in tubes without anticoagulants. The samples taken in tubes without anticoagulants were centrifuged at 3000 rpm for 15 minutes and the serum samples were separated and stored at -20 ^{o}C until analysis. NO concentration was measured in a spectrophotometer according to the method reported by Miranda et al. (17). In this method, nitrate was converted to nitrite with vanadium (III) chloride. The reaction of nitrite with sulfanilamide in acidic medium with N-(1-Naphthyl) ethylene diamine dihydrochloride resulted in the formation of a complex diazonium compound. This colored complex was measured at 540 nm. After the nitrate and nitrite levels were determined separately, the sum of the two indicates the amount of NO. Serum MDA concentration was determined according to the method reported by Yoshioka et al. (18). The formed MDA forms a pink complex with thiobarbituric acid and the absorbance of this solution is measured spectrophotometrically at 535 nm to determine the degree of lipid peroxidation.

TSA is the sum of free, PBSA and LBSA (19). Serum TSA and LBSA analyses were measured colorimetrically (Epoch, Biotek, USA) using the methods described by Sydow (20) and Katopodis and Stock (21), respectively, and the absorbances obtained were evaluated from the standard curve prepared with N-acetyl neuraminic acid. PBSA concentration was calculated by subtracting LBSA from TSA.

Statistical Analysis

SPSS 20.0 package program was used to evaluate the study data. Independent Sample T-test was used to compare the groups.

RESULTS

Clinical examination revealed rumen atony, dyspnea, cough, shallow and rapid respiration and tachycardia in the heart (Table 1). In addition, auscultatory examination revealed wheezing in the lung sounds and widespread dullness in the lung percussion area. Radiographic examination revealed numerous cysts with regular borders showing opacities in the lungs. When the cattle infected with hydatid cysts and the control group were compared, it was determined that TSA, LBSA, MDA and NO levels increased compared to the control group, while PBSA levels increased but were insignificant (Table 2).

Table 1. Physical examination findings of hydatid cyst infected cattle and control group

Parameters	Control	Infected	P
Rectal temperature (°C)	38.12±0.06	38.67±0.12	NS
Breaths/min	22.97±1.72	32.05±2.53	P<0.01
Heart beats/min	64.69±5.29	92.24±3.50	P<0.01

NS: Non significant

Table 2. Sialic acid and oxidative stress parameter levels in clinically healthy and hydatid cyst infected cattle

Parameters	Control	Infected	P
TSA (mg/dL)	71.32±2.66	92.37±3.11	P<0.001
LBSA (mg/dL)	32.15±2.34	44.83±2.21	P<0.001
PBSA (mg/dL)	39.17±3.71	47.54±3.08	NS
NO (µmol/L)	23.64±1.44	18.45±1.66	P<0.05
MDA (µmol/L)	2.12±0.09	3.32±0.19	P<0.01

TSA: Total sialic acid, LBSA: Lipid-bound sialic acid, PBSA: Protein-bound sialic acid, NO: Nitric oxide, MDA: Malondialdehyde, NS: Non significant

DISCUSSION AND CONCLUSION

Hydatid cyst is a chronic zoonotic parasitic infection that causes economic losses such as destruction of consumable organs, especially the liver and lungs, decrease in the amount and quality of meat, milk, and wool, delayed growth, and decreased birth rate (2,22). Although the disease is seen all over the world, it is more common in countries where eradication programs and preventive medical services are inadequate (23).

APR is formed in the animal tissues where it is located, depending on the cellular and humoral response, and changes occur in APP synthesis in the liver. The level of sialic acid in the structure of APPs also increases in parallel with the increase in APP concentration (12). TSA, which has an increased concentration, is a very important biomarker in the diagnosis of inflammatory diseases (8). Studies have reported that TSA levels increase in diseases such as neonatal diarrhea (24), tuberculosis (25), anaplasmosis and theleriosis (26), echinococcosis (12), hypodermosis (8), aspiration pneumonia (27), leptospirosis (28) and botulinum (29). In the study, TSA levels also increased and the increase may probably be due to increased sialoprotein synthesis in the liver as a result of APR, which develops due to inflammation and tissue damage.

NO is a free radical that is produced as a result of the reaction catalyzed by nitric oxide synthase and mediates many physiological or pathological events such as regulation of cellular conduction in the organism and acting as a receptor in the structure of membranes. It has been reported that NO produced by macrophages, neutrophils and mast cells has anti-inflammatory, antimicrobial and antitumoral functions and its concentration increases in many bacterial, viral and parasitic infections (14-16,30). In two separate studies conducted on animals infected with foot and mouth (14,16), it was reported that NO concentration increased in animals with foot and mouth. In addition, Özkan et al. (31) found that NO levels increased in a study they conducted in cattle with traumatic pericarditis. In addition, Atakişi et al. (15) reported an increase in NO concentration in another study they conducted on cattle with traumatic reticuloperitonitis. The NO level also increased in the study and the reason for the increase may be due to the increased activity in mononuclear cells such as monocytes and macrophages stimulated in the disease.

Tissue damage, inflammation and infections occurring in the organism activate many mononuclear cells such as monocytes and macrophages. As a result of this activation, mononuclear cells consume excessive oxygen and ultimately cause the formation of free radicals such as hydrogen peroxide and superoxide anion. Therefore, oxidative stress occurs and products such as MDA, which cause damage to tissues and organs, accumulate as a result of the oxidative stress (9,10). Studies have reported that the oxidant-antioxidant balance is disrupted and oxidative stress occurs in bacterial/viral and parasitic diseases such as hypodermosis (8,32), pneumonia (33) and sheeppox (34,35). It has been reported that parasitic infections in particular cause damage to cells and tissues due to the increase in free radicals in host cells (11). In a study conducted on sheep infected with *Toxoplasma gondii*, it was reported that oxidative stress caused by the parasite may play a role in the pathogenesis of the disease (36). In addition, in a study conducted on cattle with cystic echinococcosis, they reported that serum MDA levels increased due to increased lipid peroxidation in infected cattle (37). In another study conducted on sheep infected with *Dicrocoelium dendriticum* and hydatid cysts, they reported that endoparasites may cause oxidative stress (38). In the study, serum MDA levels increased due to the increase in lipid peroxidation, and the reason for the increase may be due to the increased activity in mononuclear cells against the parasite.

In conclusion, when the data obtained from the study were evaluated, it was determined that TSA, LBSA, NO and MDA levels were increased in animals with hydatid cyst compared to the control group, while PBSA levels increased but were statistically insignificant. It was concluded that the determination of serum TSA concentration and oxidative stress parameters in the cattle infected with hydatid cysts could be used as auxiliary biochemical parameters in determining and monitoring the severity of inflammation.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

KB took part in the study planning and MD sample collection. The writing of the study and final checks were carried out with the contributions of all authors.

ETHICAL STATEMENT

This study was started after receiving the ethics committee approval of Kafkas University Animal Experiments Local Ethics Committee (2021/011).

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ARAŞTIRMA MAKALESİ

Dondurularak Muhafaza Edilen Van Gölü İnci Kefalinde (*Chalcalburnus tarichi*, Pallas 1811) Ambalajlamanın ve Muhafaza Süresinin Kalite Değişiklikleri Üzerine Etkisi

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Öz

Bu araştırmada bütün ve temizlenmiş inci kefalinin vakumsuz ve vakumlu ambalajlarda -35°C'de dondurularak, -18°C'de 150 gün süreyle muhafazası sırasında meydana gelen mikrobiyolojik, kimyasal ve duyusal değişiklikler incelenmiştir. Toplam aerob psikrofil mikroorganizma (TAPM) ve laktik asit bakteri (LAB) sayıları muhafaza süresinin sonlarına doğru ~1-2 log kob/g seviyesine kadar düşmüştür. Maya-küfler 60. günden ve koliform grubu bakteriler de 15. günden sonra hiçbir grupta belirlenememiştir. Ayrıca incelenen numunelerde fekal streptokoklar ve *Pseudomonas* spp.'ye rastlanmamıştır. Muhafaza süresince mikroorganizmaların üremesi sınırlı kaldığından bütün halde vakumsuz ambalajlanan numuneler hariç diğer gruplarda toplam uçucu bazik azot (TVB-N) miktarları genellikle artmamıştır. Ancak tiyobarbitürik asit (TBA) miktarları ve peroksit sayıları muhafaza süresince düzensiz bir seyir izleyerek tüm gruplarda artmıştır. pH değerleri tüm gruplarda 6.16-6.66 arasında tespit edilmiştir. Duyusal analiz puanlarına göre temizlenmiş gruplardaki numuneler muhafaza süresinin sonuna kadar "çok iyi" kalite sınıfına girmiştir. Muhafaza süresinin sonunda vitamin A/D_3 miktarları sırasıyla <1 µg/100 g ve ~10 µg/100 g olarak belirlenmiştir. Sonuç olarak; baş ve iç organları çıkarıldıktan sonra vakumlu bir şekilde ambalajlanan inci kefalinin dondurularak 150 gün süreyle kaliteli bir şekilde muhafaza edilebileceği belirlenmiştir. Dolayısıyla, dondurarak muhafazada inci kefalini temizleyerek vakumla ambalajlamanın en uygun yöntem olduğu kanaatine varılmıştır.

Anahtar Kelimeler: Dondurarak muhafaza, duyusal, inci kefali (*Chalcalburnus tarichi*), kimyasal, mikrobiyolojik, vakumlu ambalajlama

The Effect of Packaging and Storage Period on Quality Changes in Lake Van Pearl Mullet (*Chalcalburnus tarichi*, Pallas 1811) Preserved by Freezing

Abstract

In this research, microbiological, chemical, and sensory changes that whole and cleaned pearl mullets frozen at -35°C in non-vacuumed/vacuumed packages and stored at -18°C for 150 days were examined. Total aerobic psychrophile microorganism (TAPM) and lactic acid bacteria (LAB) counts decreased to ~1-2 log cfu/g towards the end of the storage period. Yeast and molds couldn't be detected in any group after the 60th day and coliforms couldn't be detected in any group after the 15th day. Additionally, fecal streptococci and *Pseudomonas* spp. were not found in the samples examined. Since the growth of microorganisms remained limited during storage, total volatile basic nitrogen (TVB-N) amounts did not generally increase in other groups, except for whole and non-vacuumed package samples. However, thiobarbituric acid (TBA) amounts and peroxide counts increased in all groups, following an irregular course during storage. pH values were determined between 6.16-6.66 in all groups. According to the sensory analysis scores, the samples in the cleaned groups were in the "very good" quality class until the end of the storage period. At the end of the storage period, vitamin A/D_3 amounts were determined as <1 µg/100 g and ~10 µg/100 g, respectively. Consequently; it was determined that the pearl mullet, which was vacuumed-package after removing the head and internal organs, could be frozen and preserved in good quality for 150 days. Therefore, it was convinced that cleaning-vacuum packaging is the most appropriate method for preserving pearl mullet by freezing.

Key Words: Chemical, freezing preservation, microbiological, pearl mullet (*Chalcalburnus tarichi*), sensory, vacuum packaging

GİRİŞ

Nüfusun hızlı bir şekilde artmasının yanında dünyanın birçok bölgesinde kırmızı ve beyaz et üretiminin azalması, besleyici değeri yüksek olan su ürünlerinin insanların protein ihtiyacının karşılanmasında ne kadar önemli olduğunu göstermektedir. Su ürünleri arasında önemli bir yer tutan, kolay sindirilebilen ve yüksek kaliteli protein içeriğine sahip olan balık; içerdiği yağ asitleri, vitaminler ve mineral maddeler yönünden de önemli bir gıdadır (1,2). Balık etinin pH değeri ve rutubet miktarının yüksek olması ile bağ dokusundaki zayıflık, bu gıdayı diğer birçok et türüne göre bozulmaya karşı daha hassas duruma getirmektedir (3,4). Balıklarda meydana gelen bozulmalar; avlanma yöntemi, balığın yakalandığı andaki durumu, avlama mevsimi ve bölgesi, suyun sıcaklığı ve kirliliği gibi birçok faktörün yanı sıra balığın kimyasal bileşimi, balıkta bulunan mikroorganizmaların türü ve yoğunluğu, balıkların muhafazaya alınmadan önce geçen süre ve sıcaklık derecesi ile ambalajlama şekliyle de yakından ilgilidir (5-7).

Daha uzun süre dayanabilen ve kaliteli bir ürün elde edilebilmesi için balığın taze olması ve en iyi muhafaza yöntemlerinden biri olan dondurma işleminin tekniğine uygun olarak yapılması gerekmektedir (5,8,9). Dondurulmuş balıklarda ürünlerin yapısında bulunan su, buz kristallerine dönüştüğünden mikroorganizmalar tarafından kullanılamaz ve çok düşük sıcalıklarda biyokimyasal ve mikrobiyolojik gelişmeler de iyice yavaşladığından ürünler daha uzun süre muhafaza edilir (8,10-12). Balıkların dondurulması ile balığın pazar arzı dengelenebileceği gibi, taze balığın bulunmadığı dönemlerde de tüketicilerin kaliteli balığa ulaşmaları mümkün olabilecektir.

İnci kefali (*Chalcalburnus tarichi*, Pallas 1811) yüksek alkaliliğe sahip Van Gölü'nde yaşayan ve bölgede sevilerek tüketilen bir balıktır. Yıl içindeki fiyatları avlanan miktara göre değişen, bol avlandığı dönemlerde çok düşük fiyatlara satılan ve belirli dönemlerde de olsa arzın talepten fazla olması nedeniyle zayi olan inci kefalinin dondurularak muhafaza edilmesi suretiyle değerlendirilmesinin ekonomimize sağlayacağı pozitif katkı tartışmasızdır.

Farklı şekillerde ve sürelerde muhafaza edilen balıklar üzerine yapılan bazı çalışmalarda (13-23) mikrobiyolojik ve kimyasal faktörlerden kaynaklanan kalite değişiklerinin tespit edildiği bildirilmiştir. Ayrıca bazı çalışmalarda da (24,25) balıkların vakumlanarak ambalajlanmasının depolama süresince ürünün kimyasal ve duyusal özellikleri üzerine olumlu etkiler gösterdiği belirtilmiştir.

Bu araştırma, bütün halde ve bazı kısımları (baş, iç organlar) çıkarılıp vakumlu/vakumsuz olarak ambalajlandıktan sonra dondurularak muhafaza edilen inci kefalinde meydana gelen kalite değişiklikleri ile bu balıklar için uygun olan ambalajlama şekli ve muhafaza süresini belirlemek amacıyla yapılmıştır.

MATERYAL VE METOT

Bu araştırmada ticari amaçla balıkçıların Van Gölü'nden avladıkları inci kefali zaman geçirmeden soğutuculu araçlarla laboratuvara getirilmiş ve farklı işlemlere alınarak ambalajlanmıştır. Balıkların bir kısmı bütün halde (grup A), bir kısmı da baş ve iç organları ayrılarak soğuk suyla yıkandıktan (grup B) sonra strafor kaplarda streç filmle kaplanarak vakumsuz olarak ambalajlanmıştır. Ayrıca, bütün haldeki balıklar (grup C) ve baş/iç organları çıkarılan balıklar (grup D) da vakumlanarak ambalajlanmıştır. Böylelikle dört farklı grupta da muhafaza süresinin belirlenen yedi gününde (1., 15., 30., 60., 90., 120. ve 150. gün) analizlerin üç paralel olarak yapılabilmesi için her gruptan en az 21 adet olmak üzere toplamda 84 adet ambalajlı ürün oluşturulmuştur. Ambalajlanan numuneler -35°C'de dondurulduktan sonra -18°C'de muhafaza edilerek belirlenen günlerde mikrobiyolojik, kimyasal ve duyusal kalite parametreleri yönünden incelenmiştir. Kolaylıkla numune alınabilmesi amacıyla dondurulmuş balıklar buzdolabının soğutucu kısmında bir gün bekletilmiş ve farklı ambalajlardaki 10-12 adet balığın en az beş tanesinin dorsal kaslarından karıştırılarak numuneler alınmıştır.

Mikrobiyolojik Analizler

Aseptik koşullar altında steril stomaher torbasına alınan 10 g numune ve 90 mL tamponlanmış peptonlu su (Oxoid, CM0509), stomaherde (SJIA-04C) homojenize edilerek 10^{-8}'e kadar desimal dilüsyonlar hazırlanmış ve mikrobiyolojik ekimler yapılmıştır. Toplam aerob psikrofil mikroorganizma (TAPM) sayımında Plate Count Agar (Oxoid, CM0325) kullanılmış ve ekim yapılan petriler 7°C'de 7-10 gün süreyle inkübasyona bırakılarak üreyen koloniler sayılmıştır. Laktik asit bakterilerinin (LAB) sayımında M17 Agar (Oxoid, CM0785) kullanılmış ve çift kat ekim yapılan petriler 35°C'de 48 saat inkübasyona bırakıldıktan sonra 1-2 mm çapındaki beyaz kolonilerin sayımı yapılmıştır. Maya-küf sayımında %10'luk steril tartarik asit ilave edilerek pH'sı 3.50'ye ayarlanan Potato Dextrose Agar (Oxoid, CM0139) kullanılmış, ekim yapılan petriler 20-25°C'de 5-7 gün süreyle inkübasyona bırakılmış ve inkübasyon sonunda üreyen tipik koloniler sayılmıştır. Koliform grubu bakterilerin sayımı için Violet Red Bile Agar (Oxoid, CM0107) kullanılmış, çift kat ekim yapılan petriler 37±1°C'de 24-48 saat süreyle inkübasyona bırakılmış ve 0.5 mm çapındaki kırmızı kolonilerin sayımı yapılmıştır. Fekal streptokokların sayımında Slanetz & Bartley Agar (Oxoid, CM0377) kullanılmış ve ekim yapılan petriler 37°C'de 24 saat süreyle inkübasyona bırakılarak değerlendirilmiştir. *Pseudomonas* spp.'nin sayımı için Pseudomonas Agar Base (Oxoid, CM0559) kullanılmış, sterilize edilerek 50°C'ye kadar soğutulan besiyerine Pseudomonas Selective Supplement (Oxoid, SR0103) ilave edilmiş ve ekim yapılan petriler 25°C'de 48-72 saat süreyle inkübasyona bırakılarak değerlendirilmiştir (26).

Kimyasal Analizler

Numunelerdeki toplam uçucu bazik azot (total volatile basic nitrogen, TVB-N) miktarı (Antonacpoulos tarafından modifiye), tiyobarbitürik asit (thiobarbituric acid, TBA) miktarı ve peroksit sayısı (Hadorn ve ark. tarafından modifiye), Varlık ve ark. (27) tarafından bildirilen yöntemlere göre belirlenmiştir. pH değerleri Honikel (28)'e göre pH-metrede (Hanna, pH 211) tespit edilirken, vitamin A ve vitamin D_3 miktarları da Miller ve Yang (29) tarafından bildirilen yüksek performanslı sıvı kromatografisi (High Performance Liquid Chromatography, HPLC) yöntemine göre belirlenmiştir.

Duyusal Analizler

Duyusal analizler Paulus ve ark. (30) tarafından geliştirilen yönteme göre yapılmış ve puanlama araştırma süresince genel olarak aynı kişilerden oluşan beş panelist tarafından gerçekleştirilmiştir. Numuneler renk, koku, lezzet ve genel kabul

edilebilirlik kriterleri dikkate alınarak hedonik skalaya göre (9.00-7.00 puan "çok iyi", 6.99-4.01 puan "iyi", 4.00 puan "tüketilebilir", <4.00 puan "bozulmuş") değerlendirilmiştir.

İstatistiksel Analizler

Bu araştırmadaki örneklemi oluşturan Van Gölü'nden ticari olarak avlanmış inci kefali, bütün ve temizlenmiş olarak vakumlu/vakumsuz ambalajlanma durumlarına göre gruplara ayrıldıktan sonra -18°C'de 150 gün süreyle muhafaza edilmiştir. Yapılan mikrobiyolojik, kimyasal ve duyusal analizler neticesinde belirlenen bulgular, bağımsız değişkenlerin etkilerini aynı anda test edebilen faktöriyel deneme desenine göre istatistiksel analiz sistemi (Statistical Analysis System, SAS) programında değerlendirilmiştir (31). Bu şekilde bulguların ortalama ve standart sapma değerleri ile muhafaza süresince (1., 15., 30., 60., 90., 120., 150. gün) balıkların bazı kalite özelliklerinde meydana gelen değişimlerin istatistiksel yönden önemi (p<0.05) belirlenmiştir.

BULGULAR

Dondurularak muhafaza edilen inci kefali numunelerinin mikrobiyolojik analiz bulguları (log kob/g) Tablo 1'de, mikrobiyolojik analiz bulgularındaki değişimler de Tablo 2'de gösterilmiştir. Farklı şekillerde işlenen numunelerdeki TAPM, LAB, maya-küf ve koliform grubu bakteri sayıları muhafaza süresince genellikle azalmış, koliform grubu bakteriler muhafaza süresinin 15. gününden ve maya-küfler 60. gününden sonra grupların hiçbirinde belirlenmemiş (Tablo 1), ayrıca incelenen numunelerde muhafaza süresince hiçbir grupta *Pseudomonas* spp. ve fekal streptokoklara rastlanmamıştır. TAPM sayısı yönünden bütün ile temizlenmiş ve vakumsuz ile vakumlu numuneler arasındaki fark ve muhafaza süresinin etkisi istatistiksel olarak önemli (p<0.05) bulunurken, LAB sayısı yönünden ise bütün ile temizlenmiş numuneler arasındaki fark ve zamanın etkisi önemli (p<0.05) bulunmuştur (Tablo 2).

Tablo 1. Dondurularak muhafaza edilen inci kefalinin mikrobiyolojik analiz bulguları (log kob/g)

Analizler	Zaman (gün)	GRUPLAR			
		A	B	C	D
TAPM	1	3.90±0.18 ^{a}	3.50±0.12 ^{a}	3.49±0.31 ^{a}	2.89±0.45 ^{a}
	15	3.21±0.52 ^{ab}	2.50±0.10 ^{a}	3.43±0.34 ^{a}	2.98±0.34 ^{a}
	30	3.41±0.24 ^{ab}	3.02±0.12 ^{a}	3.66±0.55 ^{a}	3.06±0.06 ^{a}
	60	2.82±0.52 ^{ab}	3.68±0.00 ^{a}	4.30±0.05 ^{a}	3.08±0.30 ^{a}
	90	2.93±0.03 ^{Bab}	<2.30 ^{Db}	3.61±0.26 ^{Aa}	2.36±0.06 ^{Cab}
	120	2.40±0.10 ^{Bb}	2.46±0.16 ^{Ba}	3.98±0.06 ^{Aa}	2.50±0.10 ^{Bab}
	150	0.76±0.76 ^{c}	0.76±0.76 ^{b}	1.22±1.22 ^{b}	1.53±0.76 ^{b}
LAB	1	2.16±0.49 ^{ABa}	1.59±0.06 ^{Bab}	2.68±0.31 ^{Aa}	1.36±0.22 ^{Bb}
	15	<1.00 ^{b}	0.98±0.49 ^{bcd}	0.63±0.63 ^{c}	1.42±0.22 ^{b}
	30	1.59±0.11 ^{ABa}	0.33±0.33 ^{Ccd}	2.35±0.28 ^{Aa}	1.33±0.20 ^{Bb}
	60	2.40±0.02 ^{a}	1.44±0.72 ^{abc}	1.99±0.04 ^{ab}	1.69±0.09 ^{b}
	90	2.19±0.04 ^{a}	2.42±0.13 ^{a}	2.33±0.03 ^{a}	2.41±0.05 ^{a}
	120	2.32±0.02 ^{Aa}	2.02±0.03 ^{Bab}	1.81±0.06 ^{Cab}	1.46±0.08 ^{Db}
	150	0.43±0.43 ^{b}	<1.00 ^{d}	0.96±0.49 ^{bc}	<1.00 ^{c}
Maya-küf	1	0.76±0.76	3.30±0.00 ^{a}	3.30±0.00 ^{a}	2.30±0.00 ^{a}
	15	1.95±0.98	2.30±0.00 ^{ab}	<2.30 ^{c}	2.30±0.00 ^{a}
	30	1.79±0.89	1.69±0.85 ^{ab}	<2.30 ^{c}	0.86±0.86 ^{ab}
	60	2.30±0.00	0.86±0.86 ^{b}	2.45±0.15 ^{b}	2.30±0.00 ^{a}
	90	<2.30	<2.30 ^{b}	<2.30 ^{c}	<2.30 ^{b}
	120	<2.30	<2.30 ^{b}	<2.30 ^{c}	<2.30 ^{b}
	150	<2.30	<2.30 ^{b}	<2.30 ^{c}	<2.30 ^{b}
Koliformlar	1	1.88±0.34 ^{ABa}	2.28±0.16 ^{Aa}	1.46±0.08 ^{Ba}	1.82±0.19 ^{ABa}
	15	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}
	30	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}
	60	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}
	90	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}
	120	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}
	150	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}	<1.00 ^{b}

A: Vakumsuz-bütün; **B:** Vakumsuz-baş ve iç organları çıkarılmış; **C:** Vakumlu-bütün; **D:** Vakumlu-baş ve iç organları çıkarılmış; **TAPM:** Toplam aerob psikrofil mikroorganizmalar; **LAB:** Laktik asit bakterileri; **^{ABC}:** Aynı satırdaki farklı harfler gruplar arasında farklılığı gösterir (p<0.05); **^{abcd}:** Aynı sütundaki farklı harfler zamanlar arasında farklılığı gösterir (p<0.05)

Tablo 2. Dondurularak muhafaza edilen inci kefalinin mikrobiyolojik analiz bulgularındaki değişimler (log kob/g)

Grup-zaman	n	TAPM	LAB	Maya-küf	Koliformlar
Muamele					
Bütün	42	3.05±0.19 ^{a}	1.68±0.15 ^{a}	0.68±0.20	0.23±0.09
Temiz	42	2.37±0.17 ^{b}	1.31±0.13 ^{b}	0.75±0.27	0.29±0.11
Ambalaj					
Vakumsuz	42	2.46±0.20 ^{b}	1.39±0.15	0.80±0.26	0.29±0.11
Vakumlu	42	2.97±0.17 ^{a}	1.59±0.13	0.63±0.20	0.23±0.09
Zaman (gün)					
1	12	3.44±0.16 ^{a}	1.94±0.20 ^{ab}	1.86±0.61 ^{a}	1.86±0.12 ^{a}
15	12	3.03±0.18 ^{a}	0.76±0.23 ^{d}	2.09±0.54 ^{a}	<1.00 ^{b}
30	12	3.29±0.15 ^{a}	1.38±0.26 ^{c}	1.08±0.39 ^{b}	<1.00 ^{b}
60	12	3.44±0.27 ^{a}	1.83±0.24 ^{b}	1.80±0.39 ^{ab}	<1.00 ^{b}
90	12	2.22±0.41 ^{b}	2.34±0.04 ^{a}	<2.30 ^{c}	<1.00 ^{b}
120	12	2.83±0.20 ^{ab}	1.90±0.09 ^{ab}	<2.30 ^{c}	<1.00 ^{b}
150	12	1.07±0.39 ^{c}	0.35±0.18 ^{d}	<2.30 ^{c}	<1.00 ^{b}

TAPM: Toplam aerob psikrofil mikroorganizmalar; **LAB:** Laktik asit bakterileri; **n:** Ambalajlı numune sayısı; **^{abcd}:** Aynı sütundaki farklı harfler balıkların temizlenmesi/vakumlanmasına göre oluşturulan gruplar ve muhafaza zamanları arasındaki farklılığı göstermektedir (p<0.05)

Numunelerin kimyasal analiz bulguları ve pH değerleri Tablo 3'te, kimyasal analiz bulgularındaki ve pH değerlerindeki değişimler Tablo 4'te; vitamin A/D_3 miktarları ve duyusal analiz puanları Tablo 5'te, vitamin A/D_3 miktarlarındaki ve duyusal analiz puanlarındaki değişimler ise Tablo 6'da sunulmuştur. Muhafaza süresince bütün bir şekilde vakumsuz olarak ambalajlanan (A grubu) inci kefali numuneleri hariç olmak üzere diğer gruplarda TVB-N miktarları genellikle artmamış, ancak TBA miktarları ve peroksit sayıları muhafaza süresince düzensiz bir seyir izleyerek tüm gruplarda (A, B, C, D) artmıştır. İncelenen numunelerdeki pH değerleri de 6.16-6.66 arasında belirlenmiştir (Tablo 3). Bu araştırmada TBA miktarları, peroksit sayıları ve pH değerleri bakımından bütün ile temizlenmiş ve vakumsuz ile vakumlu numuneler arasındaki fark ve zamanın etkisi önemli (p<0.05) bulunmuştur (Tablo 4). Yapılan duyusal analiz puanlamalarına göre temizlenmiş gruplardaki (B, D) numunelerin muhafaza süresince "çok iyi" kalite sınıfına girdiği görülmüştür (Tablo 5). Numunelerdeki vitamin A miktarları bakımından sadece muhafaza süresinin istatistiksel olarak önemli (p<0.05) olduğu belirlenirken, Vitamin D_3 ve duyusal analiz puanları bakımından ise hem bütün ve temizlenmiş balıklar arasındaki farkın hem de zamanın etkisinin önemli (p<0.05) olduğu görülmüştür (Tablo 6).

Tablo 3. Dondurularak muhafaza edilen inci kefalinin kimyasal analiz bulguları ve pH değerleri

Analizler	Zaman (gün)	GRUPLAR			
		A	B	C	D
TVB-N	1	10.73±0.46 ^{c}	10.73±0.46 ^{b}	10.73±1.23 ^{ab}	10.26±0.46 ^{b}
(mg/100 g)	15	15.86±1.23 ^{Aa}	12.60±0.00 ^{Ba}	12.13±0.46 ^{Bab}	12.60±0.80 ^{Bab}
	30	12.13±0.46 ^{bc}	11.66±0.46 ^{ab}	13.06±0.93 ^{a}	12.13±0.46 ^{ab}
	60	10.60±0.41 ^{BCc}	12.20±0.40 ^{ABab}	10.40±0.41 ^{Cb}	12.66±0.75 ^{Aab}
	90	11.66±0.46 ^{bc}	11.20±0.80 ^{ab}	11.66±0.46 ^{ab}	13.06±0.46 ^{a}
	120	13.53±0.46 ^{Ab}	10.73±0.46 ^{Bb}	12.13±0.46 ^{ABab}	12.60±0.80 ^{ABab}
	150	13.53±0.46 ^{b}	11.66±0.46 ^{ab}	11.20±0.80 ^{ab}	10.73±1.23 ^{ab}
TBA	1	2.37±0.35 ^{Abc}	1.46±0.18 ^{Bd}	2.00±0.17 ^{ABb}	1.72±0.16 ^{ABbc}
(mg MA/kg)	15	1.13±0.17 ^{ABc}	0.47±0.16 ^{Ce}	1.40±0.26 ^{Abc}	0.58±0.02 ^{BCd}
	30	2.46±0.80 ^{Abc}	1.08±0.24 ^{ABd}	1.05±0.26 ^{ABc}	0.82±0.20 ^{Bd}
	60	2.74±0.19 ^{Bb}	3.55±0.31 ^{Ac}	2.17±0.04 ^{BCb}	1.93±0.23 ^{Cb}
	90	2.70±0.05 ^{Ab}	2.76±0.24 ^{Ac}	1.66±0.11 ^{Bbc}	1.37±0.14 ^{Bc}
	120	3.55±0.21 ^{Bb}	6.42±0.28 ^{Ab}	1.79±0.12 ^{Dbc}	2.55±0.19 ^{Ca}
	150	5.01±0.80 ^{Ba}	11.51±0.93 ^{Aa}	5.09±0.51 ^{Ba}	2.65±0.10 ^{Ca}
Peroksit	1	2.33±0.16 ^{Bc}	1.56±0.42 ^{Bf}	3.38±0.82 ^{ABb}	4.80±0.76 ^{Aa}
(mmol O_2 kg)	15	2.40±0.61 ^{c}	1.24±0.01 ^{f}	1.92±0.43 ^{b}	2.07±0.33 ^{c}
	30	4.44±0.48 ^{ABab}	4.22±0.30 ^{ABe}	5.69±0.60 ^{Aa}	4.01±0.40 ^{Bab}
	60	3.70±0.06 ^{Bbc}	6.35±0.16 ^{Ad}	2.69±0.21 ^{Cb}	1.74±0.23 ^{Dc}
	90	4.21±0.87 ^{Bab}	9.42±0.36 ^{Aa}	3.36±0.18 ^{BCb}	2.20±0.34 ^{Cc}
	120	4.57±0.35 ^{Bab}	8.21±0.36 ^{Ab}	3.75±0.29 ^{BCab}	2.89±0.37 ^{Cbc}
	150	5.70±0.19 ^{ABa}	7.25±0.16 ^{Ac}	4.03±1.25 ^{Bab}	4.48±0.37 ^{Ba}
pH	1	6.57±0.02 ^{a}	6.55±0.00 ^{a}	6.59±0.01 ^{b}	6.58±0.01 ^{a}
	15	6.59±0.03 ^{Ba}	6.56±0.01 ^{Ba}	6.66±0.00 ^{Aa}	6.53±0.02 ^{Ba}
	30	6.47±0.01 ^{Ab}	6.41±0.03 ^{Ab}	6.20±0.01 ^{Ce}	6.28±0.02 ^{Bcd}
	60	6.18±0.03 ^{c}	6.16±0.01 ^{c}	6.17±0.02 ^{e}	6.24±0.02 ^{d}
	90	6.48±0.02 ^{b}	6.43±0.01 ^{b}	6.44±0.02 ^{cd}	6.44±0.01 ^{b}
	120	6.44±0.03 ^{b}	6.41±0.01 ^{b}	6.47±0.02 ^{c}	6.40±0.01 ^{b}
	150	6.44±0.03 ^{Ab}	6.43±0.02 ^{Ab}	6.39±0.02 ^{ABd}	6.33±0.02 ^{Bc}

A: Vakumsuz-bütün; **B:** Vakumsuz-baş ve iç organları çıkarılmış; **C:** Vakumlu-bütün; **D:** Vakumlu-baş ve iç organları çıkarılmış; **TVB-N:** Total volatile basic nitrogen (toplam uçucu bazik azot); **TBA:** Tiyobarbitürik asit; **^{ABCD}:** Aynı satırdaki farklı harfler gruplar arasında farklılığı gösterir (p<0.05); **^{abcdef}:** Aynı sütundaki farklı harfler zamanlar arasında farklılığı gösterir (p<0.05)

Tablo 4. Dondurularak muhafaza edilen inci kefalinin kimyasal analiz bulgularındaki ve pH değerlerindeki değişimler

Grup-zaman	n	TVB-N (mg/100 g)	TBA (mg MA/kg)	Peroksit (mmol O_2/kg)	pH
Muamele					
Bütün	42	12.10±0.27	2.51±0.20 ^{b}	3.73±0.21 ^{b}	6.43±0.02 ^{a}
Temiz	42	11.77±0.19	2.78±0.44 ^{a}	4.32±0.40 ^{a}	6.41±0.01 ^{b}
Ambalaj					
Vakumsuz	42	12.06±0.25	3.37±0.43 ^{a}	4.68±0.38 ^{a}	6.43±0.02 ^{a}
Vakumlu	42	11.81±0.22	1.91±0.17 ^{b}	3.36±0.21 ^{b}	6.41±0.02 ^{b}
Zaman (gün)					
1	12	10.61±0.32 ^{c}	1.89±0.14 ^{d}	3.02±0.44 ^{c}	6.57±0.00 ^{a}
15	12	13.30±0.55 ^{a}	0.90±0.13 ^{e}	1.91±0.21 ^{d}	6.58±0.01 ^{a}
30	12	12.25±0.30 ^{b}	1.35±0.27 ^{e}	4.59±0.27 ^{b}	6.34±0.03 ^{d}
60	12	11.46±0.36 ^{bc}	2.60±0.20 ^{c}	3.62±0.52 ^{c}	6.19±0.01 ^{e}
90	12	11.90±0.32 ^{b}	2.12±0.19 ^{cd}	4.80±0.86 ^{ab}	6.45±0.00 ^{b}
120	12	12.25±0.39 ^{b}	3.58±0.53 ^{b}	4.85±0.62 ^{ab}	6.43±0.01 ^{bc}
150	12	11.78±0.47 ^{b}	6.07±0.03 ^{a}	5.36±0.47 ^{a}	6.40±0.01 ^{c}

TVB-N: Total volatile basic nitrogen (toplam uçucu bazik azot); **TBA:** Tiyobarbitürik asit; **n:** Ambalajlı numune sayısı; **^{abcde}:** Aynı sütundaki farklı harfler balıkların temizlenmesi/vakumlanmasına göre oluşturulan gruplar ve muhafaza zamanları arasındaki farklılığı göstermektedir (p<0.05)

Tablo 5. Dondurularak muhafaza edilen inci kefalinin vitamin A/D_3 miktarları (yaş doku) ve duyusal analiz puanları

Analizler	Zaman (gün)	GRUPLAR			
		A	B	C	D
Vitamin A (µg/100 g)	1	28.20±2.97 ^{a}	27.83±5.08 ^{a}	37.40±2.45 ^{a}	31.83±3.16 ^{a}
	15	25.00±2.88 ^{ab}	22.00±0.50 ^{ab}	24.06±2.96 ^{b}	22.00±0.50 ^{b}
	30	18.75±3.75 ^{b}	15.75±5.75 ^{b}	9.00±1.50 ^{c}	8.75±1.25 ^{c}
	60	10.43±4.30 ^{c}	6.46±0.78 ^{c}	7.36±1.34 ^{c}	6.76±0.50 ^{c}
	90	0.56±0.21 ^{d}	0.33±0.03 ^{c}	0.33±0.08 ^{d}	0.50±0.05 ^{d}
	120	0.23±0.13 ^{d}	0.26±0.03 ^{c}	0.23±0.08 ^{d}	0.43±0.03 ^{d}
	150	0.23±0.03 ^{d}	0.20±0.10 ^{c}	0.20±0.05 ^{d}	0.33±0.06 ^{d}
Vitamin D_3 (µg/100 g)	1	83.33±4.91 ^{Ba}	71.00±5.85 ^{Ba}	108.33±11.66 ^{Aa}	71.33±5.23 ^{Ba}
	15	64.00±14.29 ^{ab}	58.00±1.52 ^{b}	57.66±4.70 ^{b}	48.50±3.50 ^{b}
	30	58.33±9.27 ^{b}	64.50±6.50 ^{ab}	53.33±3.75 ^{bc}	51.00±1.00 ^{b}
	60	37.66±1.45 ^{c}	34.66±2.90 ^{c}	38.33±7.26 ^{cd}	46.66±4.40 ^{b}
	90	21.00±1.73 ^{cd}	18.66±1.33 ^{d}	21.66±1.76 ^{de}	25.66±2.96 ^{c}
	120	12.33±1.45 ^{d}	11.66±0.88 ^{d}	10.66±0.66 ^{e}	11.33±0.88 ^{d}
	150	11.66±0.88 ^{Ad}	12.66±0.33 ^{Ad}	9.00±0.57 ^{Be}	8.66±0.88 ^{Bd}
Duyusal analiz*	1	8.80±0.20 ^{a}	9.00±0.00 ^{a}	8.60±0.24 ^{a}	9.00±0.00 ^{a}
	15	8.20±0.20 ^{a}	8.20±0.20 ^{ab}	7.80±0.48 ^{abc}	8.20±0.37 ^{ab}
	30	7.75±0.25 ^{bc}	8.00±0.40 ^{b}	7.75±0.25 ^{abc}	8.25±0.25 ^{ab}
	60	7.80±0.37 ^{bc}	8.00±0.31 ^{b}	7.40±0.24 ^{bcd}	8.00±0.31 ^{b}
	90	7.75±0.25 ^{bc}	7.75±0.25 ^{b}	8.25±0.25 ^{ab}	8.25±0.25 ^{ab}
	120	7.00±0.40 ^{ABcd}	7.75±0.47 ^{Ab}	6.50±0.28 ^{Bd}	7.75±0.25 ^{Ab}
	150	6.75±0.25 ^{d}	7.75±0.25 ^{b}	6.75±0.47 ^{cd}	7.50±0.28 ^{b}

A: Vakumsuz-bütün; **B:** Vakumsuz-baş ve iç organları çıkarılmış; **C:** Vakumlu-bütün; **D:** Vakumlu-baş ve iç organları çıkarılmış; ***:** 9.00-7.00 puan "çok iyi", 6.99-4.01 puan "iyi", 4.00 puan "tüketilebilir", <4.00 puan "bozulmuş"; **^{AB}:** Aynı satırdaki farklı harfler gruplar arasında farklılığı gösterir (p<0.05); **^{abcde}:** Aynı sütundaki farklı harfler zamanlar arasında farklılığı gösterir (p<0.05)

Tablo 6. Dondurularak muhafaza edilen inci kefalinin vitamin A/D_3 miktarlarındaki (yaş doku) ve duyusal analiz puanlarındaki değişimler

Grup-zaman	n	Vitamin A (µg/100 g)	Vitamin D_3 (µg/100 g)	Duyusal analiz*
Muamele				
Bütün	42	11.39±2.01	41.95±4.77 [a]	7.69±0.11 [b]
Temiz	42	9.84±1.87	36.89±3.76 [b]	8.12±0.08 [a]
Ambalaj				
Vakumsuz	42	10.85±1.86	39.36±4.10	7.93±0.09
Vakumlu	42	10.42±2.03	39.67±4.61	7.88±0.11
Zaman (gün)				
1	12	31.31±1.91 [a]	83.50±5.56 [a]	8.85±0.08 [a]
15	12	23.38±1.06 [b]	57.81±3.91 [b]	8.10±0.16 [b]
30	12	12.61±1.95 [c]	56.60±3.19 [b]	7.93±0.14 [b]
60	12	7.75±1.09 [d]	39.33±1.15 [c]	7.80±0.15 [b]
90	12	0.43±0.06 [e]	21.75±2.35 [d]	8.00±0.12 [b]
120	12	0.29±0.04 [e]	11.50±0.46 [e]	7.25±0.21 [c]
150	12	0.24±0.03 [e]	10.50±0.59 [e]	7.18±0.18 [c]

***:** 9.00-7.00 puan "çok iyi", 6.99-4.01 puan "iyi", 4.00 puan "tüketilebilir", <4.00 puan "bozulmuş"; **n:** Ambalajlı numune sayısı; [abcde]**:** Aynı sütundaki farklı harfler balıkların temizlenmesi/vakumlanmasına göre oluşturulan gruplar ve muhafaza zamanları arasındaki farklılığı göstermektedir (p<0.05)

TARTIŞMA VE SONUÇ

Dondurularak muhafaza edilen gıdalarda mikroorganizmalara göre değişmekle birlikte üreme yavaşlamakta veya durmakta, ancak enzimatik faaliyetler azalmakla birlikte devam etmektedir (6,10,32,33). Bu araştırmada da farklı şekillerde işlenerek ambalajlanan ve dondurularak muhafaza edilen inci kefalindeki mikroorganizma sayılarının genellikle azaldığı gözlenmiştir. Numunelerin başlangıçta ~3 log kob/g seviyesinde olan TAPM sayıları, bütün halde vakumlanarak ambalajlanan grup (C) hariç olmak üzere 120. gün 2 log kob/g ve 150. gün ~1 log kob/g seviyesine kadar düşmüştür (Tablo 1). Bu araştırmada TAPM sayısı yönünden bütün ile temizlenmiş ve vakumsuz ile vakumlu numuneler arasındaki fark ve zamanın etkisi önemli (p<0.05) bulunmuştur (Tablo 2). Bu araştırmadaki bulgulara benzer şekilde dondurularak muhafaza edilen bütün ve temizlenmiş hamsi (15) ile vakumlanmış derili/derisiz aynalı sazan filetolarında da (24) psikrofil mikroorganizma sayılarının düştüğü bildirilmiştir. Psikrofil mikroorganizmaların 10^7-10^8 kob/g seviyelerine ulaştığında balıkların bozulmuş olarak değerlendirileceği belirtilmektedir (5). Tablo 1 incelendiğinde tüm gruplarda belirlenen bu mikroorganizma sayılarının belirtilen seviyelerden daha düşük olduğu görülmektedir.

Tüm gruplarda düzensiz bir seyir izleyen LAB sayıları muhafaza süresince başlangıçta olduğu gibi 1-2 log kob/g seviyelerinde tespit edilmiş (Tablo 1), bütün ile temizlenmiş numuneler arasındaki fark ve muhafaza süresinin etkisi önemli (p<0.05) bulunmuştur (Tablo 2). Bu araştırmada elde edilen bulgular, bütün ve temizlenmiş bir şekilde dondurularak muhafaza edilen inci kefali (21) ve sazan (22) üzerine yapılan çalışmalarda bildirilen bulgulara benzerdir. Başlangıçta en fazla 3.30±0.00 log kob/g olarak belirlenen maya-küf sayısı muhafaza süresinin 60. gününe kadar düzensiz bir seyir izlemiş ve daha sonraki günlerde grupların hiçbirinde maya-küf tespit edilmemiştir (Tablo 1). Kietzmann ve ark. (34) balıklardaki maya-küflerin daha çok karasal kökenli olduğunu ve genellikle taşıma/işleme aşamalarında bu mikroorganizmaların balıklara bulaşabileceğini belirtmişlerdir. Bu araştırmada da muhafazanın başlangıcında maya-küflerin belirlenmesinde, gölden avlanmış balıkların direkt tekne içinde veya kontamine olmuş tahta kasalarda taşınmasının etkili olabileceği düşünülmektedir.

İncelenen tüm gruplarda koliform grubu bakterilere sadece 1. gün rastlanmış (1-2 log kob/g), daha sonraki günlerde ise numunelerde bu bakteri yönünden herhangi bir üreme olmadığı (<1.00 log kob/g) görülmüştür (Tablo 1). Dondurularak muhafaza edilen vakumla ambalajlanmış aynalı sazan filetoları üzerine yapılan bir araştırmada da (24), koliform grubu bakteri sayısının muhafaza süresince azaldığı bildirilmiştir. Ayrıca bu araştırmada incelenen inci kefali numunelerinde fekal streptokoklar ile *Pseudomonas* spp.'ye rastlanmamıştır. Benzer bir şekilde dondurularak muhafaza edilen bütün ve temizlenmiş sazanlarda da muhafaza süresince fekal streptekokların tespit edilmediği belirtilmiştir (22).

TVB-N balıktaki mikroorganizmaların faaliyetleriyle meydana gelen parçalanma ürünleridir (5), ancak bu araştırmada muhafaza süresince dondurulan numunelerde mikroorganizmaların üremesi sınırlı kaldığından A grubu hariç diğer gruplarda TVB-N miktarları genellikle artmamıştır (Tablo 3). Bu nedenle incelenen numuneler, Kietzmann ve ark. (34)'nın sınıflandırmasına göre "iyi kalite" sınıfına girmiştir. Bu araştırmanın bulgularına benzer şekilde aynalı sazan filetolarında beşinci aya kadar (24), bütün ve temizlenmiş sazanlarda dördüncü aya kadar (22) bu değer yönünden önemli bir artış olmadığı, bütün ve fileto halinde buzda muhafaza edilen alabalıklarda da (35) TVB-N miktarlarının düzensiz bir seyir izlediği ve muhafazanın sonunda bile balıkların yüksek kalite sınıfında kaldığı belirtilmiştir. Ancak dondurularak muhafaza edilen temizlenmiş vakumlu/vakumsuz lüfer (14), berlam balığı (16), sudak filetoları (17), bütün/temizlenmiş inci kefali (21), kemani vatoz (36) ve havuz sazanı (37) üzerine yapılan çalışmalarda bu araştırmada elde edilen bulgulardan farklı olarak muhafaza süresince TVB-N miktarlarının arttığı belirtilmiştir. Araştırmalar arasındaki farklılıkların incelenen ürünlerin yaşadıkları habitat, muhafaza süreleri ve ürünlerin işlenmesi sırasında oluşan kontaminasyonlara bağlı olarak gelişen mikroorganizma faaliyetlerinden kaynaklandığı düşünülmektedir.

Bu araştırmada TBA miktarları ve peroksit sayısı muhafaza süresince genellikle düzensiz bir seyir izlemiş ve bu değerlerin bütün gruplarda arttığı görülmüştür. En yüksek değerler B grubunda belirlenirken, en düşük değerler ise D grubunda belirlenmiştir (Tablo 3). Yapılan farklı çalışmalarda Perez-Villarreal ve Howgate (13) ile Simeonidou ve ark. (16) berlam balıklarında, Karaçam ve Boran (15) bütün ve temizlenmiş hamsilerde, Olgunoğlu ve ark. (17) sudak filetolarında, Çaklı ve ark. (18) da sardalyada muhafaza süresince TBA miktarının yükseldiğini bildirmişlerdir. Ayrıca Perez-Villarreal ve Howgate (13) ile Karaçam ve Boran (15) da inceledikleri örneklerde muhafaza süresince peroksit sayılarının arttığını belirtmişlerdir. Bu araştırmada TBA miktarı ve peroksit sayısı bakımından bütün ile temizlenmiş ve vakumsuz ile vakumlu numuneler arasındaki fark önemli (p<0.05) bulunmuş, ayrıca temizlenmiş ve vakumsuz numunelerdeki bulguların daha yüksek olduğu görülmüştür (Tablo 4). TBA miktarı ve peroksit sayısının temizlenmiş ve vakumlanmamış numunelerde bütün ve vakumlu numunelerdekinden daha yüksek çıkması, bu numunelerin daha fazla oksijene maruz kalarak oksidasyona uğramasından kaynaklanmış olabilir.

İncelenen inci kefali numunelerinde 6.16-6.66 arasında değişen pH değerleri muhafaza süresince düzensiz bir seyir izleyerek muhafazanın son günlerinde bütün gruplarda düşmüştür (Tablo 3). pH değerleri bakımından bütün ile temizlenmiş ve vakumsuz ile vakumlu numuneler arasındaki fark istatistiksel olarak önemli (p<0.05) bulunmuş (Tablo 4), ancak belirlenen değerlerin grupların hiç birinde tüketilebilirlik sınırı olan 6.80-7.00 değerlerini (10,38) aşmadığı görülmüştür. Bu araştırmanın bulgularından farklı olarak dondurularak muhafaza edilen hamsi (15) ve alabalık (35) üzerine yapılan çalışmalarda örneklerdeki pH değerlerinde önemli bir değişiklik olmadığı belirtilmiştir. Ancak lüfer (14) ve kemani vatozun (36) incelendiği çalışmalarda ise pH değerlerinin muhafaza süresince arttığı bildirilmiştir.

Numunelerde başlangıçta 30 µg/100 g civarındaki vitamin A miktarları 60. güne kadar düzenli bir şekilde azalmış (~10 µg/100 g) ve bütün gruplarda daha sonraki günlerde bu miktar 1 µg/100 g'ın altına kadar düşmüştür (Tablo 5). Vitamin A miktarları bakımından bütün ile temizlenmiş numuneler arasındaki fark ve vakumsuz ile vakumlu numuneler arasındaki fark önemsiz bulunurken, zamanın etkisinin önemli (p<0.05) olduğu görülmüştür (Tablo 6). Vitamin D_3 miktarları da düzenli bir şekilde düşerek tüm gruplarda muhafaza süresinin sonunda ~10 µg/100 g olarak belirlenmiştir (Tablo 5). Bu vitamin bakımından vakumsuz ve vakumlu numuneler arasındaki fark önemsiz, bütün ve temizlenmiş balıklar arasındaki fark ve muhafaza süresinin etkisi istatistiksel olarak önemli (p<0.05) bulunmuştur (Tablo 6). İnsanların günlük vitamin A ihtiyacının 375-1000 µg ve vitamin D_3 ihtiyacının ise 5-10 µg olduğu belirtilmektedir (1). Buna göre inci kefali vitamin D_3 yönünden iyi bir kaynak olarak görünse de, vitamin A için aynı durum söz konusu değildir.

Duyusal analiz puanları bütün gruplarda azalmakla birlikte bu puanların sadece 9.00 (çok iyi) ile 6.50 (iyi) arasında değiştiği gözlenmiştir (Tablo 5). Benzer bir şekilde aynalı sazan filetolarında (24) da muhafaza süresinin son günlerine kadar duyusal analiz puanlarında önemli bir azalma olmadığı bildirilmiştir. Duyusal analiz puanlarına göre bütün ile temizlenmiş numuneler arasındaki fark ve zamanın etkisi istatistiksel olarak önemli (p<0.05) bulunmuştur (Tablo 6). Panelistler tarafından yapılan puanlamaya göre A grubundaki numuneler 120. güne kadar "çok iyi" ve 150. güne kadar "iyi", B ve D grubundaki numuneler muhafazanın sonuna kadar "çok iyi" ve C grubundaki numuneler ise 90. güne kadar "çok iyi" ve 120. güne kadar "iyi" kalite sınıfına girmiştir. Bu araştırmada duyusal analiz puanlarında belli bir azalma olurken, TVB-N ve TBA miktarlarının duyusal analiz bulgularını büyük ölçüde desteklediği görülmüştür. Huss (5) da, yapılan çalışmalarda genel olarak incelenen örneklerin TBA miktarları ile duyusal analiz bulguları arasında korelasyonların gözlemlendiğini bildirmiştir. Ancak bu araştırmada numunelerde belirlenen peroksit sayıları ile duyusal analiz bulguları arasında tam bir korelasyon bulunmamaktadır. Özellikle B grubunda 60. günden sonra peroksit sayısı yüksek çıkmasına rağmen, aynı dönemde duyusal analiz puanlarında düşüş olmakla birlikte numunelerin "çok iyi" kalite sınıfına girdiği görülmüştür. Bu nedenle inci kefalinde sadece peroksit sayısına bakılarak bir kalite sınıflandırması yapılmasının uygun olmayacağı düşünülmektedir. Nitekim, hidroperoksitlerin kokusuz ve tatsız bileşikler olması nedeniyle peroksit sayısının analizi yapılan ürünün gerçek duyusal kalitesiyle ilişkilendirilemeyeceği ve bu değerin doğrudan bir kalite kriteri olarak yorumlanmasının doğru olmadığı belirtilmektedir (5). Ayrıca Taliadourou ve ark. (20) tarafından analizler arasında her zaman tam bir korelasyon olmasının mümkün olamayacağı, balıkların tazeliğinin gerçek anlamda belirlenebilmesi için mikrobiyolojik, kimyasal ve duyusal analiz bulgularının birlikte değerlendirilmesinin önemli olduğu vurgulanmıştır.

Bölgede inci kefalinin pazarlanma şekli en önemli sorunlardan birini oluşturmaktadır. Bu balıklar gerekli muhafaza tedbirleri alınmadan günlük olarak pazara sürülmekte, aynı gün satılamayan balıklar önemli ölçüde zayi olmakta veya ertesi gün çok düşük fiyatlara satılmaktadır. Çoğu zaman balıklar sokaklarda açık havada ve sıcağın altında arabalarda satılmaya çalışılmakta, bazen de satılamayan balıklar yeni tulumuş balıklarla karıştırılarak pazarlanmaktadır. Bunlardan dolayı, halk sağlığı potansiyel risklerle karşı karşıya kalmakta ve ekonomik kayıplar meydana gelmektedir (39). Bu durum da soğuk hava depolarının ne kadar önemli olduğunu ortaya koymakta ve bölgede önemli bir sorunu gözler önüne sermektedir.

Bölge halkı avlanan inci kefalini muhafaza etmek için çok eski bir depolama ve saklama şekli olan tuzla muhafaza yöntemini devam ettirmekte ve bu yöntem en yaygın kullanılan muhafaza şekli olarak karşımıza çıkmaktadır. Bunda yöre halkının yıllardan beri süregelen alışkanlıklarını devam ettirmek istemesinin yanında, buna alternatif olabilecek soğutma ve dondurma imkânlarının kısıtlı olmasının da etkisi olabilir.

Yöredeki tüketiciler uzun yıllardan beri balıkları, bol bulunduğu mevsimlerde en pratik muhafaza şekli olan tuzlayarak muhafaza etmektedir. Günümüzde avlanan inci kefalinin %40'ının tuzlu olarak tüketildiği, piyasadan toplanan ve geleneksel yöntemlerle tuzlanan balıkların hijyenik kalitelerinin çok kötü ve neredeyse tamamının bozulmuş durumda olduğu, ayrıca bu balıkların sağlık sorunlarına yol açabileceği bildirilmiştir (40). Kılınççeker ve Küçüköner (19) tuzlu balıklardaki TBA ve TVB-N değerlerinin sırasıyla 3.31-5.05 mg/kg ve 43.60-173.04 mg/100 g arasında değiştiğini, tuz içeriğinin ise oldukça yüksek (%15.10-18.26) olduğunu, özellikle sağlık sorunları yaşayan kişilerin tuzlu balık tüketiminde dikkatli ol-

maları gerektiğini bildirmişlerdir. Hijyenik ve kimyasal kaliteleri iyi olsa bile tüketilen balıkların muhafazasında kullanılan yüksek miktarlardaki tuz başlı başına bir sağlık problemi oluşturmaktadır. Gıdalarla birlikte alınan fazla miktardaki tuzun özellikle yüksek tansiyon ile özafagus ve mide kanserine yol açtığı (41,42), Van ve çevresinde de yüksek tansiyon ve sindirim sistemi kanserinin yaygın olarak görüldüğü bildirilmiştir (43,44). Sarı ve ark. (40), yörede yüksek tansiyonun yaygın olarak görülmesi ile tuz oranı oldukça fazla olan tuzlu balık tüketimi arasında önemli bir ilişkinin olduğunu belirtmişlerdir. Hijyenik olmaması ve bazı sağlık riskleri taşıması nedeniyle yöre halkına tuzlu balık tüketiminin zararları sık aralıklarla düzenlenecek seminer ve konferanslarla anlatılarak vakit geçirmeden insanların bilinçli bir şekilde tuzlu balık tüketimini terk etmesi veya azaltması sağlanmalıdır. Ayrıca yapılacak kapsamlı çalışmalarla bölge halkının kaliteli balık tüketebilmesini sağlayacak alternatif muhafaza yöntemleri devreye konulmalıdır. Uzun bir raf ömrüne sahip olan ve sağlık açısından risk taşımayan dondurarak muhafazanın da bu konuda en etkili ve yaygınlaştırılması gereken önemli bir yöntem olduğu düşünülmektedir.

Gölden avlanan inci kefalinin önemli bir kısmı taze olarak tüketilmektedir. Bu araştırmada baş ve iç organları çıkarılarak temizlenmiş ve bütün haldeki inci kefalinin vakumlu ve vakumsuz ambalajlarda dondurularak muhafaza edilme imkânları denenmiş ve muhafaza süresince meydana gelen kalite değişimleri incelenmiştir. Bu araştırmada inci kefalinin baş ve iç organlarını çıkarmak suretiyle depolanmasının bu balıkların dayanma sürelerini arttırdığı belirlenmiştir. Ayrıca, inci kefalinin işlenmiş bir şekilde dondurularak tüketime sunulmasının balık popülasyonunun korunması, halk sağlığı açısından potansiyel risklerin azaltılması ve ekonomik kalkınma açısından önemli sayılabilecek birçok faydasının da olabileceği düşünülmektedir. Nitekim; kışın avlanan fazla miktardaki balıkların zayi olması önlenebilir ve üreme dönemi balıkçılığı engellenerek Van Gölü'ndeki balık popülasyonu korunabilir. İnci kefali endüstriye kazandırılarak katma değeri yüksek yeni istihdam alanları oluşturulabilir, değişik şekillerde stok yapılabilmesi ve oluşan rekabete bağlı olarak piyasada fiyat istikrarı sağlanabilir ve bölge ekonomisi canlanabilir. Balığın baş ve iç organlarının çıkarılarak temizlenmesi tüketiciler tarafından rağbet görebilir ve temizleme zahmeti ortadan kalkacağı için zamandan tasarruf sağlanarak balık tüketimi teşvik edilebilir. İşlenmiş ürünler daha az yer kaplayacağından taşıma/pazarlama işlemleri kolaylaşabilir ve ambalajlanmış ürünler de dış etkilerden korunduğu için balıklar en az kalite kaybı ile tüketicilere sunulabilir. İşlenmiş veya işlenmemiş inci kefalinin dondurulması ile bu balığın pazar arzı dengelenebileceği gibi, taze balığın bulunmadığı zamanlarda depolanmış iyi kalitede balığın piyasaya sunulması ile tüketici talebi karşılanabilir. Böylelikle bölge halkının kaliteli ve ucuz bir protein kaynağı olan inci kefalinden daha uzun yıllar yararlanması sağlanabilir. Ancak bütün bu faydaların sağlanabilmesi için ilgili kuruluşlar denetimlerini arttırmalı, bilgilendirme faaliyetlerini yaygınlaştırmalı ve bölgede mevcut haliyle devam eden hijyenik olmayan pazarlama şekli terk edilerek bu balıklar modern satış yerlerinde satılmalıdır.

Sonuç olarak; inci kefalinin temizlenerek ve vakumlu bir şekilde ambalajlanmasının uygun bir yöntem olduğu ve bu şekilde işlenen balıkların dondurularak 150 gün süreyle kaliteli bir şekilde muhafaza edilebileceği belirlenmiştir. Bölgede inci kefalinin muhafazasında eskiden beri kullanılan sağlıksız bir yöntem olan tuzla muhafazaya alternatif bir yöntem olarak balıkların dondurularak muhafazası önerilebilir. Böylelikle tüketiciler bölgede avlanmanın yasak olduğu dönemlerde bile duyusal özelliklerini kaybetmemiş inci kefaline kolaylıkla ulaşabilecektir.

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Yazarlar tarafından beyan edilecek bir çıkar çatışması yoktur.

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ES araştırmanın planlanmasında; ES, KE ve HS numunelerin alınması ve işlenmesinde görev aldı. Analizlerin yapılması, araştırmanın yazılması ve son kontroller bütün yazarların katkılarıyla gerçekleştirildi.

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ARAŞTIRMA MAKALESİ

Alternatif Silaj Bitkilerinin AHP-TOPSIS Yöntemi Kullanarak Değerlendirilmesi

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Öz

Mısır silajı ruminant hayvanların beslenmesinde kullanılan önemli bir yem bitkisidir. Ancak iklim değişikliğine bağlı olarak artan kuraklık probleminin ilerleyen yıllarda bu bitkinin silajlık olarak kullanımını kısıtlaması beklenmektedir. Dolayısıyla mısır bitkisine alternatif silajlık bitkilerin belirlenmesi önemli bir konu haline gelmiştir. Bu çalışmada silaj bitkisi seçiminde yeşil ot verimi, kuru madde (KM) oranı, pH, ham protein (HP) ve sindirilebilir KM oranı kriterlerine dayanarak bir analitik hiyerarşi prosesi (AHP) modeli kullanılmıştır. AHP analizinde yeşil ot veriminin %30, KM'nin %21, sindirilebilir KM'nin %19, pH'nın %18 ve HP'nin %11 oranında etkili olduğu belirlenmiştir. Bu kriterler İdeal Çözüme Benzerliğe Göre Tercih Sırası Tekniği (TOPSIS) analizine entegre edilerek skorlama yapıldığında mısır, yonca, sorgum, ayçiçeği ve buğday hasılının sırasıyla 0.729, 0.715, 0.618, 0.513 ve 0.273 puana sahip oldukları tespit edilmiştir. Sonuç olarak mısır silajından sonraki en iyi seçeneğin yonca silajı olabileceği, ancak bu modellemenin belirli bölgelere özgü saha çalışmaları yapılarak su kullanım verimliliği ve kuraklığa dayanıklılık kriterleri göz önüne alınarak yapılmasının daha etkili sonuçlar vereceği kanısına varılmıştır.

Anahtar Kelimeler: AHP, mısır, silaj, TOPSIS

Evaluation of Alternative Silage Crops Using AHP-TOPSIS Method

Abstract

Maize silage is an important forage plant used to feed ruminant animals. However, due to climate change, increasing drought problems are expected to restrict the use of this plant as silage in the future. Hence, finding alternative plants for silage besides corn has become a significant concern. In this study, an Analytical Hierarchy Process (AHP) model was used based on the criteria of green grass yield, dry matter (DM), pH, crude protein (CP), and digestible DM for selecting silage plants. The AHP analysis determined that green grass yield was 30%, DM was 21%, digestible DM was 19%, pH was 18%, and HP was 11% effective. When these criteria were integrated into Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) analysis and scoring were performed, and it was determined that maize, alfalfa, sorghum, sunflower, and whole crop wheat had 0.729, 0.715, 0.618, 0.513, and 0.273 points, respectively. Following maize silage, alfalfa silage emerged as the subsequent favorable choice. However, it was concluded that this modelling would yield more effective results if field studies specific to certain regions were conducted and water use efficiency and drought resistance criteria were considered.

Key Words: AHP, maize, silage, TOPSIS

GİRİŞ

Yem bitkilerinin silaj şeklinde muhafazası, hasattan sonra depolama sırasında besin maddesi kaybını en aza indirebildiği için önemli ve popüler bir yem depolama yöntemi haline gelmiştir (1). Mısır (*Zea mays* L.), yüksek verimliliği ve besin kalitesi nedeniyle sığırlar için en önemli yem bitkisidir. Dolayısıyla kolay fermente edilebilir bir enerji kaynağı olan mısır silajı dünyanın birçok yerindeki yetiştiriciler tarafından kullanılmaktadır (2). Hava sıcaklığının yüksek seyrettiği dönemlerde meydana gelen susuzluk stresi, yoğunluğa ve süreye bağlı olarak sürgün büyümesinde ve yaprak alanında önemli azalmalara neden olduğu için mısır silajının verimini ve kalitesini düşürebilir (3). Dolayısıyla iklim değişikliğinin mısır üretiminin sekteye uğratma riskini artıracağı öngörüldüğünden (4) daha kurak koşullarda, daha az su gerektiren, yem üretimi ve gübre yönetimini entegre eden üretim sistemine uyan ve tarımsal sürdürülebilirliği koruyan alternatif silajlık yem bitkileri arayışı sürmektedir (5).

Yemlik sorgum (*Sorghum bicolor* L.), mısır yetiştirmek için yeterli yağışın olmadığı bölgelerde mısırın alternatifi olarak kullanılmaktadır. Çünkü mısırla karşılaştırıldığında daha yüksek su kullanım verimliliği ve kuraklık stresine dayanıklılık gibi çeşitli avantajlara sahiptir (6). Yemlik sorgum, genellikle biçilerek ya da doğrudan otlatılarak kullanılmasının yanı sıra yağışın mısır büyümesini sınırladığı yerlerde silajlık olarak değerlendirilebilir (7). Ayçiçeği (*Helianthus annuus* L.) yetiştiriciliği genellikle yağmur suyuna dayalı olarak yapıldığı için mısıra göre daha az suya ihtiyaç duymaktadır. Ayçiçeği silajı mısır silajı ile karşılaştırıldığında besin maddesi yönünden büyük oranda benzerlik gösterir. İki silaj arasındaki en önemli fark ayçiçeği silajının mısır silajından daha fazla HP ve yağa sahip olmasıdır (8). Yetiştirme koşulları mısır için olumsuz olduğu durumlarda ruminantların beslenmesi için ayçiçeği silajının kullanımı, mısır silajının kullanımına bir alternatif olabilmektedir (9). Yine mısır silajına alternatif olarak buğday (*Triticum spp.*) gibi hızlı büyüme süresine sahip ürünler kullanılabilir (10). Süt olum döneminde biçilerek silajlanan buğday hasılının mısır silajına göre yaklaşık %2 oranında daha fazla HP'ye sahip olduğu bildirilmiştir (11). Ancak daha düşük metabolik enerji ve yeşil ot verimine sahip olduğundan buğday silajının maliyeti mısır silajına göre daha fazla olabilmektedir (10). Yonca silajı yüksek HP (rumende parçalanabilen protein açısından yüksek ve rumende parçalanamayan protein açısından düşüktür) oranına sahiptir. Mısır silajı, yüksek nişasta içeriği nedeniyle fermente edilebilir karbonhidratların iyi bir kaynağı olmasına rağmen düşük HP oranı bulunmaktadır (12). Diğer yandan, silajlık mısır, yoncadan daha yüksek verime ve enerji içeriğine sahip olmanın yanı sıra hasat için daha az iş gücü gerektirmektedir (13).

Farklı kriterler göz önüne alındığında alternatif silaj bitkilerinin arasından yapılacak olan tercihler karmaşık bir hale gelmektedir. Bu kriterlerin değerlendirilerek mısıra alternatif en uygun silaj bitkisinin belirlenmesi yetiştiricilerin karlı ve sürdürülebilir bir üretim yapmalarına olanak sağlayabilir.

Analitik hiyerarşi prosesi (AHP), bu tür karmaşık kararlar için bir model oluşturup farklı kriterlerin veya faktörlerin önem derecelerini değerlendirmeyi kolaylaştırarak uygun çözüm önerileri sunmaya yardımcı olan çok kriterli karar verme (ÇKKV) yöntemlerinden biridir (14). Birçok farklı alanda kullanılabilen AHP'yi diğer ÇKKV'ler den ayıran özellik hem objektif hem de subjektif fikirlerin karar sürecine katılmasına imkân sağlamasıdır (15,16). İdeal Çözüme Benzerliğe Göre Tercih Sırası Tekniği (TOPSIS) yöntemi herhangi bir dönüşüme gerek duyulmaksızın elde bulunan veri üzerinde direkt olarak uygulanabilmektedir. Bu yöntemde, seçenekler belirli kriterlerin minimum ve maksimum değerlerine göre çözüme en uygun uzaklıkları skorlanarak sıralanır. Çok kriterli süreçlerde her bir kriterin karar matrisinde bir değere sahip olduğu durumda AHP-TOPSIS yönteminin birlikte kullanımı alternatif seçeneklerin sıralamasının sayısal değerler ile oluşturulmasını sağlar (17). Bu çalışmada yemlik sorgum, ayçiçeği, buğday ve yonca silajlarının yeşil ot verimi, kuru madde (KM) oranı, pH, ham protein (HP) ve sindirilebilir KM oranı kriterlerine göre AHP-TOPSIS hibrit yöntemi yardımıyla mısır silajı ile karşılaştırılarak alternatif silaj bitkilerinin öncelik sıralamalarının yapılması amaçlanmıştır.

MATERYAL VE METOT

Verilerin Toplanması

AHP'nin diğer ÇKKV yöntemlerine göre en büyük avantajı istatistiksel olarak geçerli sonuçlar elde etmek için büyük bir örneklem boyutu gerektirmemesidir. Uzman kişi görüşlerine dayanan bu analiz için örneklem büyüklüğünün ikiden fazla olması yeterli kabul edilir (18). Bu çalışmada, AHP analizi için Türkiye'de Hayvan Besleme ve Beslenme Hastalıkları ve Tarla Bitkileri alanında doktorasını tamamlamış 13 uzman kişiyle anket çalışması yapılmıştır (Şekil 1). Anket sorularında silaj bitkisi tercih edilirken dikkat edilen kriterler arasında ikili karşılaştırma yapılması istenmiş ve bu analiz sonucunda silaj üretiminde önemli olan yeşil ot verimi (yıllık), KM oranı, pH, HP ve sindirilebilir KM oranı kriterlerinin yüzdesel ağırlıkları belirlenmiştir.

	Daha önemli								Eşit önem	Daha önemli							
	←									→							
Yeşil Ot Verimi	9 □	8 □	7 □	6 □	5 □	4 □	3 □	2 □	**1** ■	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □	Kuru Madde Oranı
Yeşil Ot Verimi	9 □	8 □	7 □	6 □	5 □	4 □	3 □	2 □	**1** ■	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □	pH
Yeşil Ot Verimi	9 □	8 □	7 □	6 □	5 □	4 □	3 □	2 □	**1** ■	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □	Ham Protein
Yeşil Ot Verimi	9 □	8 □	7 □	6 □	5 □	4 □	3 □	2 □	**1** ■	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □	Sindirilebilir Kuru Madde
Kuru Madde Oranı	9 □	8 □	7 □	6 □	5 □	4 □	3 □	2 □	**1** ■	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □	pH
Kuru Madde Oranı	9 □	8 □	7 □	6 □	5 □	4 □	3 □	2 □	**1** ■	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □	Ham Protein
Kuru Madde Oranı	9 □	8 □	7 □	6 □	5 □	4 □	3 □	2 □	**1** ■	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □	Sindirilebilir Kuru Madde
pH	9 □	8 □	7 □	6 □	5 □	4 □	3 □	2 □	**1** ■	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □	Ham Protein
pH	9 □	8 □	7 □	6 □	5 □	4 □	3 □	2 □	**1** ■	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □	Sindirilebilir Kuru Madde
Ham Protein	9 □	8 □	7 □	6 □	5 □	4 □	3 □	2 □	**1** ■	2 □	3 □	4 □	5 □	6 □	7 □	8 □	9 □	Sindirilebilir Kuru Madde

Şekil 1. İkili karşılaştırma için uzmanlara yöneltilen anket

TOPSIS analizi için mısır, sorgum, ayçiçeği, buğday hasılı ve yonca silajlarının yeşil ot verimi, KM oranı, pH, HP ve sindirilebilir KM oranları PubMed, Scopus ve Google Scholar veri tabanlarındaki literatür verilerinden ve farklı çalışmalardan elde edilen verilerin ortalamaları olarak alınmıştır (Tablo 1). Literatür verilerinin ortalamalarının alınması bu bitkilerin farklı varyeteleri arasındaki farklılıkların en aza indirilmesini sağlamıştır.

Tablo 1. Literatür verilerinden elde edilen silaj parametrelerinin ortalamaları

	Yeşil ot (t/d)	KM (g/kg)	pH	HP (g/kg)	Sindirilebilir KM (g/kg)
Mısır	8.73[1-5]	294.25[3-10]	3.86[3,4,6-10]	78.64[3-10]	627.17[3,4,6-9]
Sorgum	7.30[11-13]	265.83[12-17]	3.83[12-15]	80.83[12-17]	585.33[12-17]
Ayçiçeği	6.50[18,19]	214.50[18-21]	4.57[18,20,21]	114.75[18-21]	569.75[18-21]
Buğday	3.07[22,23,25]	334.00[22-24]	4.08[22-25]	82.00[22,24]	626.00[22-24]
Yonca	7.18[26,27]	324.00[20,28-30]	5.10[28,20]	172.5[20,28-30]	642.5[20,29,30]

[1]Bai ve ark. (19), [2]Yıldız ve ark. (20), [3]Filya (21), [4]Li ve ark. (22), [5]Yılmaz ve ark. (23), [6]Khan ve ark. (24), [7]Alvarado-Ramírez ve ark. (25), [8]Huang ve ark. (26), [9]Sırakaya (27), [10]Meeske ve Basson (28), [11]Arıcı ve Avcı (29), [12]Farhadi ve ark. (30), [13]Terler ve ark. (31), [14]Rodrigues ve ark. (32), [15]Arriola ve ark. (33), [16]McCary ve ark. (34),[17]Lv ve ark. (35), [18]Demirel ve ark. (36), [19]Erdoğan ve Yıldız (37), [20]Tan (38), [21]Santos ve ark. (39), [22]Filya (40), [23]Nadeau (41), [24]Crovetto ve ark. (42), [25]Xie ve ark. (43), [26]Keskin ve ark. (44), [27]Albayrak ve Öten (45), [28]Gao ve ark. (46), [29]Broderick (47), [30]Wang ve ark. (48). KM: Kuru madde, HP: Ham Protein, t/d: ton/dekar

AHP-TOPSIS Yöntemi

AHP, ÇKKV sürecinde hedef, kriterler (varsa alt kriterler) ve seçimler (alternatifler) arasındaki ilişkinin hiyerarşik bir yapıda modellenebilmesinde kullanılmaktadır. Birden fazla değerlendirme ölçütünün bulunduğu karar süreçlerinde, farklı kriterlerin sonuç üzerindeki etkisinin belirlenebilmesi için "kriter ağırlıkları" hesaplanarak en uygun seçime ulaşılabilmektedir. Bu modelde karar kriterlerinin alternatif seçeneklerle karşılaştırılması uzman kişiler yardımıyla gerçekleştirilmekte ve her alternatif kararın öncelik sırasının belirlenmesi sağlanmaktadır. Hedefe ulaşılması için oluşturulan hiyerarşinin her kademesindeki kriterlerin göreceli önem derecelerini belirlemek amacıyla kriterler arasında ikili karşılaştırma yapılır, ardından sonra en iyi kararı vermek için alternatifler kıyaslanır (49).

Bu çalışmada AHP modelinde yer alan kriterlerin ikili karşılaştırmaları yapılarak Denklem 1 yardımıyla kriterler için ikili karşılaştırma matrisi elde edilmiştir. Bu karşılaştırmalar uzmanlara yöneltilen anket sorularına göre kriterlerin arasındaki bağıl önem (üstünlük) derecelerinin belirlenmesi ile yapılmış ve önem derecelerinin bulunması için Saaty (49) tarafından geliştirilen 9 skaladan oluşan karşılaştırma ölçeği kullanılmıştır. Anket çalışmasından elde edilen ikili karşılaştırmaların sonuçlarının geometrik ortalaması alınmıştır.

$$A=\begin{bmatrix} 1 & \alpha_{12} & \cdots & \alpha_{1n} \\ \alpha_{21} & 1 & \cdots & \alpha_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ \alpha_{n1} & \alpha_{n2} & \cdots & 1 \end{bmatrix} \text{nxn} \quad (1)$$

Karşılaştırma matrisinde her sütundaki değer aynı sütundaki değerlerin toplamına bölünerek, elde edilen yeni değerler ile normalize matris $[b_{ij}]_{nxn}$ oluşturulmuştur (Denklem 2) (50).

i=1,2,3,….,n ve j= 1,2,3,…, n olmak üzere;

$$b_{ij} = \frac{\alpha_{ij}}{\sum_{i=1}^{n} \alpha_{ij}} \quad (2)$$

Karşılaştırma matrisi normalize edildikten sonra her satırın toplamı matris boyutuna (n, kriter sayısına) bölünerek aritmetik ortalamaları alınmıştır (Denklem 4). Bu işlemden sonra her bir satır için ayrı ayrı (kriter sayısı kadar) öncelik vektörleri (w) hesaplanmıştır (51). Bu öncelik vektörü kriterlerin önem ağırlıklarıdır.

i=1,2,3,….,n ve j= 1,2,3,…, n olmak üzere;

$$w_i = \frac{\sum_{i=1}^{n} b_{ij}}{n} \quad (4)$$

Uzman kişilerden alınan yanıtların tutarlılık oranını (CR) hesaplamak amacıyla Saaty ve Tran (52) tarafından hazırlanan RI değerlerinden yararlanılmıştır. Bu değerler n kriter sayısına sahip (1-15 arasındaki) matrisin büyüklüğüne göre verilen RI değerlerini göstermektedir.

İkili karşılaştırma matrislerinin tutarlılık oranının <0.10 olması gereklidir. Eğer bu değer sağlanamazsa uzman kişilerle tekrar görüşülerek tutarlılık oranı 0.10'dan küçük olana kadar yeniden düzenleme yapılır (53).

CR'nin hesaplanması için öncelikle normalize edilmemiş karşılaştırma matrisi (A, $[\alpha_{ij}]_{nxn}$) ile $[w_i]_{nx1}$ matrisi çarpılarak sütun vektörü (d, $[d_i]_{nx1}$) hesaplanmıştır (Denklem 5). Daha sonra aynı satırdaki sütun vektörü elemanlarının aynı satırdaki öncelik vektörü elemanlarına olan oranları toplanarak λmax hesaplanmıştır (Denklem 6). λmax kullanılarak Denklem 7'de verilen formüle göre tutarlılık indeksi (CI) değeri bulunmuştur. CI değeri RI tablosunda aynı matris boyutuna yani 5'e denk gelen RI değerine (1.12) bölünerek CR değeri elde edilmiştir (Denklem 9) (53).

i=1,2,3,….,n ve j= 1,2,3,…, n olmak üzere;

$$[\alpha_{ij}]_{nxn} \times [w_i]_{nx1} = [d_i]_{nx1} \quad (5)$$

$$\lambda_{\max} = \frac{\sum_{i=1}^{n} \frac{d_i}{w_i}}{n} \quad (6)$$

$$CI = \frac{(\lambda_{max} - n)}{(n-1)} \quad (7)$$

Temelleri Hwang ve Yoon (54) tarafından atılan TOPSIS yöntemi daha sonra Hwang ve ark. (55) tarafından genişletilmiş ve yeni TOPSIS yöntemi geliştirilmiştir. AHP'ye göre farklılığı nitel verilerin nicel verilere dönüştürülmesi gibi bir aşaması olmaması ve direkt olarak toplanan ya da elde bulunan nicel veriler üzerinde uygulanabilmesidir. Bu ÇKKV yönteminin araştırmacılar tarafından ilgi görme nedeni karmaşık algoritmalar ve matematiksel işlemler içermemesinin yanı sıra problemin çözümünde geometrik olarak negatif ideal çözüme en uzak pozitif ideal çözüme ise en yakın mesafede olan değerleri elde etmeye yönelik olmasıdır (56).

İlk olarak Tablo 1'deki literatür verilerinden 5x5 boyutlu bir karar matrisi (A) oluşturulmuştur. Matrisin normalize edilmesi için karar matrisinin her sütunundaki bütün elemanların kareleri alınarak toplanmıştır. Her satırdaki kriter

değeri sütun elemanlarının kareleri toplamının karekökü ne bölünerek normalize edilmiştir (Denklem 8) (57).

i=1,2,3,….,m ve j= 1,2,3,…, n olmak üzere;

$$b_{ij} = \frac{\alpha_{ij}}{\sqrt{\sum_{l=1}^{m} \alpha_{ij}^2}} \quad (8)$$

Ağırlıklandırma için AHP analizinden elde edilen veriler kullanılmıştır. Normalize edilmiş matriste aynı sütunda bulunan her bir değer aynı sütunun yani kriterin ağırlıklandırma derecesi ile çarpılarak normalize W matrisi (V) elde edilmiştir (Denklem 9).

$$W=\begin{bmatrix} w_1 b_{11} & w_2 b_{12} & \cdots & w_n b_{1n} \\ w_1 b_{21} & w_2 b_{22} & \cdots & w_n b_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ w_1 b_{m1} & w_2 b_{m2} & \cdots & w_n b_{mn} \end{bmatrix} = V = \begin{bmatrix} v_{11} & v_{12} & \cdots & v_{1n} \\ v_{21} & v_{22} & \cdots & v_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ v_{m1} & v_{m2} & \cdots & v_{mn} \end{bmatrix} \quad (9)$$

Alternatif seçiminde fayda sağlayacak pozitif ideal çözüm değerleri (Denklem 10) ve dezavantaj sağlayacak negatif ideal çözüm değerleri bulunmuştur (Denklem 11) (58).

i=1,2,3,….,m ve j= 1,2,3,…, n olmak üzere;

$$V^+ = \left(max_j^{vij}\right) = (v_1^+, v_2^+, v_3^+ \ldots . v_m^+) \quad (10)$$

$$V^- = \left(min_j^{vij}\right) = (v_1^-, v_2^-, v_3^- \ldots . v_m^-) \quad (11)$$

V^+ ve V^- değerleri belirlendikten sonra negatif ideal çözüme en uzak, pozitif ideal çözüme ise en yakın olan mesafeler bulunmuştur. Pozitif ideal uzaklık formülü için Denklem 12 ve negatif ideal uzaklık formülü için Denklem 13 kullanılmıştır. Bu denklemlere göre V matrisinin satırındaki her değer ile sütunun (kriterin) pozitif ideal veya negatif ideal çözüm değerleri arasındaki farkların karelerinin toplamının karekökü hesaplanmıştır. Bu hesaplama sonucunda alternatif (karar noktası) sayısı kadar S_i^- ve S_i^+ işlemi gerçekleştirilmiştir.

$$S_i^+ = \sqrt{\sum_{j=1}^{n} (V_{ij} - v_j^+)^2} \quad (12)$$

$$S_i^- = \sqrt{\sum_{j=1}^{n} (V_{ij} - v_j^-)^2} \quad (13)$$

İdeal çözüme olan göreli yakınlık hesaplanırken (C_i^+) negatif ideal çözüme en uzak olan alternatif bulunmuştur. Bu da pozitif ideal çözüme en yakın olan alternatifin bulunmasını sağlamıştır. Tüm alternatiflerin S_i^+ ve S_i^- değerleri Denklem 14'te gösterildiği gibi hesaplanarak sıralama (C_i^+) değerleri bulunmuştur. C_i^+ değerlerine göre negatif ideal çözüme olan uzaklıklar sıralanmıştır (59).

$$C_i^+ = \frac{S_i^-}{S_i^+ + S_i^-} \quad (14)$$

BULGULAR

Uzmanların yaptığı karşılaştırmaların geometrik ortalamaları alındıktan sonra elde edilen karşılaştırma matrisi ve sorulara verilen yanıtların tutarlılık oranı Tablo 2'de verilmiştir. Yapılan karşılattırmaların tutarlılık oranı 0.09 olarak bulundu.

Tablo 2. AHP analizinde kullanılan karşılaştırma matrisi, kriter ağırlıkları (w_i) ve tutarlılık oranı (CI/RI).

	Yeşil Ot Verimi	KM	pH	HP	Sindirilebilir KM	d_i	λmax	CI	CI/RI
Yeşil Ot Verimi	1.00	1.81	1.18	2.74	2.15	1.62	5.40	0.10	0.09
KM Oranı	0.55	1.00	2.63	1.93	0.47	1.16			
pH	0.84	0.38	1.00	2.05	1.09	0.95			
HP	0.36	0.52	0.49	1.00	0.89	0.59			
Sindirilebilir KM	0.47	2.14	0.92	1.12	1.00	1.08			
w_i	**0.30**	**0.21**	**0.18**	**0.11**	**0.19**				

Wang ve ark. (48). KM: Kuru madde, HP: Ham Protein. d_i: sütun vektörü, λmax: öz vektör, CI: tutarlılık indeksi, RI: rassal indeks.

Karşılaştırma matrisi normalize edildikten sonra öncelik vektörleri hesaplanmıştır (Tablo 2). Buna göre silaj bitkisi seçilirken bakılan kriterlerden yeşil ot veriminin %30, KM'nin %21, sindirilebilir KM'nin %19, pH'nın %18 ve HP'nin %11 oranında etkili olduğu belirlenmiştir. Kriterlerin ağırlık oranları TOPSIS analizinde literatür verilerinin ağırlıklandırılması için kullanılarak Tablo 3'teki normalize matris elde edilmiştir.

Tablo 3. TOPSIS analizi sonuçları

	Yeşil ot (t/d)	KM (g/kg)	pH	HP (g/kg)	Sindirilebilir KM (g/kg)	S^+	S^-	C^+
Mısır	0.172	0.097	0.073	0.035	0.088	0.044	0.117	0.729
Sorgum	0.144	0.088	0.073	0.036	0.083	0.055	0.089	0.618
Ayçiçeği	0.128	0.071	0.087	0.051	0.080	0.067	0.070	0.513
Buğday	0.060	0.110	0.077	0.036	0.088	0.119	0.045	0.273
Yonca	0.141	0.107	0.097	0.076	0.091	0.039	0.098	0.715
	Mak	Mak	Min	Mak	Mak			
V^+	0.172	0.110	0.073	0.076	0.091			
V^-	0.060	0.071	0.097	0.035	0.080			

KM: Kuru madde, HP: Ham Protein, S+: pozitif ideal çözüm, S^-: pozitif ideal çözüm, C^+: sıralama değerleri, V^+: maksimum fayda, V^-: minimum fayda.

Yeşil ot verimi, KM, HP ve sindirilebilir KM için en yüksek değerler V^+, pH için en düşük değerler V^+ olarak belirlenmiştir. Tüm silaj alternatiflerinin S_i^+ ve S_i^- değerleri hesaplanarak sıralama skorları (C_i^+) bulunmuştur (Tablo 3). Sıralama değerlerine göre 0.729 puanla mısır bitkisinin en iyi silaj seçeneği olduğu ve bunu sırasıyla 0.715 puanla yoncanın, 0.618 puanla sorgumun, 0.513 puanla ayçiçeğinin ve 0.273 puanla buğday hasılının takip ettiği belirlenmiştir.

TARTIŞMA VE SONUÇ

Uzmanların yaptıkları ikili karşılaştırmalara göre yeşil ot veriminin yüksek olması bir bitkinin silaj materyali olarak seçilmesini önemli ölçüde etkileyeceğini göstermektedir. Önceki çalışmalarda silajlık mısırın yeşil ot verimine bakıldığında dekar başına ortalama 8,73 tonluk bir üretimin olabildiği görülmektedir (19-23). Dolayısıyla mısırın silaj bitkileri arasında birinci sırada olmasında yüksek yeşil ot veriminin etkili olduğu söylenebilir. Diğer yandan sorgum bitkisinin yoncadan daha yüksek yeşil ot verimine sahip olmasına rağmen seçim sıralamasında geride kalması yeşil ot veriminin tek başına etkili olmadığını ve diğer kriterlerin bu seçimi etkilediğini göstermiştir.

Yonca silajının KM, HP ve sindirilebilir KM kriterleri ele alındığında sorgum silajından daha üstün olması bu silajın mısırdan sonra ikinci sırayı almasını sağlamıştır. Yüksek besin değerlerine sahip olan ve dünya çapında en önemli yem bitkisi olarak bilinen yonca, kuru ot veya silajl için yetiştirilmektedir (60). Mısırın silajlık olarak yetiştirilmesi, birim su başına yoncaya kıyasla yaklaşık iki kat daha yüksek brüt CO_2 asimilasyon oranına ulaşarak su kullanımında çok daha verimli bir seçenek haline gelir. Bu da mısırın su kullanım verimliliğinin yoncaya göre %70 daha fazla olmasını sağlamaktadır (13). Diğer yandan, silajlık mısırın yoncadan daha yüksek verime sahip olması ve daha az iş gücü gereksinimine duyması, mısır silajının yoncaya göre öncelikli olarak seçilmesinin nedenlerindendir (61).

Sorgum bitkisinin su, azot, fosfor ve potasyum kullanım etkinliği mısıra göre daha fazla olduğu için yeşil ot verimi düşük olmasına rağmen göreceli olarak daha fazla biyokütle üretebilmektedir (62). Elde edilen bulgulara benzer şekilde Arriola ve ark. (33) mısırın sorgum silajına göre daha yüksek KM'ye sahip olduğunu bildirmişlerdir. Dolayısıyla sorgum silajının KM'sinin düşük olması bu silajın AHP-TOPSIS analizine göre yonca silajından sonra seçilebilecek ikinci alternatif olmasında etkili olmuştur. Sorgum bitkisinin ADF oranı genel olarak mısıra göre daha fazla olduğundan ruminantlardaki sindirilme derecesi düşük olabilmektedir (63). Sindirilebilir KM değerinin diğer bitkilere göre daha düşük olması, sorgumun öncelik sırasında önemli ölçüde etkili olmuştur. Bu çalışmada, sorgum silajı yonca silajından sonraki alternatif olsa da, sorgumun su kullanım etkinliğinin yüksek olması (62) yonca silajından daha öncelikli olarak kullanılmasını sağlayabilmektedir. Ancak bu bitkilerin su kullanım verimleri ile ilgili nicel bilgi yetersizliği ilgili kriterin TOPSIS analizine eklenmesini kısıtlamıştır.

Mısır silajıyla karşılaştırıldığında HP oranının yüksek olması ayçiçeği silajını öne çıkarmaktadır (38). Diğer yandan ayçiçeği bitkisinin düşük KM ve yüksek ADF içeriği pH değerinin yüksek olmasına neden olabilmekte ve bu bitkinin silolanması sırasında problemlere yol açabilmektedir (37,38). Bu nedenlere bağlı olarak mevcut çalışmada AHP-TOPSIS analizi sonucunda ayçiçeği silajı mısır silajına alternatif olabilecek silajlar arasında üçüncü sırada yer almıştır. Ayçiçeğinin soğuk ve sıcak koşullara mısıra göre daha iyi uyum sağlaması, bu bitkiyi mısıra göre avantajlı kılabilmektedir. Özellikle yetersiz sulama koşulları altında ayçiçeği bitkisi, sıcaklık stresine dayanıklılık göstermektedir (64). Yine kuraklığa dayanıklılık ile ilgili nicel araştırma verilerinin eksikliği nedeniyle, bu çalışmada ilgili kriterin kullanımı mümkün olmamıştır.

KM bakımından en yüksek değere sahip olan silaj buğday hasılı silajı olmasına rağmen (40,41,43) yeşil ot verimin silaj seçimini büyük oranda etkilemesi, bu silajın en son alternatif olarak değerlendirilmesine neden olmuştur.

Sonuç olarak yeşil ot verimi, KM oranı, pH, HP ve sindirilebilir KM kriterleri kullanılarak yapılan AHP-TOPSIS analizinde mısıra alternatif silajlık bitki seçim sıralamasının yonca, sorgum, ayçiçeği ve buğday hasılı şeklinde olduğu bulunmuştur. Bu sıralamada yeşil ot veriminin büyük oranda etkili olduğu belirlense de kuraklığa dayanıklılık ve su kullanım verimliliği kriterlerinin nicel veri yetersizliği nedeniyle modele dahil edilememesi sıralamanın geçerliliğini kısmen sınırlamaktadır. Bu eksikliğin giderilmesi ve belli bölgelere uygun silajlık bitkinin seçilmesinin sağlanması için o bölgeye özgü saha çalışmalarının yapılması ve elde edilen veriler kullanılarak yeni AHP-TOPSIS modelinin oluşturulması gerekmektedir.

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ARAŞTIRMA MAKALESİ

İklim Değişikliğinin Asıl Sorumlusu Hayvancılık Sektörü Mü?

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Öz

Mevcut çalışmada dünya geneli, gelişmiş ekonomiler ve nüfusu fazla olan ülkelerle birlikte Türkiye'de sera gazı salınım miktarları ile hayvancılığın payının karşılaştırmalı olarak ortaya konulması amaçlanmıştır. Çalışma materyali olan ham veriler FAO'dan temin edilmiş, gerekli hesaplamalar yapılmış ve deskriptif istatistikler halinde sunulmuştur. Çalışma bulgularına göre, Dünya'da 2021 yılında toplam 51.3 milyar ton olan CO_{2e} sera gazı salınımının %29'u Çin, %11.5'i ABD ve %1.2'si Türkiye tarafından gerçekleşmiştir. Dünya'da toplam sera gazı salınımı içerisinde hayvancılığın payı %8.2 olarak hesaplanmıştır. Dünya genelinde son 30 yılda kişi başına düşen toplam sera gazı salınımı ortalama olarak 6.4 ton olup, bu değer ABD'de 22 ton, AB-27'de 10.1 ton, Çin'de 6.7 ton, Hindistan'da 2.2 ton ve Türkiye'de 5.3 ton olarak Dünya ortalamasının altında gerçekleşmiştir. Hayvancılık kaynaklı sera gazı salınımları incelendiğinde, 1991-2021 yılları arasında gerek Dünya geneli, gerekse incelenen ülkeler bazında kişi başına düşen ortalama salınımların devamlı azalma eğiliminde ve <1 ton/kişi olduğu anlaşılmaktadır. Sonuç olarak, toplum sağlığı için stratejik konumda olan hayvancılık sektörünün iklim değişikliği üzerindeki payının son derece düşük olduğu tespit edilmiştir. Hayvancılık sektörünün iklim değişikliğinin sebepleri arasında ön sıralarda gösterilmesinin doğru/sorumlu bir yaklaşım olmadığı ve aksine asıl sorunun kaynağı olan sektörlerin (sanayi, enerji, fosil yakıtlar gibi) göz ardı edilmesine yol açtığı söylenebilir.

Anahtar Kelimeler: Ekonomi, hayvancılık, iklim değişikliği, sera gazı, üretim

Is The Livestock Sector Mainly Responsible for Climate Change?

Abstract

The aim of the study is to compare the amount of greenhouse gas emissions and the share of livestock in Türkiye together with the world in general, developed economies and countries with large populations. The raw data, which is the material of the study, was obtained from FAO, necessary calculations were made and presented as descriptive statistics. According to the findings of the study, of the total 51.3 billion tonnes of CO_{2e} greenhouse gas emissions in the world in 2021, 29% was emitted by China, 11.5% by the USA and 1.2% by Turkey. The share of livestock in total greenhouse gas emissions in the world is calculated as 8.2%. In the last 30 years, the total greenhouse gas emissions per capita in the world has been 6.4 tonnes on average and this value has been 22 tonnes in the USA, 10.1 tonnes in the EU-27, 6.7 tonnes in China, 2.2 tonnes in India and 5.3 tonnes in Turkey, which is below the world average. When the greenhouse gas emissions from livestock are examined, it is understood that the average emissions per capita between 1991 and 2021 both in the world in general and on the basis of the countries examined are in a continuous decreasing trend and <1 ton/capita. As a result, it has been determined that the share of the livestock sector, which is in a strategic position for public health, on climate change is extremely low. It can be said that showing the livestock sector at the forefront among the causes of climate change is not a correct/responsible approach and leads to ignoring the sectors (such as industry, energy, fossil fuels) that are the source of the real problem.

Key Words: Climate change, economy, greenhouse gas, livestock, production

GİRİŞ

Dünya nüfusunun 8 milyarı geçmiş olması, beslenmenin temel ihtiyaç olarak önemini her geçen gün artırmaktadır. Bu bağlamda gıda üretimi başlığı altında yer alan hayvansal ürünlere olan ihtiyaç da artmaktadır. Dünya'da büyükbaş hayvan birimi (BBHB) cinsinden yaklaşık 2 milyar olan hayvan varlığı dikkate alındığında ortalama olarak kişi başına 0.25 hayvanın düştüğü söylenebilir (1). Bunun yeterliliğini ve dengeli dağılıp dağılmadığını sorgulamak gerekir. Eğer yeterli ve dengeli olmuş olsa idi, kısmen gelişmiş ülkeler hariç yeryüzünde sağlıklı ve dengeli beslenme sorunu (malnutrisyon) ve açlık gündeme gelmezdi. FAO verilerine göre Dünya'da yetersiz beslenme oranı %9.1'dir. Yaklaşık 50 yıl önce kişi başına düşen hayvan varlığının %40 daha fazla olması, geçmişten günümüze hayvan varlığından ziyade insan varlığının ve dolayısıyla ihtiyaçların (konut, araç, fabrika vs.) arttığını göstermektedir. Yine son 50 yılda dünyanın ortalama sıcaklığı da 1-4.5 ºC artış göstermiştir (2). Yani esasında sorunun temelinde yatan sebep, hayvan varlığından ziyade artan insan sayısı ve insan faaliyetleridir. Aksine kişi başına düşen hayvan varlığı giderek azalmaktadır. Sanayi devrimi sonrası artan nüfus; daha fazla araç, yakıt, gıda, konut, enerji ihtiyacı neticesinde daha fazla sera gazı salınımı ve iklim değişikliği demektir.

Bu durumda asıl meselenin; devamlı değişen iklimle birlikte, artan insan nüfusunun sağlıklı/dengeli beslenmesi sorunu olduğu ortaya çıkmaktadır (3). Hayvancılık, en temel ihtiyaç olan beslenme sorununa çözüm üreten taraftadır. Ancak bazı kesimler tarafından hayvansal üretim sonucu ortaya çıkan sera gazları nedeniyle iklim değişikliğinde önemli oranda etkisinin olduğu ileri sürülerek hayvan sayılarının ve üretimin azaltılması yönünde tedbirlerin alınması gündeme getirilmektedir (4-6).

Bu yaklaşıma objektif perspektif getirmesi açısından mevcut çalışmada, dünya geneli, gelişmiş ekonomiler ve nüfusu fazla olan ülkelerle birlikte Türkiye'de sera gazı salınım miktarları ile hayvancılığın payının karşılaştırmalı olarak ortaya konulması amaçlanmıştır.

MATERYAL VE METOT

Çalışma materyali olan ham veriler FAO'dan temin edilmiştir (1). Verilere ait deskriptif istatistikler (oran, yüzde) oluşturulan tablolar halinde sunulmuştur.

Sera gazı salınım miktarları, genel kabul gören şekliyle "Karbon Ayak İzi" olarak hesaplanmakta, ortak bir değer olması ve karşılaştırmaları kolaylaştırmak bakımından karbondioksit eşdeğerine (CO_{2e}) dönüştürülmektedir. Dolayısıyla CO_{2e} değeri, iklim değişikliğinin boyutunu ortaya koymada temel alınan başlıca standart birim olarak kabul edilmektedir (7,8). Çalışmada sera gazı salınım miktarları CO_{2e} olarak verilmiştir.

BULGULAR

Çeşitli ülkelerde toplam ve hayvancılık kaynaklı sera gazı salınım miktarları Tablo 1'de verilmiştir.

Dünya'da 2021 yılında toplam 51.3 milyar ton olan CO_{2e} sera gazı salınımının %29'u Çin ve %11.5'i ABD tarafından gerçekleşmiştir. Türkiye'nin toplam salınım içerisindeki payı sadece %1.2'dir. Dünya'da toplam sera gazı salınımı içerisinde hayvancılığın payı %8.2 iken, en yüksek Hindistan'da (%12.8) ve en düşük Çin'de (%2.3) bulunmuştur. Türkiye'de bu değer %7.2 ile Dünya ve AB-27'nin gerisinde kalmıştır (Tablo 1).

Tablo 1.Çeşitli ülkelerde toplam ve hayvancılık kaynaklı sera gazı salınım miktarları, 2021

Ülkeler	Toplam salınım miktarları, milyar ton CO_{2e}	Hayvancılık kaynaklı salınım miktarları, milyon ton CO_{2e}	Hayvancılığın payı, %
Dünya	51.3	4200.8	8.2
AB-27	3.6	289.4	8.0
ABD	5.9	267.5	4.5
Çin	14.9	337.2	2.3
Hindistan	4.0	512.4	12.8
Türkiye	0.6	43.1	7.2

Ülkelerin yıllara göre kişi başına düşen sera gazı salınım miktarları Tablo 2'de sunulmuştur. Son 30 yılda Dünya genelinde kişi başına düşen sera gazı salınımı ortalama olarak 6.4 ton olup, bu değer AB-27'de 10.1 ton, ABD'de 22 ton, Çin'de 6.7 ton, Hindistan'da 2.2 ton ve Türkiye'de 5.3 ton olarak Dünya ortalamasının altında gerçekleşmiştir. İncelenen dönemde AB-27 ve ABD'de kişi başına düşen toplam salınım miktarları devamlı olarak azalırken (%30 civarında azalma), özellikle Çin'de (%192.3 artış) ve kısmen Türkiye (%78.8 artış) ve Hindistan'da (%64.4 artış) artışın olduğu dikkati çekmektedir.

Tablo 2. Ülkelerin yıllara göre kişi başına düşen sera gazı salınım miktarları (1991-2021)

Ülkeler	Salınım Miktarı, ton	1991	1996	2001	2006	2011	2016	2021	Ortalama
Dünya	Toplam	6.25	6.08	5.94	6.54	6.83	6.64	6.66	6.4
	Hayv. kaynaklı	0.67	0.62	0.58	0.57	0.55	0.54	0.53	0.6
	Hayv. Payı, %	10.72	10.20	9.76	8.72	8.05	8.13	7.96	9.1
AB-27	Toplam	11.59	11.19	10.77	10.68	9.49	8.87	8.05	10.1
	Hayv. kaynaklı	0.94	0.82	0.77	0.72	0.68	0.66	0.65	0.7
	Hayv. Payı, %	8.11	7.33	7.15	6.74	7.17	7.44	8.07	7.4
ABD	Toplam	24.64	24.58	24.11	23.30	20.78	19.02	17.54	22.0
	Hayv. kaynaklı	1.04	1.02	0.92	0.89	0.83	0.80	0.79	0.9
	Hayv. Payı, %	4.39	4.15	3.82	3.82	3.99	4.21	4.50	4.1
Çin	Toplam	3.49	4.20	4.21	6.79	9.04	9.07	10.20	6.7
	Hayv. kaynaklı	0.33	0.32	0.30	0.28	0.25	0.24	0.23	0.3
	Hayv. Payı, %	9.45	7.62	7.13	4.12	2.77	2.65	2.25	5.1
Hindistan	Toplam	1.74	1.86	1.88	2.03	2.38	2.69	2.86	2.20
	Hayv. kaynaklı	0.47	0.44	0.41	0.40	0.39	0.37	0.36	0.40
	Hayv. Payı, %	31.70	27.20	21.75	19.72	16.49	13.84	12.81	20.50
Türkiye	Toplam	3.97	4.36	4.39	5.10	5.86	6.45	7.10	5.3
	Hayv. kaynaklı	0.59	0.52	0.42	0.37	0.35	0.41	0.51	0.5
	Hayv. Payı, %	14.86	11.93	9.57	7.25	5.97	6.36	7.18	9.0

Diğer taraftan, 1991-2021 arasında hayvancılık kaynaklı sera gazı salınımları incelendiğinde gerek Dünya geneli, gerekse incelenen ülkeler bazında kişi başına düşen ortalama salınımların devamlı azalma eğiliminde ve <1 ton/kişi olduğu, toplam salınım içerisinde hayvancılığın payının da Hindistan hariç tek haneli değerlerde (<%10) olduğu görülmektedir (Tablo 2).

Üretilen farklı hayvansal ürünlere göre sera gazı salınım miktarları Tablo 3'te verilmiştir. Dünya genelinde 2021 yılında hayvansal üretim kaynaklı toplam 4 milyar ton CO_{2e} olan sera gazı salınım miktarının %57.2'si büyükbaş eti, %21'i büyükbaş sütü, %11.2'si küçükbaş eti, %5'i domuz eti, %2.8'i küçükbaş sütü, %1.6'sı tavuk eti ve %1.2'si yumurta üretiminden kaynaklanmaktadır. Diğer bir ifadeyle toplam salınımın %73.4'ü kırmızı et, %23.8'i süt ve %2.8'i de kanatlı alt sektöründe gerçekleşmiştir. Hayvansal üretim kaynaklı toplam sera gazı salınımı en fazla olan ülke 511.5 milyon ton CO_{2e} ile Hindistan olup, 327.4 milyon ton CO_{2e} ile Çin ikinci sırada yer almıştır. Türkiye'nin hayvansal üretim kaynaklı sera gazı salınımının Dünya genelinden aldığı pay %1.1'dir (Tablo 3).

Tablo 3. Üretilen farklı hayvansal ürünlere göre sera gazı salınım miktarları (milyon ton CO_{2e})

Ülkeler	Büyükbaş Eti*	Büyükbaş Sütü*	Küçükbaş Eti**	Küçükbaş Sütü**	Tavuk Eti	Yumurta	Domuz Eti	Toplam
Dünya	2310.6	849.7	453.7	113.4	65.8	49.3	200.5	4043.0
AB-27	122.8	91.2	12.6	9.8	2.9	5.1	41.3	285.7
ABD	167.1	52.5	1.9	1.2	3.8	3.1	29.2	258.8
Çin	130.2	43.2	61.3	10.5	6.9	16.3	59.0	327.4
Hindistan	250.0	204.4	41.6	9.8	0.9	3.0	1.8	511.5
Türkiye	15.7	13.0	7.2	5.4	1.0	0.7	-	43.1
Pay, %	0.7	1.5	1.6	4.8	1.5	1.4	-	1.1

*Sığır+manda; **koyun+keçi

Ülkelere göre hayvansal ürün üretiminde salınan sera gazı yoğunlukları Tablo 4'te verilmiştir. 2021 yılı verilerine göre özellikle sığır ve koyun eti üretiminde salınan sera gazı yoğunluklarının daha yüksek, inek sütü ve kanatlı ürünlerinde daha düşük seviyelerde olduğu görülmektedir (Tablo 4).

Tablo 4. Ülkelere göre hayvansal ürün üretiminde salınan sera gazı yoğunlukları (kg CO_{2e} /kg)

Ülkeler	Sığır Eti	İnek Sütü	Koyun Eti	Koyun Sütü	Tavuk Eti	Yumurta	Domuz Eti
Dünya	28.3	0.9	24.4	5.9	0.5	0.6	1.7
AB-27	17.8	0.6	22.8	2.5	0.3	0.8	1.7
ABD	13.1	0.5	22.0	-	0.2	0.5	2.3
Çin	12.4	0.8	11.6	8.0	0.4	0.5	1.1
Hindistan	29.2	1.1	51.6	9.2	0.3	0.4	5.7
Türkiye	10.6	0.6	14.4	3.6	0.4	0.5	-

TARTIŞMA VE SONUÇ

İklim değişikliği ile hayvancılığın etkileşiminden bahsetmeden önce hayvansal ürünlerin insan için önemine ve yapısı gereği hayvansal üretimin zorluğuna kısaca değinmek gerekmektedir. Her şeyden önce hayvansal ürünler içerdiği esansiyel aminoasitler (triptofan = serotonin salınımı, fenilalanin = dopamin sentezi, izolösin= O_2 ve hemoglobin vs.) nedeniyle insanın "sağlıklı/dengeli" beslenmesi için hayatın her evresinde (çocukluk, ergenlik, gebelik, doğum, yaşlılık gibi) büyüme, gelişme, zekâ, muhakeme, doğru karar verme ve entelektüel düşünce yapısının gelişimi için tüketilmesi gereken besinlerdir. Bu açıdan bakıldığında hayvansal ürünler insan için özetle "sağlık ve mutluluk" demektir. Hayvansal üretim, kâr amacıyla yapılan ancak canlı bünye ile gerçekleştirildiğinden başlı başına zor ve zahmetli bir faaliyet alanıdır. Dünya ve Türkiye'de özellikle Covid-19 pandemisi sonrası enflasyonist ortamın oluşması (9), spekülatif kazançların artması, riski düşük ve zahmeti az alternatif yatırım araçlarının (borsa, altın, döviz, faizler gibi) getirisinin fazla olduğu piyasa yapısında canlı bir materyalle üretim yapmak, hatta "doğayı kirletiyorsunuz", "iklimi değiştiriyorsunuz" baskı ve suçlamalarına maruz kalmak, haksızlık ve üretmeden tüketmenin mümkün olmadığı gerçeğini göz ardı etmektir. Hayvansal üretim sonucu sanılan kadar olmasa da sera gazı salınımı olduğu yadsınamaz bir gerçektir ama hayvansal üretimin bütün zorluğu ile birlikte, devamlı artan insan varlığının devamı için gerekli/zorunlu bir faaliyet alanı olduğu unutulmamalıdır. Mevcut durumda salınan sera gazlarının ve değişen iklimin sorumlusu olarak hayvansal üretimin gösterilmesi bir paradoks oluşturmaktadır.

Hayvancılık kaynaklı en önemli sera gazları, enterik fermentasyon ve toprağa atılan/depolanan gübre (dışkı) nedeniyle oluşan metan gazı (CH_4) ve nitroz oksit gazıdır (N_2O). Çalışma bulgularına göre, Dünya genelinde hayvancılık kaynaklı sera gazı salınım miktarları %8.2'dir. Geri kalan %91.8 oranındaki salınım başta enerji/yakıt tüketimi olmak üzere gıda, bitkisel üretim, sanayi, orman yangınları (anız dahil) ve atıklar gibi diğer alanlardan kaynaklanmaktadır. Verilen bilgiler ışığında, iklim değişikliğinden hayvancılık sektörünü sorumlu tutma anlayışı sorunlu bir bakış açısıdır. Son yıllarda yapılan bazı çalışmalarda (4-6), yazılı, görsel ve sosyal medyada iklim değişikliğinden hayvancılık sektörünün asli sorumlu olduğu ve üretimin azaltılması gerektiği gibi olumsuz algı yaratma çabalarının, bilinçlenerek ve bilinçlendirilerek

ortadan kaldırılabileceği düşünülmektedir. Ayrıca hayvancılık sektörünün asli görevi olan insanların sağlıklı ve dengeli beslenmedeki rolü ve vazgeçilmezliği de sektörün tüm paydaşları tarafından öne çıkarılmalıdır. Yaratılmaya çalışılan algının ancak bütüncül bir yaklaşımla üstesinden gelinebileceği ve zaten zor günlerden geçen sektörün daha fazla yıpranmadan artan nüfusu besleme görevini yerine getirme konusunda önünün açılması gerekmektedir.

Artan sera gazı salınımlarının yol açtığı iklim değişikliği; turizm, sağlık, inşaat, dış ticaret, lojistik, sigortacılık gibi sektörleri etkilediği gibi, özellikle az gelişmiş ve gelişmekte olan ülkelerin milli gelirlerinde önemli paya sahip olan tarım-ormancılık ile hayvancılık sektörlerini de etkileyerek ekonomilerine olumsuz yansımaları olmaktadır (10).

Dünya'da azalan su kaynakları, kuraklık, sel, dolu vs. gibi ekstrem iklim olayları zaten başta yem üretimi olmak üzere sektörün önünde hastalıklar, üretim ve verimlilik konularında aşılması gereken ciddi sorunların (11-12) olduğu bir aşamada üretimi sınırlandıracak ilave yaklaşımların sektöre uzun vadede çözümü zorlaştıracak, sürdürülebilirliğe engel olacak daha büyük darbeler vuracağı göz ardı edilmemelidir. Az gelişmiş ve gelişmekte olan ülkelerin üretimini sınırlandırarak gelişmiş ülke ekonomilerine bağımlı yaşamalarının da önünü açacak bu ve benzeri yaklaşımlara karşı dikkatli ve tedbirli olmak gerekmektedir. Aksi halde hayvansal ürünlerde gelişmiş ekonomilerin tekelinde ve ithalata dayalı bir arz yapısının oluşması, iç/yerel kaynaklarla üretimin cazibesini yitirmesi ile birlikte yerli ve milli olma hedefinin piyasalarda irreversibl olarak gerçekleşmemesi riski taşımaktadır. Bu risk hayvansal üretim yapan ve geçimini bu alandan sağlayan nüfusun büyüklüğü düşünüldüğünde hiç de azımsanmayacak düzeydedir.

Diğer taraftan, iklim değişikliği nedeniyle (kuraklık, sel, dolu vs.) yem sektörü olumsuz etkilenmekte (13) ve bu durum bir taraftan hayvancılıkta üretim maliyetlerinin artmasına, ürün fiyatlarının yükselmesine ve arzın güvence altına alınamamasına neden olurken, diğer taraftan artan talebi karşılamak için sık sık canlı hayvan ve et ithalatını zorunlu hale getirmektedir.

Gerek Dünya genelinde gerekse Türkiye'de hayvan sayıları toplamda artıyor gibi görünse de kişi başına düşen sayı azalıyor. Üretim miktarındaki artışların önemli bir bölümü sağlanan verimlilik artışından kaynaklanmaktadır. Dolayısıyla gerek hayvansal üretim kaynaklı sera gazı salınımının toplam salınımdan aldığı payın düşüklüğü, gerekse artan nüfus ve hayvansal ürünlerin insan beslenmesindeki hayati rolü birlikte düşünüldüğünde sektörün yapılanarak üretime devam etmesi en akılcı yaklaşım olacaktır.

İklim değişikliği sorunu bireysel, ülkesel veya bölgesel değil, insanlığın genel sorunu olduğundan çözüm için tüm dünya olarak bütüncül yaklaşım gerekmektedir. Çözüm için ana başlıklar halinde; salınımı azaltan, geri dönüşüme imkân sağlayan ve salınımı ikame eden (telafi) önlemler alınması önerilmektedir.

Sonuç olarak, iklim değişikliği üzerinde toplum sağlığı için son derece önemli konumda olan hayvancılık sektörünün payının/sorumluluğunun oldukça düşük olduğu görülmekte olup, iklim değişikliğinin önlenebilmesi için hayvancılık dışında yer alan sanayi, enerji, fosil yakıtlar gibi diğer sektörlere yoğunlaşılması ve mercek tutulması gerektiği düşünülmektedir.

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Bu araştırmanın yürütülmesinde herhangi bir kuruluştan destek alınmamıştır.

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Yazarlar tarafından beyan edilecek bir çıkar çatışması yoktur.

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RESEARCH ARTICLE

Galectin-1 and -3 Expression in the Testis and Epididymis of Anatolian Ground Squirrels *(Spermophilus xanthoprymnus)* during Non-Breeding Periods of Pre-Hibernation and Hibernation

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Abstract

This study aims to investigate the expression patterns of Galectin-1 (Gal-1) and Gal-3 in the testis and epididymis of Anatolian ground squirrel (*Spermophilus xanthoprymnus*) during nonbreeding pre-hibernation and hibernation periods. Hibernation is a physiological state characterized by a reduction in metabolic rate and body temperature. Gal-1 and -3 are implicated in many biological functions. Twelve squirrels were used in this study. Followed by routine tissue processing, tissue samples underwent immunohistochemical procedure. Histological examination and statistical analysis were performed. Immunohistochemical investigation revealed that Gal-1 expression during pre-hibernation was confined to peritubular myoid cells and vascular smooth muscle cells, with no expression observed in Sertoli or spermatogenic cells. Gal-1 in the epididymis was localized to smooth muscle cells encircling the epithelium and within blood vessel walls, exhibiting markedly elevated expression across the caput, corpus, and cauda regions. During hibernation, testicular and epidydimal Gal-1 expression exhibited a considerable reduction. During pre-hibernation, Gal-3 exhibited a unique pattern, with expression noted in the seminiferous epithelium and Leydig cells. Gal-3 was detected in the epithelial cells throughout the epididymis, with greater intensity in specific epithelial cells. During hibernation, Gal-3 expression increased in Sertoli cells, spermatogonia, and spermatocytes within the testis, while exhibiting diminished intensity in the epididymal epithelium across all regions. The findings suggest that Gal-1 and -3 may be involved in seasonal reproductive adaptability during nonbreeding pre-hibernation and hibernation. Further research could clarify their specific molecular functions in hibernating species.

Key Words: Anatolian ground squirrel, epididymis, galectin-1, galectin-3, testis

Üreme Dışı Aktif ve Hibernasyon Dönemlerinde Anadolu Yer Sincabı (*Spermophilus xanthoprymnus*) Testis ve Epididimisinde Galektin-1 ve -3 Ekspresyonu

Öz

Bu çalışma, üreme dışı aktif ve hibernasyon dönemlerinde Anadolu yer sincabı (*Spermophilus xanthoprymnus*) testis ve epididimisinde Galectin-1 (Gal-1) ve Gal-3 ekspresyonunu araştırmayı amaçlamaktadır. Hibernasyon, metabolik hız ve vücut sıcaklığındaki azalma ile karakterize fizyolojik bir durumdur. Gal-1 ve Gal-3 birçok biyolojik fonksiyonda rol oynamaktadır. Bu çalışmada on iki sincap kullanılmıştır. Rutin doku işleme tabi tutulduktan sonra, doku örneklerine immünohistokimyasal boyamalar yapıldı. Boyamalar histolojik olarak incelenip istatistiksel analizleri yapıldı. İmmünohistokimyasal inceleme, aktif dönemde Gal-1 ekspresyonunun peritübüler miyoid hücreler ve vasküler düz kas hücreleriyle sınırlı olduğunu, Sertoli veya spermatogenik hücrelerde ekspresyon gözlemlenmediğini ortaya koymuştur. Epididimisde Gal-1, epiteli çevreleyen düz kas hücrelerinde ve kan damarı duvarlarında lokalize olmuş, kaput, korpus ve kauda bölgelerinde belirgin şekilde yüksek ekspresyon sergilemiştir. Hibernasyon sırasında testis ve epididimal Gal-1 ekspresyonunda önemli bir azalma gözlenmiştir. Aktif dönemde Gal-3 kendine has bir ekspresyon göstermiş olup seminifer epitel ve Leydig hücrelerinde gözlemlenmiştir. Gal-3, epididimis boyunca epitel hücrelerinde tespit edilmiş, belirli epitel hücrelerinde daha yoğun olarak bulunmuştur. Hibernasyon sırasında Gal-3 ekspresyonu testisteki Sertoli hücrelerinde, spermatogonyumlarda ve spermatositlerde artarken, tüm bölgelerde epididimal epitelde yoğunluk azalmıştır. Bulgular, Gal-1 ve Gal-3'ün üreme dışı aktif dönem ve hibernasyon sırasında mevsimsel üreme adaptasyonunda rol oynayabileceğini göstermektedir. Hibernasyona yatan türlerde spesifik moleküler işlevlerini açıklığa kavuşturmak için daha fazla çalışmaya ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Anadolu yer sincabı, epididimis, galektin-1, galektin-3, testis

INTRODUCTION

Hibernation is a distinctive physiological adaptation that involves a profound suppression of metabolic activity, a significant decrease in body temperature, and the downregulation of various physiological processes, enabling animals to conserve energy and endure extended periods of environmental stress, such as cold temperatures and limited food resources (1). Seasonal reproduction is an adaptation strategy observed in numerous wild animals. This technique aligns reproductive efforts with the season most conducive to the survival and growth of progeny. Male seasonal breeders exhibit coordinated phases of testicular maturation and regression during the reproductive cycle. This results from the annual environmental fluctuations and energy constraints they encounter (2-4).

Galectins, a group of β-galactoside-binding proteins, are crucial regulators of diverse biological functions, such as cell growth, programmed cell death, and immune system modulation (5). These proteins possess a conserved carbohydrate recognition domain (CRD) and a core consisting of 130 amino acids (6). The lectin family is characterized by two fundamental traits: a high affinity for galactosides and significant similarity in their amino acid sequences (7). These attributes are the principal distinguishing characteristics. Researchers have identified fifteen different galectins in mammals, labeled gal-1 through gal-15 based on their specificity. Galectins can be categorized into three fundamental types: prototype galectins, chimeric galectins, and tandem repeat galectins. The classifications are predicated on the structural composition of the galectins (8). The expression of Gal-1 undergoes dynamic control throughout the spermatogenic cycle (9,10). At the luminal pole of the rat seminiferous epithelium, this lectin has heightened expression throughout the spermiation stages (VI–VIII) and is predominantly expressed in Sertoli cells during stages X–XII of the cycle (10). This phase is marked by its occurrence on the apical projections of Sertoli cells, the heads of mature spermatids, and the residual cytoplasmic bodies of the spermatids. Upon the conclusion of the eighth phase of spermiation, Gal-1 expression is reinstated at the basal region of Sertoli cells. As germ cell differentiation progresses, its expression progressively extends throughout the entire cell (11,12). Furthermore, the expression of Gal-1 has been identified in the Leydig cells of rats (9). While many galectin family members, such as Gal-1, are known to promote apoptosis, Gal-3 exhibits the opposite effect by acting as an anti-apoptotic molecule (13). It has been demonstrated that in rats, Gal-3 is expressed in Leydig cells, peritubular myoid cells, interstitial CD68-positive macrophages, Sertoli cells, smooth muscle cells, and the epididymal epithelium. Moreover, the expression profile of Gal-3 appears to undergo significant modulation across distinct stages of postnatal development (9).

Anatolian ground squirrels (*Spermophilus xanthoprymnus*) are communal, diurnal rodents that predominantly consume herbivorous diets and engage in burrowing behavior. From late summer to early spring, these rodents hibernate within subterranean burrows. The Anatolian ground squirrel exhibits sexual activity from mid-March to late-April, followed by an extended period of sexual inactivity from late-April to mid-March, and hibernates from late August to mid-March (14-16).

By investigating the expression dynamics of Gal-1 and -3 in the testis and epididymis of Anatolian ground squirrels during the nonbreeding pre-hibernation and hibernation periods, this study aims to uncover the molecular mechanisms underlying reproductive quiescence and subsequent reactivation. Understanding these patterns will provide deeper insights into the adaptive strategies employed by hibernating mammals to synchronize their reproductive cycles with environmental changes, thus enhancing our knowledge of mammalian reproductive biology and potentially informing conservation strategies for hibernating species.

MATERIAL AND METHODS

We collected samples from six animals in the nonbreeding pre-hibernation period and six animals in the hibernation period of the Anatolian ground squirrel (*Spermophilus xanthoprymnus*) to obtain testicular and epididymal tissues. To ensure the preservation of cellular and structural integrity, the tissues were promptly excised and fixed in Bouin's solution following euthanasia. After being dehydrated in a graded series of alcohol concentrations, the fixed specimens were processed through methyl benzoate and benzol for clearing, then embedded in paraffin to enable microtome sectioning.

Immunohistochemistry

The presence and localization of Gal-1 and -3 in the tissue samples were both detected using immunohistochemistry. Deparaffinized and rehydrated sections were exposed to citric acid retrieval solution (pH 6.0) and heated in a microwave to facilitate antigen retrieval. Following the cooling process to 20-25°C, the sections were thoroughly rinsed with PBS to ensure the removal of any residual reagents and to prepare them for subsequent steps. In order to inhibit endogenous peroxidase activity, the samples were rinsed after being treated with 3% H_2O_2 in PBS for 20 minutes in a dark environment. To minimize non-specific binding and enhance the specificity of antibody interactions, the slides were pre-incubated with 10% normal goat serum for 10 minutes at room temperature. Using Gal-1 Polyclonal (1:400, Novus Biologicals, NBP1-89791) and Gal-3 Monoclonal (1:400, Novus Biologicals, NB300-538) antibodies, primary antibody incubation was performed overnight at 4°C. After that, the samples were treated with a secondary antibody, followed by enzyme-conjugated streptavidin to enhance signal detection. In positive cells, the antibody-antigen complexes were visualized using AEC (3-amino-9-ethylcarbazole) chromogen solution, which resulted in a red coloration. Afterward, the samples were counterstained with Gill's II hematoxylin. The primary antibody was replaced with PBS in the negative control, and the same procedures were subsequently adhered to. Colon tissue was employed as a positive control. Images were captured from the sections using an Olympus BX51 research microscope with an integrated DP72 digital camera and then analyzed.

Quantitative Evaluation of Immunohistochemical Staining

This study quantitatively assessed the staining intensities of Gal-1 and -3 using the method outlined by a previous study

(17). Quantitative analyses of staining intensities for Galectin-1 and Galectin-3 were conducted using images acquired from the testis and epididymis. The intensity of immunostaining was quantified for the entire image. The testis and epididymis images of squirrels were obtained at 400X magnification during the nonbreeding pre-hibernation and hibernation periods. Ten random images were utilized for each group. Relevant images were imported into ImageJ (version 1.51, Java 1.8.0_112, https://imagej.nih.gov/ij/) and processed using the "color deconvolution" plug-in, where the staining of hematoxylin and AEC was separated into three distinct panels with the only hematoxylin, with the only AEC image, and with the only background image. Threshold values were set for only the AEC images. Subsequently, parameters for area and area fraction, namely the percentage of staining area referred to as "staining intensity," were set. After that, the area and percentage of the staining area were calculated for each image.

Statistical Analysis

GraphPad Prism 7 for Windows (Version 7.04) was employed to conduct statistical analyses. The software was used to quantitatively evaluate the staining intensity (% staining area) by importing percentage values for the stained areas. Student's t-test was conducted to compare staining intensity of the testis, caput, corpus, and cauda epididymis between the pre-hibernation and hibernation periods. Data are presented as mean ± SEM, and significance was considered at $p<0.05$.

RESULTS

Colon tissues were utilized as positive controls. Gal-1 expression was localized to the smooth muscle cells within the mucosa and vascular structures, whereas Gal-3 was prominently expressed in the intestinal epithelial cells (Figure 1).

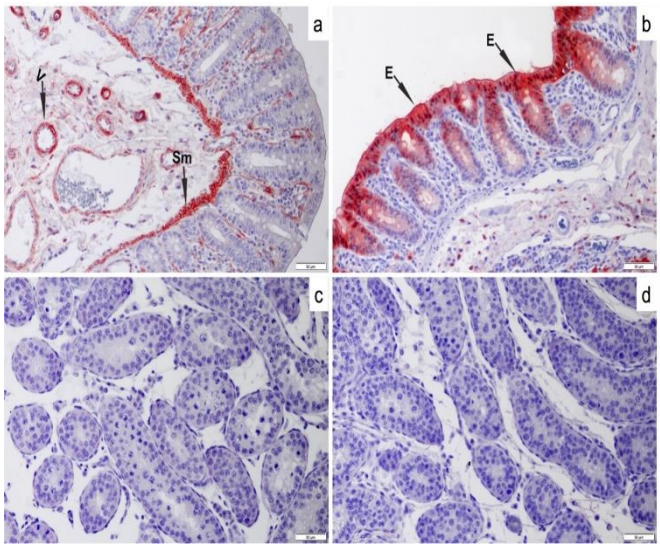

Figure 1. Colon tissue as a positive control for Gal-1 (a) and Gal-3 (b). Testis as a negative control (c, d). E: Intestinal epithelium. Sm: smooth muscle cells. V: vessel walls. Bar: 50 um.

Gal-1 Immunostaining

In the pre-hibernation period, Gal-1 expression was not observed in the seminiferous epithelium of the testis, specifically in either Sertoli cells or spermatogenic cells. A positive reaction was observed in peritubular myoid cells and in the walls of blood vessels within the intertubular area. No positive reaction was detected in the epithelial cells of the caput, corpus, or cauda regions of the epididymis. However, we identified a positive Gal-1 reaction in the smooth muscle cells surrounding the epididymal epithelium and in the walls of blood vessels located within the connective tissue (Figure 2).

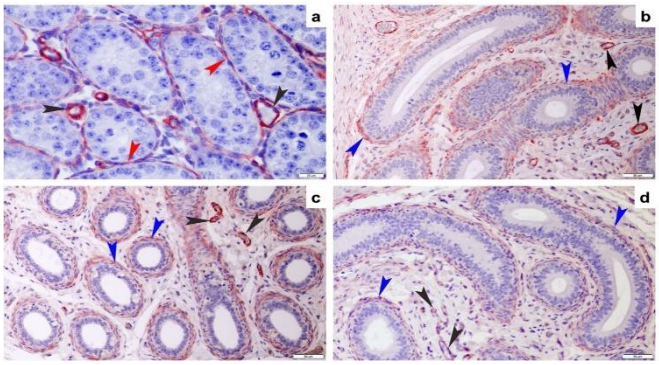

Figure 2. Immunohistochemical localization of Gal-1 in the testis (a), and in the distinct segments of the epididymis: caput (b), corpus (c), and cauda (d) during the non-breeding period of pre-hibernation. Black arrowhead: Vessel walls. Red arrowhead: Peritubular myoid cells. Blue arrowhead: Muscle layer in the ductal wall of epididymis. Bar: 20 um (a), 50 um (b, c, d).

During the hibernation period, the pattern of Gal-1 immunostaining closely resembled that observed during the pre-hibernation phase. In the testis, Gal-1 expression was predominantly localized to the peritubular myoid cells and vascular smooth muscle cells. Similarly, in the epididymis, Gal-1 immunoreactivity was evident in the smooth muscle cells surrounding the epididymal epithelium and in the vascular walls within the connective tissue, consistent with pre-hibernation findings (Figure 3).

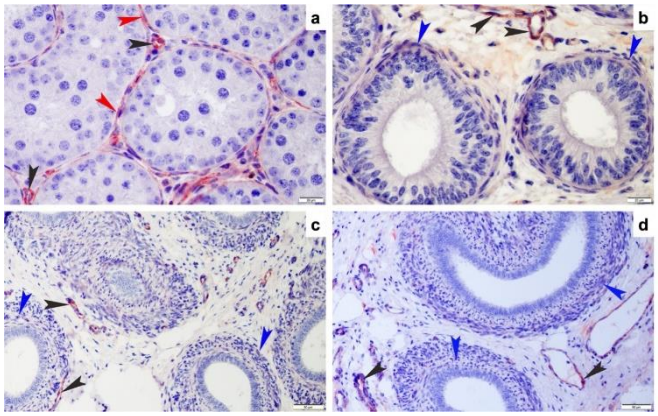

Figure 3. Immunohistochemical localization of Gal-1 in the testis (a), and in the distinct segments of the epididymis: caput (b), corpus (c), and cauda (d) during hibernation. Black arrowhead: Vessel walls. Red arrowhead: Peritubular myoid cells. Blue arrowhead: Muscle layer in the ductal wall of epididymis. Bar: 20 um (a, b), 50 um (c, d).

Analysis of Gal-1 expression in testicular and epididymal tissues revealed significant variations between pre-hibernation and hibernation periods. In the testis, Gal-1 levels were notably elevated during the pre-hibernation phase ($p<0.01$). In the caput region of the epididymis, Gal-1 levels were markedly elevated in pre-hibernation ($p<0.0001$). In

the corpus region, Gal-1 levels were markedly elevated during the pre-hibernation phase (p<0.0001). Similarly, in the cauda region, Gal-1 expression was significantly higher in pre-hibernation compared to the hibernation period (p<0.0001). (Figure 4a).

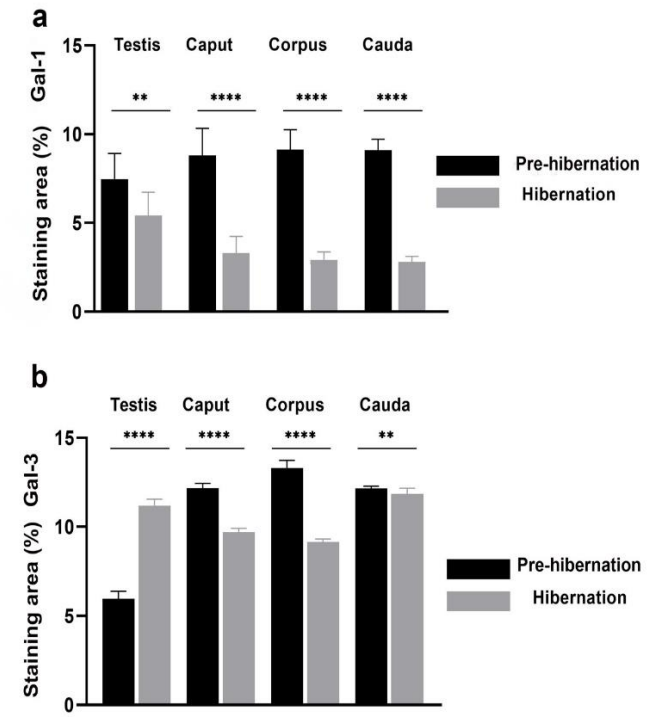

Figure 4. Quantitative evaluation of Gal-1 (a) and Gal-3 (b) immunostaining during the pre-hibernation and hibernation periods. Statistical significance is denoted as follows: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Gal-3 Immunostaining

In the pre-hibernation period, Gal-3 immunostaining showed a distinct pattern from that of Gal-1, with expression observed within the seminiferous epithelium of the testis. Positive Gal-3 expression was also detected in Leydig cells located in the intertubular area. In the epididymis, intense Gal-3 staining was noted in the epithelial cells of the caput, corpus, and cauda regions. Interestingly, intraepithelial some cells within the epididymal epithelium exhibited more intense Gal-3 expression compared to other cell types in this tissue (Figure 5).

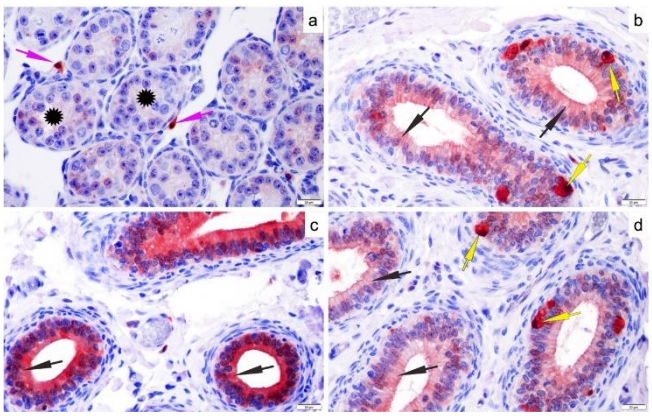

Figure 5. Immunohistochemical localization of Gal-3 in the testis (a), and in the distinct segments of the epididymis: caput (b), corpus (c), and cauda (d) during non-breeding period of pre-hibernation. Asterisk: Seminiferous epithelium. Black arrow: Epididymal epithelium. Yellow arrow: Some intraepithelial cells staining intensely. Purple arrow: Leydig cell. Bar: 20 um.

During the hibernation period, Gal-3 immunoreaction was observed to increase within the seminiferous epithelium of the testis, displaying more intense staining compared to the pre-hibernation period. Gal-3 immunostaining was specifically detected in Sertoli cells, spermatogonia, and spermatocytes. Positive Gal-3 expression was also observed in Leydig cells situated in the intertubular region. In the epididymis, Gal-3 expression was present in the epithelial cells of the caput, corpus, and cauda regions, similar to the pre-hibernation period, but with reduced staining intensity (Figure 6).

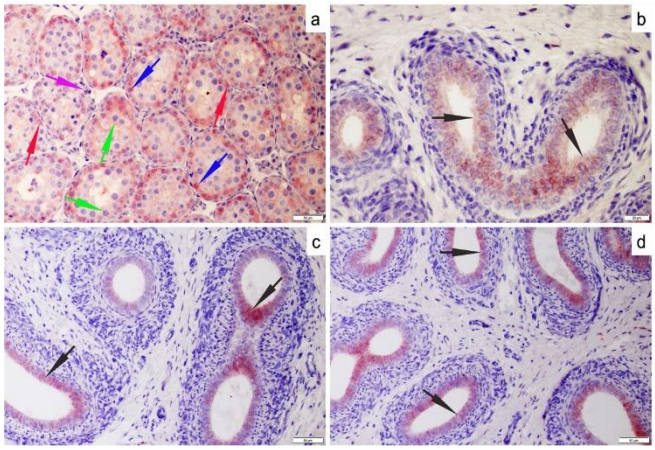

Figure 6. Immunohistochemical localization of Gal-3 in the testis (a), and in the distinct segments of the epididymis: caput (b), corpus (c), and cauda (d) during hibernation. Black arrow: Epididymal epithelium. Red arrow: Sertoli cells. Purple arrow: Leydig cell. Blue arrow: Spermatogonium. Green arrow: Spermatocytes. Bar: 20 um (b), Bar: 50 um (a, c, d).

During the hibernation period, Gal-3 expression in the testis was observed to be significantly higher compared to the pre-hibernation period (p<0.01). In contrast, in the epididymis, Gal-3 expression was significantly higher in the pre-hibernation period across all regions. Specifically, the caput region showed a highly significant decrease in Gal-3 expression during hibernation (p<0.0001), as did the corpus region (p<0.0001), while the cauda region also demonstrated a significant reduction (p<0.01) in expression during hibernation compared to pre-hibernation levels (Figure 4b).

DISCUSSION AND CONCLUSION

This study elucidates the unique expression patterns of Gal-1 and -3 in the testis and epididymis of the Anatolian ground squirrel throughout pre-hibernation and hibernation phases. The findings indicate that Gal-1 and -3 have distinct functions in modulating the adaptive response of reproductive system to hibernation, essential for seasonal breeders.

Our results show that Gal-1 expression in the testis was confined to vascular smooth muscle cells and peritubular myoid cells, with no expression in Sertoli or spermatogenic cells. In the epididymis, Gal-1 was detected in the smooth muscle cells surrounding the epithelium and in blood vessel walls. Quantitative analysis revealed significantly higher expression levels of Gal-1 in the epididymis during the pre-hibernation period across all regions (caput, corpus, and cauda) compared to hibernation (p<0.0001). In a study conduc-

ted on rats, unlike in our findings, Gal-1 expression was observed in Sertoli cells, particularly with intense immunoreactivity in the apical regions of Sertoli cells and in the heads of mature spermatids during spermiation. Following spermiation, Gal-1 expression was detected in the basal segments of Sertoli cells and gradually extended throughout as germ cell differentiation progressed (10). In humans, a high concentration of Gal-1 was observed in peritubular myoid cells, consistent with our findings (12). Özbek et al. (9), in contrast to our findings, reported positive Gal-1 expression in Sertoli and Leydig cells, while observing no Gal-1 reactivity in peritubular myoid cells in rat (9). This expression variation implies that Gal-1 may fulfill species-specific functions within the reproductive system, potentially adapting to the distinct physiological and structural requirements of each species.

The testis and epididymis exhibited divergent patterns of Gal-3 expression during the hibernation period. Gal-3 immunoreactivity was substantially increased in the testis, with intense expression observed in Sertoli cells, spermatogonia, and spermatocytes, suggesting a pronounced presence within the seminiferous epithelium. Conversely, the epididymis exhibited a substantial decrease in Gal-3 expression in all regions-corpus, cauda, and caput-compared to pre-hibernation levels. The quantitative analysis demonstrated that the decrease in Gal-3 immunoreactivity in the epididymis was statistically significant ($p<0.0001$ for caput and corpus, $p<0.01$ for cauda), indicating a substantial decrease in expression as the tissue transitions to a quiescent state. These findings align with certain aspects of previous research, though some differences highlight species-specific variations in Gal-3 expression. Khorsandi and Orazizadeh (18) observed Gal-3 expression in Leydig cells and peritubular myoid cells, but not in Sertoli cells of mouse testes. This contrasts with our findings, where Sertoli cells showed significant Gal-3 immunoreactivity, particularly during hibernation. Similarly, Deschildre et al. (19) observed Gal-3 expression in Sertoli cells in rats, but noted the absence of Gal-3 in spermatocytes and spermatids. Our results, however, revealed Gal-3 immunoreactivity in both spermatogonia and spermatocytes, especially during hibernation. Additionally, Özbek et al. (9) reported no Gal-3 expression in spermatogenic cells, while we detected immunoreactivity in both Sertoli cells and certain spermatogenic cells. Similar to Gal-1, Gal-3 may exhibit species-specific differences in its expression patterns and functional roles within the testis. The increase in Gal-3 immunostaining in Sertoli cells and spermatogenic cells within the seminiferous epithelium during hibernation could be linked to known anti-apoptotic properties of Gal-3 (20). In conditions of reduced energy availability and environmental stress, as seen in hibernation, the role of Gal-3 in inhibiting apoptosis may be essential for cellular survival. This anti-apoptotic activity likely contributes to the preservation of germ cell integrity by protecting against cell death during prolonged metabolic suppression.

The epididymis is categorized into three separate regions: Caput, corpus, and cauda. The caput and corpus are chiefly responsible for spermatozoa maturation, whilst the cauda functions as a reservoir for mature sperm. Moreover, in rats, it is lined by an epithelium consisting primarily of principle and basal cells, along with less prevalent cell types such as apical, narrow, and halo cells (21). It has been reported that Gal-3 is intensely expressed in certain epithelial cells (22). Studies conducted in various species, such as rats (9), and bulls (23), have reported intense Gal-3 expression in the epididymal epithelium, with particularly high expression in the distal regions of the epididymis. Özbek et al. (9) suggest that the presence of Gal-3 in the corpus and cauda regions indicates an important role for this protein in the maturation and storage of spermatozoa. In our study, the observed decrease in Gal-3 expression in the epididymal epithelium during hibernation may reflect a reduction in the need for this function, as there may not be sufficient spermatozoa available for storage during this period, consistent with the hypothesis proposed by Özbek et al. (9).

This study demonstrates distinct expression patterns of Gal-1 and Gal-3 in the testis and epididymis of the Anatolian ground squirrel. Gal-1 was primarily localized in peritubular myoid cells and vessel wall in testis, with higher expression in pre-hibernation, suggesting a structural role adapted to seasonal reproductive demands. Gal-3 showed increased expression in Sertoli and spermatogenic cells during hibernation, likely due to its anti-apoptotic properties. In the epididymis, Gal-3 was more intense in pre-hibernation, particularly in distal regions, indicating a role in sperm maturation and storage, which declines during hibernation as reproductive activity reduces. Further research could clarify their specific molecular functions in hibernating species.

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CONFLICT OF INTEREST

The authors state no conflicts of interest related to this study.

AUTHOR CONTRIBUTIONS

Mehmet ÖZBEK: Writing, editing, methodology, investigation. Mustafa ÖZTOP: Writing, validation, visualization, editing.

ETHICAL STATEMENT

All animal experiments in this study were performed in accordance with the ethical guidelines established by the Erciyes University Animal Experiments Local Ethics Committee. Approval for the procedures was granted under the ethical approval number 15/140, Kayseri.

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DERLEME
REVIEW

Next Generation Vaccines

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Abstract

For centuries, mankind has been aware that prevention is more valuable than cure and has sought appropriate ways to do so. The adventure of vaccination, known as the most effective protection method, began with studies against smallpox. It continued when Edward Jenner administered the vaccinia virus to a child in 1796, which he received from a woman infected by a cow. Louis Pasteur observed that the virus administered in 1798 eliminated the smallpox virus after a few months, so the smallpox vaccine was first discovered and applied. The concept of inactivated vaccines emerged during the collaboration between Robert Koch and Louis Pasteur. Inactivated vaccines against plague, cholera, and typhoid emerged around the end of the nineteenth century. In 1948, the first combined vaccine against diphtheria, tetanus and pertussis was produced. After the second half of the 20th century, new applications began to be introduced. Then, cell culture studies for viral vaccines started. The effect of advancing technology began to be felt in vaccines over time and new generation vaccine studies started. With cloning, the foundation of recombinant vaccines and thus new-generation vaccines was laid. Scientists focused on next-generation vaccine studies and introduced vaccines such as viral vector-based vaccines, RNA-based vaccines, Subunit vaccines, Virus-like particle vaccines and Marker vaccines into vaccine technology.

Anahtar Kelimeler: New generation vaccines, recombinant protein, vaccine types

Yeni Nesil Aşılar

Öz

İnsanlık yüzyıllardır önlemenin tedavi etmekten daha iyi olduğunu biliyor ve bunu başarmanın yollarını arıyor. En etkili korunma yöntemi olarak kabul edilen aşılama serüveni çiçek hastalığına ilişkin çalışmalarla başladı. Bu durum, 1796'da Edward Jenner'ın, bir inekten enfekte olan bir kadından aldığı aşı virüsünü bir çocuğa vermesiyle devam etti. Louis Pasteur 1798 yılında uygulanan virüsün çiçek virüsünü birkaç ay sonra ortadan kaldırdığını gözlemlemiş ve böylece ilk kez çiçek aşısı bulunup kullanılmıştır. Robert Koch ve Louis Pasteur tarafından sürdürülen bu süreç, inaktive aşı kavramının ortaya çıkmasına yol açtı. 19. yüzyılın sonlarına doğru veba, kolera ve tifoya karşı inaktif aşılar geliştirildi. 1948 yılında difteri, tetanoz ve boğmacaya karşı ilk kombine aşı üretildi. 20. yüzyılın ikinci yarısından sonra yeni uygulamalar ortaya çıktı. Viral aşılar için hücre kültürü çalışmaları başladı. Zamanla teknolojik gelişmelerin aşılar üzerindeki etkisi hissedildi ve yeni nesil aşılar üzerinde çalışmalar başladı. Klonlama, rekombinant aşıların ve yeni nesil aşıların temelini attı. Bilim insanları yeni nesil aşı çalışmalarına odaklanarak viral vektör bazlı aşılar, RNA bazlı aşılar, alt birim aşılar, virüs benzeri parçacıklı aşılar ve marker aşılar gibi aşıları aşı teknolojisine kazandırdı.

Key Words: Aşı çeşitleri, rekombinant protein, yeni nesil aşılar

INTRODUCTION

The process of bringing and applying the agent or agents that will cause infection to the formulation to be given to the organism by various methods and creating immunization against those agents after the application is called "vaccination" and the biological substances used for this process are called "vaccine" (1). The first vaccination history back almost 4 centuries (2). While people did not even know the definition of microorganisms, they struggled for immunization. After being infected, the impossibility of treatment together with the difficulties of the period and the occurrence of deaths even from a simple infection forced people to find ways of protection against diseases. In this context, they first tried to provide immunity against this disease by drying the crusts of the wounds of people infected with smallpox, scratching the skin of healthy people and applying them to them (3). Today, taking preventive measures against the disease rather than treating it is the priority in the fight against infections.

Vaccinology, known as vaccine science, is a multidisciplinary science. Fields such as immunology, microbiology, molecular biology, biochemistry, and statistics are closely related to this science. The first goal of vaccination is to protect against infections. However, with the recently developing science, it is also used in cases such as cancer vaccines, birth control or autoimmune diseases, allergies, such as reducing the immune response by combining new generation technologies (4). When vaccines were first administered, they were made using purified attenuated live viruses or inactivated microorganisms. Later on, more refined methods were used. Applications such as the creation of toxoid from a protein toxin and its use in treatment, the creation of purified and inactivated virus, the development and use of virus-like particles and purified polysaccharides have started to take place in science. Vaccines are usually made in a type that includes all microorganisms, purified macromolecules, combined antigens, recombinant vectors with later developing technology, synthetic peptides, or nucleic acids such as DNA RNA. With these developing vaccine types, production processes have become more technological (5).

Next Generation Vaccines

Pathogens become better understood as molecular biology and microbiological tools progress. Wolf et al. (6) discovered that mice injected with a plasmid containing a cloned protein also expressed a cloned transgenic protein in the plasmid DNA. These observations prompted the development of a new immunization approach, ushering in the age of next-generation vaccines. The first tactic employed for these novel vaccines was the DNA-based technology, followed later by the invention of viral vectors for immunization, such as adeno-associated virus (AAV), lentiviral or adenoviral vectors, and more recently RNA-based vaccines (7). With the intensive expansion of genome-based studies, different methods have started to be developed in vaccines. The advantages offered by technologies that enable the understanding of the entire genome of the microorganism have created a perspective for vaccine research. Sequencing is of great importance in determining the pathogenic profiles of similar or different types of bacteria (8). The whole genome sequence is sequenced with bioinformatics tools and the dominant pathogenic strain is identified in the field. With these genomic analyses, new-generation vaccines or antimicrobial molecules are designed against pathogenic bacteria. Genome sequences enable the identification of molecules with vaccine potential, regardless of whether the agent is produced in vivo or in vitro. In silico analysis of the genome sequence is the starting point for vaccine design. This innovative approach, which is different from conventional vaccination science, is called "Reverse vaccinology" (9). Although bioinformatics information on new potential candidate vaccines is available, in silico analysis must also be performed. When genomic data is integrated with advanced techniques like as in vivo expression technology (IVET), signature-tagged mutagenesis (STM), DNA microarrays, and proteomics, new surface antigens or virulence factors can be experimentally identifiedAll of these studies, known as "functional genomics," equip us with tremendous tools for studying the genome (10).

As a result, these innovative vaccines contain only a specific viral/bacterial antigen rather than utilizing the entire pathogen, resulting in an improved safety profile. However, developing such vaccines necessitates a more in depth understanding of viral/bacterial structures, as well as the interaction between viral/bacterial proteins and host cell receptors. Next generation vaccinations require a protracted preliminary study period before they may be developed. A reverse vaccinology strategy was used to develop a vaccine against the human pathogen Neisseria meningitidis serogroup B. A recombinant vaccine against Hepatitis B (HBV) was prepared using the subunit vaccination method, which is based on a specific immunogenic antigen, and a vaccine against whooping cough was prepared by highly purifying 3 proteins of Bordetella pertussis (11).

Recombinant Protein Vaccines

Recombinant protein vaccines use recombinant viral or bacterial structural proteins to boost the immune system. Because the immune system's humoral and cellular elements recognize and respond immunologically to specific pathogen locations (either toxins isolated from the organism or surface antigens isolated from the organism, etc.) this has led to the development of vaccinations based on pathogen components i.e. protein components that have a protective function (12). The basic strategy in recombinant vaccine technology is to clone one/several genes from different etiological agents and transfer them to bacterial, yeast, mammalian, and insect cells are capable of replicating the antigenic determinant's DNA. The important point here is that several considerations should be considered before selecting the system for antigen expression. The main features that determine the efficiency of producing the efficacy of the vaccine is influenced by various factors including the expression level of the antigen gene within the specified vector and promoter, the inclusion of a selection marker, and whether post-translational modifications are facilitated by the recombinant vector (Figure 1). The most common expression systems are bacterial extensively utilized systems because of their case of use and high-level expression capabilities. Despite the developing technology in the field of vaccination, it is still difficult to develop vaccines for persistent infections

such as HIV and mycobacteria. In such cases, the immunogenic part of the pathogen is produced as a recombinant protein and an immune response is created in the organism. The bulk of vaccines being researched now are made up of highly purified recombinant proteins or pathogen components (13). This technology enables the development of immunogenic protein-based vaccines for agents that are difficult to produce by culture. Following the identification and production of recombinant antigens of malaria and SM28 protein of schistosomiasis, which is one of the research within this scope, it enables the development of appropriate vaccine formulation by proceeding to clinical trials (14). The Hepatitis B (HBV) vaccine is one of the recombinant vaccines that has been confirmed to be effective and is currently licensed for human use. It contains the recombinant Hepatitis B viral surface antigen (HBsAg) is created by DNA transfected yeast or mammalian cells (15). Although vaccines based on recombinant proteins provide significant advantages over conventional vaccines in terms of safety and production cost; yet, most of them demonstrate limited immunogenic effects when administered alone, so effective and appropriate adjuvants should be used to create a strong and long-lasting immunological response (16). It is much more difficult to stimulate a cellular immunological response against intracellular pathogens using conventional vaccination techniques. Live attenuated pathogen vaccinations are can elicit such a response, can cause possible risks that cannot be ignored, such as increased virulence and pathogenicity in vulnerable hosts, although not frequently. Recombinant vaccines, on the other hand, are based on the expression of one or more defined antigens by plasmids or apathogenic bacterial/viral vectors to stimulate immunity against the pathogen and are administered with adjuvants (17). When considering recombinant protein vaccines, e.g. diphtheria or tetanus toxoid vaccines, vaccines based on purified macromolecules allow protection from the major risks mentioned, for example, undesired pollutants may be co-purified, and toxoids may be converted into dangerous forms. This approach also addresses the issue of a lack of purified antigenic components in enough quantities (18). Some recombinant proteins have low immunogenicity and aluminum salt, currently the only immunological adjuvant licensed for human use, is sometimes insufficient. Therefore, to improve the efficacy of such vaccines, molecular biology methods can be used to characterize more effective adjuvants. Early studies on the design of adjuvants mostly focused on the use of cytokines and especially one of them, interferon g (IFN-g). IFN-g, one of the most studied cytokine adjuvants, induces an immune response even when given alone to the organism.

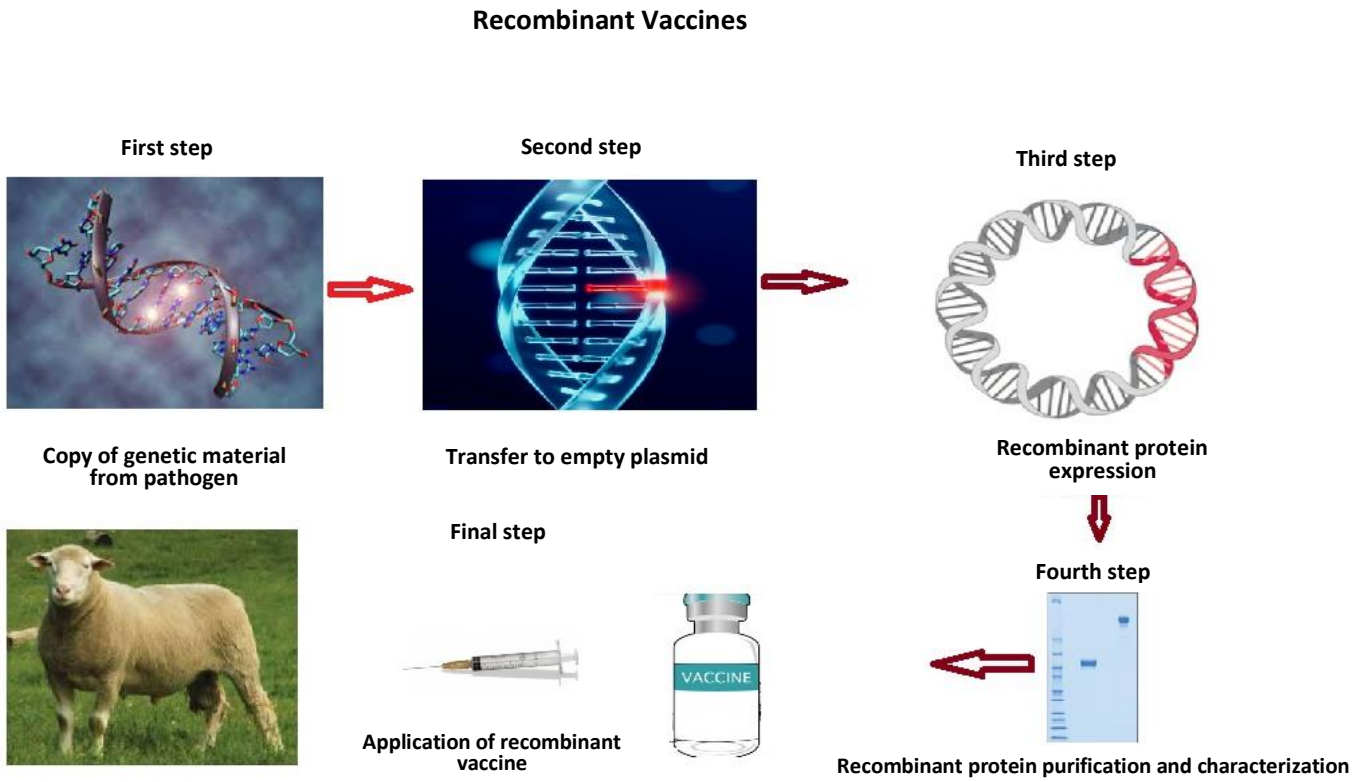

Figure 1. Recombinant vaccine production and application step

Viral Vector Vaccines

Viral vectors are considered potential tools for genetic treatments and vaccinationsThe idea of a viral vector was initially proposed in 1972. Jackson et al. (19) genetically engineered simian vacuolating virus 40 (SV40) to produce recombinant DNA. Vectors' immunogenic power is based on viruses' ability to infect cells (19). Moss et al. (1982) demonstrated the use of vaccinia virus as a transient gene expression vector. In general, viral vectors provide benefits such as (a) extremely effective gene transduction, (b) highly selective gene delivery to target cells, and (c) production of a strong immune response and strengthening of cellular immunity (20). Recombinant viral vectors offer therapeutic potential by facili-

tating intracellular antigen production, prompting robust cytotoxic T-lymphocyte (CTL) responses, thereby eliminating infected cells (21).

RNA Based Vaccines

Vaccinations based on nucleic acid were developed long ago with the intention of establishing a class of vaccines that would be easy to create, safe, and successful. RNA based vaccine study explores two distinct forms of RNA: non-replicating mRNA and self-replicating RNA generated by viruses. Self-replicating RNAs encode both the antigen and the viral replication machinery, allowing for intracellular RNA amplification and profuse protein production. This contrasts with conventional mRNA-based vaccinations, which only encode the antigen with 5' and 3' UTRs. Till the late 2000s, emphasis was concentrated on the development of DNA-based techniques due to challenges such as RNA instability, inefficient immune response when administered in vivo, and promotion of excessive inflammatory reactions. Creating in vitro transcribed (IVT) messenger RNA (mRNA) is a straightforward procedure. Producing high-quality 'therapeutic' mRNA that is very expressible and doesn't cause inflammation has been a major challenge in this field until recently (22). Some critical issues, including the addition of modified nucleosides optimization of coding sequences and high-level purification of IVT mRNA have led to the development of high-performance liquid chromatography (HPLC) to purify RNA contaminants in the early 2010s (23). With this method, the toxicity of synthetic mRNA is reduced by enabling organismal self-detection, and an accurate reading of the mRNA is made possible. mRNA vaccines are a relatively new type of vaccination that shows promise for the future. This strategy is based on newly published research that show the efficacy of mRNA vaccines in treating a variety of malignancies and infectious disorders when traditional immunizations fail to provide protective immunity. Safe, effective materials for in vivo mRNA. transfer and developed protocols for high-quality mRNA production are being studied (22). One of the first COVID-19 vaccines that started clinical trials was the RNA-based vaccine, and studies were initiated with the idea that it would made a significant contribution to the fight against the pandemic of the period (24).

Subunit Vaccines

In the 1950s and 1960s, scientific studies were centered on molecular microbial genetics. In the early 1970s, new information about the role of DNA in cells, the nature of genes, the activity of phages, and the discovery of restriction enzymes led to the alteration of DNA molecules to contain foreign DNA. Recent discoveries in immunology and protein engineering have paved the development path and manufacture of recombinant subunit vaccines. In recent years, numerous approaches have been developed to synthesize recombinant DNA and transfer it to a host cell such as *Escherichia coli* or *Saccharomyces cerevisiae*, or transfer it to a baculovirus-insect cell expression system to produce recombinant proteins (25). During the following years, the focus has been on developing more expression systems with increased power to produce recombinant proteins. This biological revolution resulted in the development of a new concept of vaccines. Working on the notion that safe, inexpensive, and effective vaccine candidates may be created using particular antigens from many infectious agents, a new light was shone on the vaccine concept. The fundamental principle of the subunit vaccination involves isolating the gene encoding the vaccine and transferring it to a second non-pathogenic organism. The heterologous host produces the gene that was introduced to it. The produced gene can be purified and engineered to be administered as an immunogen employing the production host in a living vector or as pure nucleic acids in the form of a vaccine-encoding gene (26).

DNA Vaccines

Vaccines described as third-generation vaccines, genetic immunization or DNA vaccination offer innovative techniques for the prevention and treatment of a variety of bacterial and viral illnesses. DNA vaccines are composed plasmid DNA expression vectors derived from *E. coli* contain genetic instructions for desired antigens, regulated by potent viral promoters recognized by mammalian hosts. Upon injection into an animal, the plasmid DNA prompts the expression of the antigenic gene and antigen-specific immunity develops. To develop a DNA vaccine, the gene encoding the antigen from interest is introduced into the bacterial plasmid under direction of a suitable eukaryotic promoter (27). Because of the nucleotide mismatch among bacteria and eukaryotic cells, single nucleotide polymorphisms typically modify the antigenic genes to increase the effectiveness of expression of genes. The purified and detoxifying plasmid genetic material is then injected into the host animal. Plasmids picked up by suitable cells in the recipient cell cause their own transcription of genes and protein synthesis, resulting in the production of the desired antigen. The host detects the synthesized antigens as foreign and initiates an immunological reaction against it. In the last decade, DNA immunization has emerged as an effective new technique to immunoprophylaxis. It has lately been used successfully to enhance humoral and cellular immune responses in experimental animals and non-human primates (28).

Vaccines Based on Virus-Like Particles

Virus-like particle (VLP)-based vaccinations are a more secure and more efficient alternative to conventional immunizations. VLPs are new fragments of molecules used to guard and control viral illnesses. VLPs are proteins groups that consist of a number of species. Because they lack viral amino acids, they resemble the virus from which they are produced in size and appearance. Recently, many virus-like components have been built using recombination VLP technology approaches. VLPs have also been used as carrier systems to transport foreign antigen epitopes in recently produced candidate vaccines. VLPs were designed for the development of VLP-based plasmodium vaccine candidates (29), diseases caused by group A *Streptococcus* infections (21), Alzheimer's disease (30), allergic asthma, diabetes, tumor and cancer preventive reagents delivery (31). VLP technology opens new pathways for more advanced healthcare uses such as carcinoma immunotherapy, Alzheimer's disease, metabolism and chronic illnesses (32). VLPs are produced through the expression of recombinant proteins. Bacteria, yeast,

mammals, insects, and plants all express viral structural proteins. Eukaryotic cells assemble with empty capsids in vivo, but prokaryotic cells frequently assemble in vitro. More complex VLPs, consisting of multiple viral proteins and a lipid envelope, need an eukaryotes host. An in-depth analysis of the VLP expression systems is necessary for effective production. Many VLP-based vaccination candidates have been generated by advances in genetic engineering, bioengineering, and virus structural determination and these have been assessed in studies in both preclinical and clinical settings. VLP-based immunizations have made major improvements to the fight against cervical carcinoma and hepatitis B infection (33). Even though a variety of adjuvants can be used to improve the immune system reaction in vivo, such as aluminum salts (e.g., the aluminum hydroxide and aluminum phosphate), emulsions made from oil in water (e.g., Span 85 and Polysorbate 80), and AS04 (a blend of monophosphoryl lipid A and aluminum salt), many different adjuvants are used preclinically and/or clinically. Various adjuvants, such as aluminum salts (e.g., aluminum hydroxide and aluminum phosphate), fatty emulsions in water (e.g., Span 85 and Polysorbate 80), and AS04 (a mixture of monophosphoryl lipid A and aluminum salt), can be utilized to boost the immune response in vivo. There are several pre-clinical and/or clinically used additional additives. Modern vaccine design usually considers the selection of a specific adjuvant to stimulate a specific type of immune response (34).

VLP vaccines developed/under development based on recombinant protein;

1.Human papilloma virus (HPV) vaccines

2. Hepatitis B vaccines

3. Hepatitis E vaccine

4. Flu vaccine candidate

5. Norwalk virus candidate vaccine

6. Ebola and Marburg virus vaccine candidate

7. Hepatitis C candidate vaccine

8. Human immunodeficiency virus (HIV) vaccine candidate

9. Malaria candidate vaccine

Marker Vaccines

Throughout history, vaccines have been developed to prevent disease or reduce the severity of clinical manifestations of infections. However, vaccination can sometimes interfere with serologic diagnosis and determination the prevalence and incidence of infection when the antibody response after vaccination is indistinguishable from the immune response after infection. Marker vaccines have provided a solution to this issue. Using a diagnostic kit, a marker vaccine is a sort of vaccination that makes serological discrimination between vaccinated and infected animals easy but reliable. A marker vaccination is a vaccine (inactivated or live) based on delete mutations or identified proteins from bacteria to distinguish vaccination and infected individuals were separated based on adequate antibody responses. This vaccine is used in conjunction with a test that identifies antibodies against a protein that is absent in the vaccine stem (35).

A proposed marker vaccination must meet a few minimum requirements (36).

1. It should not cause immediate or prolonged the risks in vaccinated animals.

2. Not pose a risk to immunized animals or different species following genetic recombination.

3. Be simple to produce following a consistent methodology.

4. Develop permanent immunity immediately.

5. A completely immunity to all known versions of the virus should be developed.

6. The agent should not be transmitted vertically or horizontally.

7. A simple yet extremely accurate and specific differential diagnostic test should be available.

The first step in candidate marker vaccine design should be create an efficient examination for diagnosis. In this respect, the phrase "marker vaccine" is not a very clear idea, because the primary distinction between marker vaccines and standard immunizations is the ability to distinguish between vaccinated and infected animals' antibody responses. Therefore, a marker vaccine can be referred to as a difference of infectious field strain from vaccinated animals (DIVA) vaccine (37).

Generally, there are three marker vaccination techniques: (38)

1. Marker vaccine is a strategy as a "negative marker" by excluding a minimum of one immunological epitope or protein when the field strain and vaccine strain are compared.

2. "Exogenous positive marker vaccine" by involving an immunodominant antigen or protein not normally present in a potent vaccine.

3. "Intrinsic positive marker" is a marker vaccine created by involving an epitope or immunogen present in the causative agent but stimulates a different antibody response than the vaccine strain of the field strain.

So far, practically all potential marker vaccines have been developed using a "negative marker" technique. Serologic diagnostic methods produce results by testing antibody against the target protein, which is not present in the marker vaccination. Animals that tested positive had been infected. Aside from technical issues, their specificity and sensitivity are mostly influenced by the immune system's response to the potential vaccine and natural infection. The subsequent production of antibody against negative markers, or the low number of such animals following a spontaneous infection, might significantly reduce the DIVA potency of the diagnostic test. In theory, a marker vaccination containing only negative indicators is sufficient to elicit a different antibody response than spontaneous infection. If the marker vaccination includes positive and negative markers, infected or suspected animals can be discriminated from vaccinated ones more efficiently. Vaccination with a marker vaccine is an effective strategy for limiting the magnitude of infectious outbreaks (39). Serological testing can be used to diagnose immunity after receiving marker vaccinations. Incidence and frequency can be calculated among vaccinated persons. The vaccine's efficacy can be tested, allowing the vaccine to be used in conjunction with an eradication program (40).

Table 1. Advantages and disadvantages of vaccine types

Vaccine Type	Advantage	Disadvantage
Recombinant Protein Vaccines	Protects against multiple antigens with excess protein production.	Adequate immunogenic response may not always be achieved with a single protein and therefore effective adjuvants should be used.
Viral Vector Vaccines	Recombinant viral vectors, like natural infections, actively boost immunity and have innate adjuvant properties.	To produce viral vectors, appropriate cell lines must be propagated. This raises the cost of production.
Subunit Vaccines	Its structure is more stable, safer than attenuated vaccines and more suitable for large-scale production.	Their benefits are restricted by promoting humoral immunity because they are not replicative, cannot adequately induce the cellular immune response, and are supplied externally to the antigen-presenting cell.
DNA Vaccines	Increases an effective and long-lasting cellular immunological response.	It may not always provide sufficient immunity.
Vaccines Based on Virus-Like Particles	It generates a relatively better immune response due to the presence of multiple epitopes that better simulate the surface of viruses and microorganisms	Difficulties in the production process
Marker Vaccines	It works based on deletion mutants or isolated microbial proteins that allow discrimination between vaccinated and infected individuals based on their respective antibody responses.	The late emergence of antibodies against negative markers, as well as the low percentage of such animals following natural infection, can significantly decrease the diagnostic test.

CONCLUSION

Human beings, who have been struggling with epidemics that have been a major problem for humanity for centuries, have sought ways to fight even when they could not identify the disease agent. It has been seen that the most effective way in this war is related to immunization against that agent. Synthetic immunity and protection against an agent is provided by vaccines. Vaccines have always been developed to provide more effective and longer-lasting immunity since they were first developed. Traditional vaccines, which first emerged with the approach of isolating, inactivating and injecting the agent, have been tried to be made even more effective and harmless over time and with advancing technology. With the discovery of recombinant DNA technology, second and third-generation vaccines emerged. With the advancement of genomic studies, vaccines using nucleic acid, not the causative agent, have been produced. With the reflection of these advances in vaccine studies, vaccine science has reached a point where it is more effective, safer and less costly. Today, access to vaccines has become easier. The number of people who have died and been affected by the COVID-19 pandemic worldwide is enormous. Therefore, the most important issue that scientists focused on was vaccine studies. Thanks to the products of these studies, the pandemic ended. It is seen that preventing a disease is more effective than treating it. In this context, vaccines are our indispensable weapons. Every day, all the work is going on to make these weapons even more effective. With advancing technology, studies continue at full speed to make vaccines, our most valuable defense tool against infections, even more effective.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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