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RESEARCH ARTICLES

CASE REPORT

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RESEARCH ARTICLE

Determination of Methicillin Resistance in Coagulase Negative Staphylococci Isolates Obtained from Dogs

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ABSTRACT

Although coagulase negative staphylococci are opportunistic pathogens, they are also isolated from various disease cases in dogs. Methicillin resistant staphylococci also show resistance to other β-lactam group antibiotics, which limits treatment options. In this study, determination of methicillin resistance in coagulase negative *Staphylococcus* spp. isolates isolated from various samples taken from healthy and sick dogs was aimed. Agar dilution method was used for the determination of methicillin resistance in the isolates. Eighty nine (20.74%) strains were isolated from the total 429 samples by conventional bacteriological methods. Methicillin resistance was found in 19.10% of the isolates. The *mecA* gene was determined in 11 (64.70%) of the methicillin resistant isolates by PCR. In conclusion, in this study, it was concluded that methicillin resistance should be considered in the treatment of infections in dogs that caused by coagulase negative *Staphylococcus* spp. isolates, known as opportunistic pathogens.

Keywords: Coagulase Negative *Staphylococcus* spp., Dog, *MecA*, Methicillin resistance

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Köpeklerden Elde Edilen Koagulaz Negatif Stafilokok İzolatlarında Metisilin Direncinin Araştırılması

ÖZ

Koagulaz negatif stafilokoklar, fırsatçı patojen olmalarının yanı sıra köpeklerde çeşitli hastalık olgularından da izole edilmektedir. Metisilin dirençli stafilokoklar diğer β-laktam grubu antibiyotiklere karşı da direnç göstermekte ve bu durum tedavi seçeneklerini kısıtlamaktadır. Bu çalışmada, sağlıklı görünen ve hasta olduğu belirlenen köpeklerden alınan çeşitli örneklerden izole edilen koagulaz negatif *Staphylococcus* spp. izolatlarında metisilin direncinin belirlenmesi amaçlandı. İzolatlarda metisilin direnci agar dilüsyon yöntemiyle belirlendi. Toplam 429 örnekten konvansiyonel bakteriyolojik yöntemlerle 89 (%20.74) adet koagulaz negatif *Staphylococcus* spp. izole edildi. İzolatların %19.10'u metisiline dirençli bulundu. Metisilin dirençli izolatların 11 (%64.70)'inde PCR ile *mecA* geni tespit edildi. Sonuç olarak, bu çalışmada fırsatçı patojenler olarak bilinen koagulaz negatif *Staphylococcus* spp. izolatlarının köpeklerde neden olabileceği enfeksiyonların tedavisinde metisilin direncine dikkat edilmesi gerektiği kanaatine varıldı.

Anahtar Kelimeler: Koagulaz Negatif *Staphylococcus* spp., Köpek, *MecA*, Metisilin Direnci

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GİRİŞ

Stafilokoklar, insan ve hayvanların deri ve mukoz membranları ile gastrointestinal sistemlerinin doğal florasında bulunmaktadır (Misic ve ark. 2015, Bierowiec ve ark. 2016). Özellikle burun boşluğu mukozasında yer alan etkenlerin, hayvanlar ve insanlar arasında bulaşmada kritik öneme sahip olduğu vurgulanmaktadır (Quinn ve ark. 2011).

Koagulaz enzimi stafilokok türlerinin virülens özelliklerinin belirlenmesinde önemli bir kriter olarak kabul edilmekte ve stafilokok türleri, koagulaz negatif stafilokok (KNS) ve koagulaz pozitif stafilokok (KPS) olarak 2 ana gruba ayrılmaktadır (Quinn ve ark. 2011). KNS izolatlarının hastalık yapma yeteneklerinin sınırlı olduğu düşünülse de, köpeklerde yara enfeksiyonu, otitis eksterna, apse, pyoderma ve konjuktivitis vakalarından izole edilmektedir (Kloos ve Bannerman 1995, Litster ve ark. 2007, Hariharan ve ark. 2009, Suter ve ark. 2017).

Metisilin, bakterilerde hücre duvarı sentezi için gerekli olan penisilin bağlayan proteinleri (PBP) bloke ederek etkisini göstermektedir. Metisilin direnci stafilokok izolatlarında en sık rastlanan direnç mekanizması olup, hem halk sağlığını hem de hayvan sağlığını olumsuz yönde etkilemektedir (Chah ve ark. 2014, Samanta ve Bandyopadhyay 2020). KNS izolatlarında metisilin direncinin fenotipik olarak, sefoksitin (30 µg) diskinin kullanıldığı disk difüzyon testi ya da oksasilin minimial inhibitor konsantrasyon (MİK) değeri belirlenerek tespit edilmesi gerektiği belirtilmektedir (CLSI 2018).

Metisilin dirençli stafilakoklar beta laktam antibiyotiklerin daha az afinite duyduğu PBP2a'yı sentezleyerek direnç geliştirmişlerdir (Lim ve Strynadka 2002). PBP2a *mecA* geni tarafından kodlanmaktadır ve bu genin *Staphylococcus sciuri* ve *Staphylococcus fleuretti* izolatlarından *Staphylococcus aureus* izolatlarına horizontal olarak aktarıldığı bildirilmiştir (Tsubakishita ve ark. 2010). *MecA* dışında fem, aux ve hmt gibi faktörler de metisilin ya da diğer beta laktam antibiyotiklerin varlığında hücre duvarı sentezini bloke ederek dirence neden olabilmektedir (De Lencastre ve ark. 1999). Ayrıca PBP2a'da meydana gelen nokta mutasyonlar da metisilin direncinden sorumlu tutulan diğer mekanizmalardan biridir (Ba ve ark. 2013). Yapılan çeşitli araştırmalarda da sağlıklı görünen ya da klinik olarak hasta olduğu belirlenen köpeklerden *mecA* geni pozitif metisilin dirençli stafilokok suşlarının izole ve identifiye edildiği ve bu izolatların halk sağlığı açısından risk teşkil ettiği bildirilmiştir (Aslantaş ve ark. 2013, Gandolfi-Decristopharis ve ark. 2013, Elnageh ve ark. 2020, Khanal ve ark. 2021).

Bu çalışmada sağlıklı görünen ve/veya klinik olarak hasta olduğu tespit edilen köpeklerden alınan çeşitli örneklerden izole edilen KNS suşlarında metisilin direncinin fenotipik ve genotipik karakterizasyonu amaçlandı.

MATERYAL ve METOT

Materyal

Bu çalışmada, Van ve yöresinde klinik olarak sağlıklı görünen köpeklerden alınan 402 adet örnek (idrar=114, vaginal svap=81, rektal svap=115, burun svabı=57 ve kulak svabı=35) ile konjuktivitis ve otitis belirtileri gösteren köpeklerden alınan 20 adet göz svabı ve 7 adet kulak svabı örnekleri kullanıldı.

Metot

İzolasyon ve İdentifikasyon: Svap örnekleri direkt olarak, idrar örnekleri ise Papini ve ark. (2006)'nın bildirdiği yönteme göre santrifüj edildikten sonra %5-7 oranında defibrine koyun kanı katılmış Columbia blood agar (Oxoid, CM 03331, İngiltere) ve Mannitol Salt Agar (Oxoid, CM85, İngiltere) besiyerine ekilerek, aerobik ortamda 37°C'de 24 saat inkübasyon periyoduna bırakıldı. Besiyerlerinde oluşan kolonilerden saf kültürler elde edildi. Saf kültürlerden alınan koloniler, Gram boyama, katalaz ve oksidaz reaksiyonu ile Mannitol Salt Agar besiyerinde üreme sonuçlarına göre değerlendirildi (Quinn ve ark. 2011). Stafilokok olarak belirlenen izolatların koagulaz reaksiyonunun belirlenmesinde tüp koagulaz testi uygulandı (Carter 1984).

Metisiline Karşı Duyarlılığın Belirlenmesi: Araştırmada incelenen KNS izolatlarının metisiline karşı duyarlılığı agar dilüsyon yöntemiyle belirlendi (CLSI 2018). Testte oxacillin sodium salt (28211-1G, Sigma, China) kullanıldı. Antimikrobiyal madde steril distile su ile sulandırılarak, Mueller Hinton Agar (Merck 1.05437, Almanya) besiyerinde 0.125 µg/ml'den 512 µg/ml'ye kadar dilüsyonları hazırlandı. Taze kültürlerden elde edilen izolatlar, steril fizyolojik tuzlu suda 0.5 MacFarland yoğunluğuna eşit olacak şekilde süspanse edildi. Hazırlanan süspansiyondan 1'er µl alınarak antibiyotik dilüsyonlarının ilave edildiği besiyerlerine ayrı ayrı inoküle edildi. Teste referans suş olarak *Staphylococcus aureus* ATCC® 29213 kullanıldı.

MecA Geninin Belirlenmesi:

Metisiline karşı dirençli olduğu belirlenen izolatlarda *mecA* geninin varlığı gen spesifik primerlerin (Zhang ve ark. 2005) kullanıldığı PCR ile araştırıldı. PCR işlemlerinde kullanılan genomik DNA, ticari genomik DNA izolasyon kiti (GeneAll, ExgeneTM Clinic SV Mini, 108.101, Korea) kullanılarak elde edildi. Amplifikasyon protokolünde 94°C'de 10 dk ön denatürasyon aşamasını takiben toplam 35 siklusluk PCR işleminde 94°C'de 1 dk denatürasyon, 53°C'de 1 dk bağlanma ve 72°C'de 1 dk uzama aşamaları uygulandı. Final uzaması 72°C'de 10 dk olarak ayarlandı. PCR sonucu elde edilen amplikonlar %1.5'lik agaroz jelde elektroforeze tabi tutuldu. Amplikonlar DNA marker ile karşılaştırılarak UV translüminatörde

incelendi ve 147 bp'lik bant oluşturanlar *mecA* pozitif olarak kabul edildi.

BULGULAR

Çalışmada incelenen toplam 429 örnekten bakteriyolojik konvansiyonel yöntemlerle 89 (%20.74) adet koagulaz negatif *Staphylococcus* spp. izolatı elde edildi. İdrar örneklerinden 7 (%6.14), vaginal svap örneklerinden 5 (%6.17), rektal svap örneklerinden 4 (%3.47), burun svabı örneklerinden 43 (%75), kulak svabı örneklerinden 25 (%59), göz svabı örneklerinden ise 5 (%25) adet koagulaz negatif *Staphylococcus* spp. suşu izole edildi.

İncelenen 89 adet KNS suşunda belirlenen oksasilin MİK değerleri Tablo 1'de gösterildi. Yapılan değerlendirmede oksasilin için $MİK_{50}$ değeri 0.25 µg/ml, $MİK_{90}$ değeri ise 512 µg/ml olarak tespit edildi. MİK değerleri dikkate alındığında 89 izolatın 17 (%19.10)'si metisiline (oksasilin) dirençli bulundu. Metisilin dirençli izolatların çoğunluğu (%58.82) burun svabı örneklerinden elde edildi. Sağlıklı görünen ya da hastalık olgusu tespit edilen köpeklerden alınan örneklerden metisilin dirençli KNS izolasyon oranlarında istatistiksel olarak anlamlı bir ilişki tespit edilmedi ($p>0.05$).

Metisiline karşı fenotipik olarak direnç tespit edilen izolatlarda metisilin direncinden sorumlu *mecA* geninin varlığı PCR ile araştırıldı. Metisiline dirençli olduğu belirlenen 17 izolatın 11 (%64.70)'inde *mecA* geni belirlendi (Tablo 2).

Tablo 1. Koagulaz negatif *Staphylococcus* spp. izolatlarında (n=89) oksasilin MİK değerinin dağılımı.

Table 1. Distribution of oxacillin MIC value in coagulase negative Staphylococcus spp. isolates (n=89).

Antibiyotik	MİK (µg/ml)													
	0.125	**0.25**	**0.50**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**>512**
OX	41	31	4	1	0	0	0	0	0	1	0	1	**9**	1

Ox: Oksasilin
Altı Çizili Değer: $MİK_{50}$
Koyu Renkli Değer: $MİK_{90}$

Tablo 2. Metisilin (oksasilin) dirençli izolatlarda *mecA* gen pozitifliğinin dağılımı.

Table 2. Distribution of mecA gene positivity in methicillin (oxacillin) resistant isolates.

İzolat No	Kaynak	MİK (µg/ml)	*mecA*
4	Burun Svabı	512	+
5	Burun Svabı	0.50	-
6	Burun Svabı	512	+
8	Burun Svabı	512	+
9	Burun Svabı	0.50	-
27	Göz Svabı (Konjuktivitis)	>512	+
28	Kulak Svabı (Otitis)	0.50	-
35	İdrar	256	+
38	Vaginal Svap	0.50	+
55	Kulak Svabı	512	+
56	Kulak Svabı	512	-
66	Kulak Svabı	64	-
70	Burun Svabı	512	+
77	Burun Svabı	512	+
81	Burun Svabı	512	+
83	Burun Svabı	1	-
86	Burun Svabı	512	+

TARTIŞMA

Beşeri hekimlikte nazokomiyal enfeksiyonlardan sıklıkla izole edildiği bildirilen metisilin dirençli stafilokok türleri, sağlıklı hayvanların mukoz membranlarının doğal florasında bulunabildiği gibi hastalık olgularından da izole ve identifiye edilmektedir (Yiğit ve ark. 2008, Köck ve ark. 2010, Dantes ve ark. 2013). KPS suşlarının KNS suşlarına göre virülenslerinin daha yüksek olduğu

düşünülmektedir. Ancak hem beşeri hekimlikte, hem de veteriner hekimlikte KNS suşlarının neden olduğu enfeksiyonların oranları gün geçtikçe artmaktadır. KNS izolatları, antimikrobiyal direnç gelişiminde rol oynayan genleri suşlar arasında aktarma özelliğine de sahiptir (Nemeghaire ve ark. 2014, Suepaul ve ark. 2021).

Çeşitli araştırmalarda insanların sosyal hayatlarında yakın temas halinde oldukları köpeklerin, antimikrobiyal maddelere karşı dirençli olan bakteriyel etkenleri taşıyıcı konumunda olduklarına dikkat çekilmektedir (Aslantaş ve ark. 2013, Chah ve ark. 2014, Siugzdaite ve Gabinaitiene 2017, Teixeira ve ark. 2019, Elnageh ve ark. 2020).

Aslantaş ve ark. (2013) sağlıklı köpeklerden aldıkları 162 nazal svap örneğinin %15.43'ünde metisilin dirençli KNS suşunun izole edildiğini bildirmişlerdir. Benzer başka bir çalışmada da bu oran %16.7 olarak rapor edilmiştir (Gomez-Sanz ve ark. 2019). Pet hayvanlarında farklı klinik olgulardan alınan svap örneklerinden %20.51 oranında metisilin dirençli KNS izole edildiği bildirilmiştir (Göçmen ve ark. 2020). Evcil memeli hayvanlardan alınan çeşitli klinik örneklerin ise %23.88'inde metisilin dirençli KNS izole ve identifiye edildiği belirtilmiştir (Göçmen ve ark., 2018). Khanal ve ark. (2021) köpek nazal svap örneklerinden metisilin dirençli KNS suşunun izolasyon oranının %23.6, Elnageh ve ark. (2020) ise %18.75 olduğunu rapor etmişlerdir. Buna karşın Abdel-Moein ve Zaher (2020) köpeklerden aldıkları nazal svap örneklerinden sınırlı sayıda (%3.2) metisilin dirençli KNS izole ettiklerini bildirmişlerdir. Köpeklerden alınan inguinal svap, kulak svabı, rektal svap örnekleri ile pyoderma ve otitis externa vakalarında metisilin dirençli KNS izolasyon oranının ise %6-14 arasında değiştiği bildirilmektedir (Gandolfi-Decristopharis ve ark. 2013, Chah ve ark. 2014, Siugzdaite ve Gabinaitiene 2017, Teixeira ve ark. 2019).

Yapılan değerlendirmede sağlıklı ve/veya hasta olduğu belirlenen köpeklerden alınan çeşitli örneklerde metisilin dirençli KNS izolasyon oranının %3-25 arasında değiştiği belirlendi. Sunulan bu çalışmada da sağlıklı görünen ve/veya klinik olarak hastalık tespit edilen köpeklerden alınan örneklerden metisilin dirençli KNS izolasyon oranının %3.96 olduğu belirlendi. Elde edilen verinin Abdel-Moein ve Zaher (2020)'in bildirdiği sonuçlarla uyumlu olduğu, diğer çalışmalarda bildirilen izolasyon oranına göre ise düşük olduğu görüldü. Bu duruma kullanılan test yöntemlerindeki farklılıkların ya da örnekleme yapılan köpeklerde daha önceden uygulanan antibiyotik tedavi protokollerinin neden olabileceği düşünüldü.

Metisilin dirençli stafilokok izolatlarının diğer β-laktam grubu antimikrobiyal maddelere karşı da dirençli olarak rapor edilmesi gerektiği önemle vurgulanmaktadır. Bunun yanı sıra izolatlarda direnç mekanizmasının açıklanmasında *mecA* gen pozitifliğinin araştırılması gerektiği, *mecC* geninin neden olduğu metisilin dirençlilik oranının oldukça nadir olarak karşılaşıldığı belirtilmektedir (CLSI 2018). Köpeklerden alınan örneklerden izole edilen metisilin dirençli stafilakok suşlarında *mecA* gen varlığının araştırıldığı çalışmaların çoğunda izolatların tamamında ilgili genin pozitif olduğu rapor edilmiştir (Aslantaş ve ark. 2013, Gandolfi-Decristopharis ve ark. 2013, Chah ve ark. 2014, Teixeira ve ark. 2019, Khanal ve ark. 2021). Elnageh ve ark. (2020) ise metisilin dirençli KNS izolatlarının %11.11'inde *mecA* geni tespit ettiklerini bildirirken, Abdel-Moein ve Zaher (2020) izole ettikleri 1 adet metisilin dirençli KNS izolatının *mecA* negatif olduğunu belirlemişlerdir. Bu çalışmada ise 17 adet metisilin dirençli KNS izolatının çoğunda (%64.70) *mecA* geni PCR ile pozitif bulundu. Geri kalan 6 (%35.30) izolatta metisilin direncinden diğer direnç mekanizmalarının sorumlu olabileceği (Ba ve ark. 2013, CLSI 2018) düşünüldü.

SONUÇ

Sonuç olarak bu çalışmada metisilin dirençli koagulaz negatif *Staphylococcus* spp. izolatlarının köpeklerde hem doğal floradan hem de enfeksiyon olgularından izole edilebileceği belirlendi. KNS izolatlarında *mecA* geninin rol oynadığı metisilin direncine dikkat edilmesi gerektiği belirlenirken, suşların hayvan sağlığının yanı sıra halk sağlığı açıcından da önem arz edebileceği görüldü. İleriki çalışmalarda pet hayvanlarında özellikle hastalık olgularından izole edilecek suşlarda metisilin dirençliliğinin belirlenmesinin epidemiyolojik çalışmalara katkı sağlayacağı kanaatine varıldı.

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RESEARCH ARTICLE

Detection of Helminth Egg Contamination on Raw Vegetables in Afyonkarahisar, Turkey

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ABSTRACT

This study aimed to investigate the helminth egg contamination of raw vegetables grown in Afyonkarahisar. The material of the study consisted of 508 randomly selected vegetable samples, including lettuce, parsley, carrot, green onion, spinach, cress, arugula, mint, dill, and purslane, which were collected from the field between October 2018 and September 2019. According to the relevant literature, the sediments obtained after the proper washing procedure were examined under a light microscope for helminth eggs. According to the results, *Taenia/Echinococcus* eggs were found in 2 (0.39%) vegetable samples, including 1 lettuce and 1 dill. *Toxocara* spp. eggs were found in 2 (0.39%) vegetable samples, including 1 lettuce and 1 mint. *Toxascaris leonina* eggs were found in 1 (0.2%) rocket sample, and hookworm/strongylid eggs were found in 58 samples (11.42%), including 4 lettuce, 6 parsley, 3 carrots, 18 green onions, 2 spinach, 9 cresses, 4 garden rocket, 6 mint, and 6 purslanes. Moreover, *Dicrocoelium* spp. eggs were detected in 2 (3,63%) carrots and 1 (2,04%) rocket, *Moniezia* spp. eggs were detected 2 (3.63%) carrots, *Fasciola* spp.eggs were detected in 1 (2.08%) green onion. This study concluded that some vegetables sold and consumed raw in Afyonkarahisar bazaars are contaminated with helminth eggs, which are a risk to public health. It was agreed that these vegetables should be thoroughly washed and consumed in accordance with hygiene standards, otherwise serious health problems may arise.

Keywords: Afyonkarahisar, Contamination, Helminth egg, Public health, Raw vegetables

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Afyonkarahisar'da Çiğ Sebzelerde Helmint Kontaminasyonunun Tespiti

ÖZ

Bu çalışma ile Afyonkarahisar'da yetiştirilen çiğ sebzelerin helmint yumurta kontaminasyonunun araştırılması amaçlanmıştır. Çalışmanın materyalini Ekim 2018-Eylül 2019 tarihleri arasında tarladan toplandığı haliyle semt pazarlarında satışa sunulan marul, maydanoz, havuç, yeşil soğan, ıspanak, tere, roka, nane, dereotu ve semizotu olmak üzere rastgele seçilmiş toplam 508 sebze örneği oluşturmuştur. İlgili literatürler doğrultusunda uygun yıkama prosedüründen sonra elde edilen sedimentler, ışık mikroskobunda helmint yumurtaları yönünden incelenmiştir. Buna göre, 1 marul, 1 dereotu olmak üzere 2 (%0.39) sebze örneğinde *Taenia/Echinococcus* yumurtası, 1 marul, 1 nane olmak üzere 2 (%0.39) sebze örneğinde *Toxocara* spp., 1 (%0.2) roka örneğinde *Toxascaris leonina* yumurtası, 4 marul, 6 maydanoz, 3 havuç, 18 soğan, 2 ıspanak, 9 tere, 4 roka, 6 nane, 6 semizotu olmak üzere 58 örnekte (%11.42) kancalı kurt/strongylid tip yumurta bulunmuştur. Ayrıca 2 (3.63%) havuç, 1 (2.04%) roka örneğinde *Dicrocoelium* spp., 2 (3.63%) havuç örneğinde *Moniezia* spp., 1 (2.08%) yeşil soğan örneğinde *Fasciola* spp. yumurtası tespit edilmiştir. Bu çalışmada Afyonkarahisar il pazarlarında çiğ olarak satılan ve tüketilen bazı sebzelerin halk sağlığı için risk oluşturan olan helmint yumurtaları ile kontamine olduğu sonucuna varılmıştır. Bu sebzelerin yeteri kadar yıkanması ve hijyen kurallarına uyularak tüketilmesi gerekmektedir; aksi takdirde ciddi sağlık sorunları ortaya çıkabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Afyonkarahisar, Çiğ Sebzeler, Halk Sağlığı, Helmint Yumurtası, Kontaminasyon

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INTRODUCTION

Fresh vegetables and fruits are vital components of a healthy and nutritious human diet. Vegetables and fruits have been reported to reduce the risk of developing diabetes, atherosclerosis, stroke and certain types of cancer if consumed regularly and are rich in vitamins, fibre, and minerals (Van Duyn and Pivonka 2000).

Heat-treatment of vegetables, like many other foods, gradually loses nutrients that are beneficial to human health, such as vitamins and minerals. Such loss is dependent on the amount and duration of heat exposure. Therefore, consuming vegetables raw for a healthy life has become more popular recently. Water and fertilizer sources, human and animal movement in areas where vegetables are grown are critical due to many pathogenic microorganisms that can contaminate vegetables and pose problems for public health.

According to the World Health Organization, parasites are ranked sixth among the most pathogenic factors causing infectious diseases in humans (WHO 2019). Vegetables consumed without following appropriate washing protocols are considered a critical source for the transmission of parasitic diseases to humans. It is reported that intestinal parasitic outbreaks in developed or developing countries may be related to the consump-tion of contaminated vegetables (Slifko et al. 2000).

Intestinal parasites can be transmitted via the faecal-oral route by consuming of contaminated food and water with the infective forms of the parasites. Faeces, irrigation water or sewage, are all possible sources of parasitic contamination (Al-Binali et al. 2006, Newell et al. 2010). Epidemiological studies show that vegetables are contaminated with parasite eggs, larvae and cyst forms due to irrigating vegetables with untreated wastewater in epidemic areas, and humans are infected after consuming raw contaminated vegetables (Kozan et al. 2005, Ahmed and Karanis 2018).

Ascaris lumbricoides, *Enterobius vermicularis*, *Trichuris* spp., *Toxocara* spp., Trichostrongylidae and hookworm infections were observed in humans after consumption of inade-quately washed vegetables and fruits as salads in catering establishments such as schools, hospitals, restaurants, and hotels (Vázquez et al. 1997, Mesquita et al. 1999, Coelho et al. 2001, Takayanagui et al. 2001).

This study was conducted in Afyonkarahisar, which is located on lands of Aegean region of Turkey. The primary source of income in Afyonkarahisar is agriculture and animal husbandry. Agriculture is mainly based on the cultivation of cereals like wheat, barley and sunflower. Poppy and sugar beet have an important place among the industrial crops. Legumes cultivation also plays an important role. Locally grown vegetables and fruits are offered for sale throughout the year in the district bazaars. No reports of parasite contamination of vegetables were detected following the literature search, including the "Afyonkarahisar" term. This study aimed to examine helminth contamination of vegetables grown in Afyonkarahisar, which are brought to the district bazaars and sold for raw consumption without any post-harvesting processing procedures.

MATERIALS and METHODS

Study Region

This study was conducted in Afyonkarahisar, which is located on lands of Aegean region of Turkey. Afyonkarahisar is located on the 38°45'25" north latitude and 30°53'87" east longitude (Figure 1). It has a hot and dry climate in the summer, while cold and snowy in winter. It is warm and rainy in the spring and autumn, and the precipitation is mainly in the form of rain. The average annual rainfall of Afyonkarahisar is 444 mm, and the annual temperature is 11 °C. The livelihood of Afyonkarahisar, in general, is based on agriculture and livestock. Therefore, domestic vegetables and fruits are sold in the district bazaars. Agriculture is primarily of smallholder type in the lowlands, which are located near the mountains. Alongside seasonal vegetable production, fruit production is also carried out to a large extent. Vegetable production is predominate in areas where irrigated farming is carried out. (Anonymus, 2019).

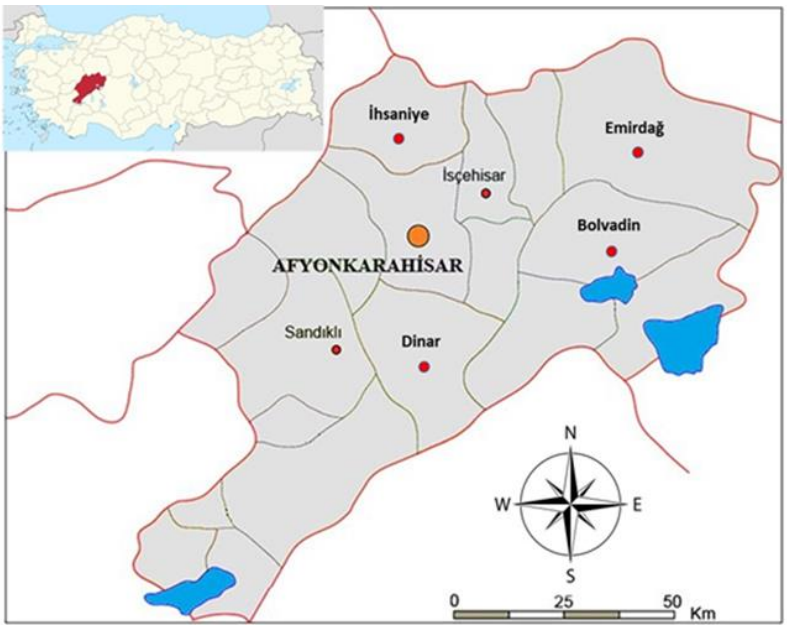

Figure 1: Study Region (Sarıışık 2021)

Sample Collection

A total of 508 samples consisting of lettuce, parsley, carrot, green onion, spinach, cress, arugula, dill, mint and purslane was collected from the established district bazaars every week between October 2018 and September 2019 on different days in Afyonkarahisar city centre and brought to the laboratory. These vegetables are mostly consumed raw as a salad.

Sample Preparation and Helminthological Examination

200 g of each vegetable was weighed in a plastic bag and washed with 2 l of physiological saline (0.95% NaCl) solution. The washing water was subjected to sedimentation for 24 h, and the upper layer of liquid was discharged, and the sediment centrifuged for 15 min at 2000 rpm. The supernatant was removed, and a drop of physiological saline containing lugol was added to the remaining sediment. The concentration of helminth eggs in the residue was investigated using the technique of Téléman Rivas modified by Bailenger (1962). Samples taken from the mixture were examined for contamination of helminth eggs using 10x and 40x magnification. Helminth eggs were detected according to laboratory manual (Bailenger 1962, Soulsby 1982, Ayres and Mara 1996, Hernández-Chavarría and Avendaño 2001).

Statistical Analysis

The chi-square test was used to compare the rates of egg types detected in the vegetable samples, and PASW Statistics 18.0.0 program was used in statistical analysis (SPSS 2009).

RESULTS

Helminth egg contamination in the examined vegetable samples is given in Table 1 and Figure 2. A total of 508 different samples made up of lettuce, parsley, carrot, green onion, spinach, cress, arugula, dill, mint and purslane were sold to the public for consumption in different retail markets in Afyonkarahisar and were analyzed for the presence of helminth eggs and larvae. The study results showed that 69 (13.58%) vegetables were contaminated with helminth eggs. These include 10% of lettuce, 9.23% of parsley, 12.71% of carrot, 39.58% of green onion, 4.4% of spinach, 16.07% of cress, 12.24% of rocket, 16.67% of mint, 2.32% of dill and 13.3% of purslane (Table 1). In green onion samples, Hookworm/Strongyle spp. eggs were significantly more than Fasciola spp. eggs ($P < 0.05$). In mint samples, Hookworm/Strongyle spp. eggs were significantly more than Toxocara spp. eggs ($P < 0.05$).

Out of the 60 samples of lettuce, 65 parsley, 55 carrots, 48 green onion, 45 spinach, 56 cresses, 49 rocket, 42 mint, 43 dills, and 45 purslanes examined, eggs of Hookworm/Strongyle spp. were detected in 6.67% of lettuce, 9.23% of parsley, 5.45% of carrot, 37.5% of green onion, 4.44% of spinach, 16.07% of cress, 8.16% of rocket, 14.29% of mint and 13.3% of purslane; Taeniid eggs in 1.66% and 2.32% of lettuce and dill, respectively; Toxocara spp. eggs in 1.66% and 2.38% of lettuce and mint, respectively; eggs of Toxascaris leonina in 2.04% of rocket only; Dicrocoelium spp. were detected in 3.63% and 2.04% of carrot and rocket respectively; Fasciola spp. were detected in 2.08% of green onion only and Moniezia spp. were detected in 3.63% of carrot only. Hookworm/Strongyle spp. eggs contaminated vegetables significantly more than the other helminth parasites($P<0.05$).

Table 1. Distribution of helminth egg detected in the examined vegetable samples in district bazaars of Afyonkarahisar.

Vegetables	Number of Samples	Hookworm/ Strongyle eggs (%)	Taeniid eggs	*Toxocara* spp. Eggs	*Toxascaris leonina* eggs	*Dicrocoelium* spp. eggs	*Fasciola* spp. eggs	*Moniezia* spp. eggs
Lettuce	60	4 (6.67[a])	1 (1.66 [a])	1 (1.66 [a])	0	0	0	0
Parsley	65	6 (9.23)	0	0	0	0	0	0
Carrot	55	3 (5.45 [a])	0	0	0	2 (3.63 [a])	0	2 (3.63 [a])
Green Onion	48	18 (37.5 [a])	0	0	0	0	1 (2.08 [b])	0
Spinach	45	2 (4.44)	0	0	0	0	0	0
Cress	56	9 (16.07)	0	0	0	0	0	0
Rocket	49	4 (8.16 [a])	0	0	1 (2.04 [a])	1 (2.04 [a])	0	0
Mint	42	6 (14.29 [a])	0	1 (2.38 [b])	0	0	0	0
Dill	43	0	1 (2.32)	0	0	0	0	0
Purslane	45	6 (13.3)	0	0	0	0	0	0
Total	508	58 (11.42 [a])	2 (0.39 [b])	2 (0.39 [b])	1 (0.2 [b])	3 (0.59 [b])	1 (0.2 [b])	2 (0.39 [b])

[a,b]Differences between odds with different letters on the same line are significant ($p<0.05$).

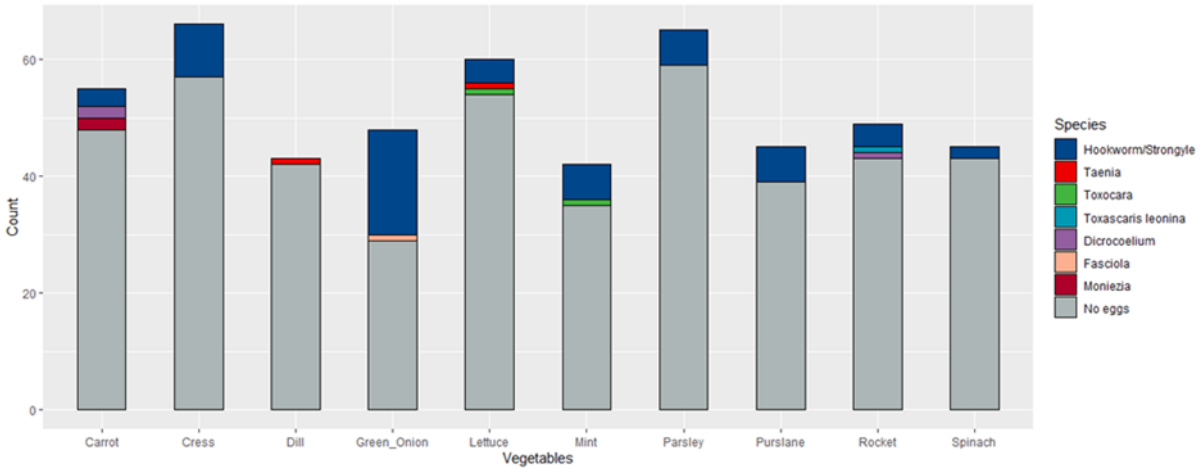

Figure 2: Distribution of helminth eggs in vegetable samples in district bazaars of Afyonkarahisar.

DISCUSSION

While 200-300 mm of the water required for vegetable production is generally met by direct precipitation, the rest is supplied from various water sources, including rivers, streams, lakes, wells and irrigation channels (Aras 2019). However, human or animal waste may mix with water sources from time to time. Animal fertilizers are also frequently used for vegetable cultivation. Both animal manure and water sources used for growing and irrigation of raw consumed vegetables could mediate the transmission of eggs belonging to various parasites of humans and animals. Some parasites like *Toxocara* spp. and *Taenia* spp. are zoonoses and pose significant risks to humans.

Five hundred eight vegetable samples were collected from the field and brought to the district bazaar without any cleaning It was identified in this study that 69 (13.19%) vegetable samples were contaminated with various helminth eggs. *Taenia* / *Echinococcus* spp., *Toxocara* spp. were found in companion animals, and helminth eggs which were seen in ruminant faeces like *Fasciola* spp., *Dicrocoelium* spp. and *Moniezia* spp. were detected in the studied samples. It is thought that infected ruminant faeces, which were not subjected to any decontamination processes, were used as fertilizer. In the case of insufficient water sources, the water required for plant vegetation can be supplied from wastewater or

streams which might be contaminated with wastewater. Vegetables could be contaminated with various helminth eggs after irrigation with waste or untreated water, which is mixed with infected human or animal faeces.

Taeniid eggs were detected on one lettuce and one dill sample (0.39%) of the 508 vegetable samples, but no statistical significance was determined ($P < 0.05$). Kozan et al. 2005 found Taeniid eggs in 3.45% of unwashed vegetable samples, and Adanır and Taşçı 2013 detected 2.7% of samples as contaminated. In other countries, Adamu et al. 2012 found six of 1130 vegetable samples (0.5%), Fallah et al. 2012, 28 of 304 vegetable samples (9.2%), Adenusi et al. 2015, 12 of 960 vegetable samples (1.25%) and Fallah et al. 2016 22 of 453 vegetable samples (4.86%) were contaminated with *Taenia/Echinococcus* eggs. Cysticercosis is caused by the larval stages of the parasite cestode *Taenia solium* infecting both humans and pigs. Ingestion of eggs which shed in the faeces of a human tapeworm carrier causes infection. These eggs are infectious and do not need a developmental stage outside the host. Humans are typically exposed to eggs by consuming of food or water contaminated with faeces containing these eggs or proglottids or through person-to-person transmission. *Taenia saginata* and *Taenia solium* are tapeworm located in the small intestine of humans. When *Taenia saginata* eggs are detected, it is thought that vegetables may have been irrigated with water contaminated with infected human faeces. However, it does not pose a risk to human health. Since there is no pork consumption in the region, *T. saginata* is ignored. While there is no evidence that human faeces contaminates the vegetable gardens, the presence of stray cats and dogs is expected in the study area. It is quite likely that the identified Taeniid type eggs belong to a tapeworm from the Taenidae family, which is found in the small intestine of cats and dogs: Echinococcus. The definitive hosts for *Echinococcus granulosus* are usually dogs, but many mammals and humans could be intermediate hosts, especially domestic ruminants (Eckert and Deplazes 2004). The infective eggs are released after disruption of segments in the faeces of dogs. Following consumption of contaminated water and food by intermediate hosts, the larval form, called a hydatid cyst, develops in various organs, especially in the liver and lungs. This has the potential to cause life-threatening disease. Therefore, the consuming of raw vegetables contaminated with Echinococcus eggs without proper washing increases the risk of developing hydatid cysts in humans (Daryani et al. 2008, Fallah et al. 2016). It is not microscopically possible to distinguish *Echinococcus* spp. based on egg morphology from other species in the Taenidae family. Therefore, it should not be ignored that these eggs seen in vegetable samples may also belong to important zoonotic *Echinococcus* spp. Ozkan et al. 2008 show that there were 2534 (13.13%) human cases in the Marmara region; 2114 (16.94%), in the Aegean region; 2578 (16.09%), Mediterranean region; 5404 (38.57%), in the Middle Anatolian region; 428 (5.70%), in the Black Sea region; 844 (6.80%), in the eastern Anatolian region; and 887 (2.75%), in the southeastern Anatolian region making a total of 14,789 cystic echinococcosis (CE) cases in Turkey. One hundred forty-two human CE cases were detected in Afyonkarahisar during 2001-2005 (Ozkan et al. 2008). In the study on the sero-epidemiology of the infection, Çetinkaya et al. detected CE 14.6% in 611 humans in Afyonkarahisar (Cetinkaya et al. 2005).

Ascarids are the most common gastrointestinal nematodes in cats and dogs. The embryo-developed eggs are excreted in the faeces of infected animals. They develop into an infective form that can survive for a few weeks to a few months in environments where temperature and humidity are favourable. Infective eggs are heat resistant from -25 °C to 36 °C and remain viable for at least one year (Overgaauw 1997). Oral intake of *Toxocara* spp. eggs is vital for human health due to the development of visceral or ocular larva migrans (Avcioglu et al. 2011). Therefore, Toxocariasis is considered one of the most important zoonoses in public health and economy (Macpherson 2013). *Toxocara* spp. eggs were found in 2 vegetable samples (0.39%), including one lettuce and one mint, and *Toxascaris leonina* egg was found in one rocket (0.2%), but no statistically significant difference was detected ($P < 0.05$). In other studies, Kozan et al. 2005 reported that *Toxocara* spp. eggs were detected in 1.48% of their examined vegetable samples, while Avcıoğlu et al. 2011 detected in 1.0% of their samples, and Adanır et al. 2013 in 2.7% of the samples in Turkey. The percentage of vegetable samples reported being contaminated with *Toxocara* spp. eggs was 3% in Vietnam, 48.3% in Nigeria, 19.2% in Poland and 2.6% in Thailand, respectively (Uga et al 2009, Klapeć and Borecka 2012, Maikai et al. 2013, Punsawad et al. 2019). Altındiş et al 2004. detected 5/4878 A. lumbricoides during 2000-2003 in Afyonkarahisar, Ak et al. 2006 detected *Ascaris lumbricoides* 4.8% in the Southeast Anatolian Region of Turkey during 2001-2003, Ulukanlıgıl et al. 2003 detected 64% in shantytown schools, 45.3% in apartment schools, 41.4% in rural schools in Şanlıurfa in 2000 (Ulukanligil et al. 2003, Altindiş et al. 2004, Ak et al. 2006). It is thought that differences between the studies could be due to the variation in the number of samples used in the study, and the geographical conditions and the growing conditions of these vegetables.

As for *Taenia* spp., it is not possible to distinguish microscopically *Toxocara canis* and *T. cati* eggs. Therefore, it can not be said that the eggs noticed in vegetable samples are either *T. canis* or *T. cati* eggs. The distinction of Toxocara eggs could be performed using molecular biological methods (Blaszkowska et al. 2011).

Strongyle type eggs with a thin shell, and oval shape, containing many blastomeres are produced by most

nematodes in the Trichostrongylidae and Strongyloides family (Toparlak and Tüzer 2000). Except for some of the nematode species in these families, distinguishing the identity of the species by egg morphology alone is very difficult due to similarity in size, shape, character and appearance (Purwati et al. 2017). In this study, strongyle eggs were detected in 58 (11.42%) of 508 vegetable samples. These eggs could have included *Ancylostoma* spp, which is very important in public health. Considering the growing conditions of vegetables, it is thought that these eggs on contaminated vegetables may belong to various parasites in the Trichostrongyloidea and Strongyloidea families. It should not be ignored that vegetables could be contaminated with strongyle type eggs of zoonotic parasites due to using ruminant faeces as fertilizer without any process and defecation of stray cats or dogs in the areas where these vegetables are grown. Carnivore sheded hookworm eggs could develop to infective larvae within one week under optimum conditions and maintain their infectivity for a long time. After bare hand contact with vegetables contaminated with these larvae, cutaneous larva migrans can occur, caused by the larvae penetrating the skin and migrating under the skin surface (Toparlak and Tüzer 2000). Therefore, hands should be washed well after touching vegetables with bare hands, and hygiene rules should be followed.

CONCLUSSIONS

In conclussion, it was determined that some vegetables collected from the field in Afyonkarahisar and brought to the district bazaars without being subjected to any cleaning process, and consumed raw, were contaminated with different types of helminths. It is thought that carnivore animals enter the areas where these vegetables are grown, or that infected human or animal faeces reach these areas directly and via contaminated irrigation waters, causing contamination in vegetables. If vegetables are not properly washed, it will be inevitable to be exposed to significant zoonotic diseases in humans. For this reason, the use of fertilizers should be in accordance with guidelines, stray animals should be prevented from entering these areas, raw consumed vegetables should be washed properly before consumption and hygiene rules should be followed.

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RESEARCH ARTICLE

The Prevalence of *Enterococcus* spp., Resistance Profiles, the Presence of the *VanA* and *VanB* Resistance Genes in Chicken Meats

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ABSTRACT

The aim of the present study was to investigate the prevalence of *Enterococcus* spp., resistance profiles, and the presence of the *VanA* and *VanB* resistance genes in the chicken meat samples that were collected from the Van market, Turkey. A total of 100 chicken meat samples were used. Among the samples, 27 (27%) were *Enterococcus* spp. positive. A total of 67 isolates were obtained from 27 chicken meat samples, which were positive for *Enterococcus* spp. Among the 67 isolates, 53 (79.10%) were identified to be *E. faecalis* and 14 (20.90%) were identified to be *E. faecium*. The analysis of antibiotic resistance revealed that 48 isolates (71.74%) exhibited resistance to multiple antibiotics and 19 isolates (28.36%) were resistant to at least one antibiotic. At least 50% of the *E. faecalis* and *E. faecium* strains were intermediately sensitive to ampicillin, penicillin, chloramphenicol, vancomycin, and gentamicin. Moreover, the presence of the *VanA* and *VanB* genes in 13 strains that were phenotypically and intermediately resistant to vancomycin was examined by PCR test. The PCR analysis revealed that no isolate had the *VanA* and *VanB* genes. As a result, the detection of *Enterococcus* spp. in chicken meat is an indication of not paying attention to hygienic conditions. At the same time, the existence of multiple antibiotic resistance in isolates obtained from these foods also suggests that phenotypically determined resistances may threaten public health.

Keywords: Chicken meat, *Enterococcus* spp. *VanA*, *VanB*

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Tavuk Etlerinde *Enterococcus* spp. Prevelansı, Direnç Profilleri, *VanA* ve *VanB* Direnç Genlerinin Varlığı

ÖZ

Türkiye'de Van ili piyasasından toplanan tavuk eti örneklerinde *Enterococcus* spp. prevelansı ve antibiyotik dirençliliği ve *VanA* ve *VanB* direnç genlerinin belirlenmesi amaçlandı. Çalışmada 100 adet tavuk eti örneği kullanıldı. Bunların 27'si (%27) *Enterococcus* spp. pozitif bulundu. *Enterococcus* spp. için pozitif olan 27 tavuk eti örneğinden toplam 67 izolat elde edildi. Bunlardan 53'ü (%79.10) *E. faecalis*, 14'ü (%20.90) ise *E. faecium* olarak tespit edildi. Antibiyotik dirençlilikleri incelenen analizler sonucunda *Enterococcus* spp. izolatlarının 48'sinin (%71.64) iki veya daha fazla antibiyotiğe dirençli olduğu, 19'sinin (%28.36) ise en az bir antibiyotiğe dirençli olduğu tespit edilmiştir. *E. faecalis* ve *E. faecium* suşlarının en az %50'si ampisilin, penisin, kloramfenol, vankomisin ve gentamisine duyarlı ve orta düzeyde olduğu tespit edildi. Ayrıca fenotipik olarak vankomisine dirençli ve orta düzeyde olan 13 izolatta *VanA* ve *VanB* geni varlığı PCR testi ile araştırıldı. PCR testi ile analizi yapılan izolatların hiçbirinde *VanA* ve *VanB* geni tespit edilemedi. Sonuç olarak, tavuk etlerinde *Enterococcus* spp. tespit edilmesi hijyenik koşullara dikkat edilmediğinin göstergesidir. Aynı zamanda bu gıdalardan elde edilen izolatlarda çoklu antibiyotik dirençliliğinin var olması ayrıca fenotipik olarak belirlenen dirençliliklerin halk sağlığını tehdit edebileceğini düşündürmektedir.

Anahtar Sözcükler: *Enterococcus* spp., Tavuk eti, *VanA*, *VanB*

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INTRODUCTION

Enterococcus spp. are naturally found in the environment and the normal intestinal microbiota of both animals and humans. Due to fecal contamination, these species can reach media such as soils, fertilizers of animal origin, and sewer systems and, thus, contaminate waters and vegetables. The species can invade the intestinal tracts of domestic and wild animals. Thus, enterococcus can be found in any type of food from vegetables to raw meat and cheese due to cross-contamination between food processing stages (Giraffa 2002, van Schaik and Willems 2010, Boehm and Sassoubre 2014).

Enterococci were previously known as Lancefield D group streptococci or fecal streptococci. *Enterococcus* spp. are Gram-positive, oxidase and catalase negative, facultative anaerobic and homofermentative bacteria that produce lactic as the final product of glucose fermentation. Most enterococci are grow at a NaCl concentration of 6.5%, pH value of 9.6, and temperatures between 10 ºC and 45 ºC. At least 50 different *Enterococcus* species have been identified (Foulquié-Moreno et al. 2006, Semedo-Lemsaddek et al. 2010 Lawlwy et al. 2012, Bonacina et al. 2017). The most common *Enterococcus* spp. in human intestines are *Enterococcus faecalis* and, to a lesser level, *Enterococcus faecium* whereas the most common species in foods and animals are *E. cecorum*, *E. faecalis, E. hirae*, and *E. faecium* (Prieto et al. 2016).

Enterococci are accepted as the agents of a series of clinical infections that are not food-borne such as bacteriemia and endocarditis. Moreover, they are also of importance in food microbiology. Enterococci are important spoilage microorganisms, especially in meat and dairy products, are known to have virulence factors, their antibiotic resistance is known to be species-specific, and most *Enterococcus* species are not fully pathogenic (Lawlwy at al. 2012, Halkman 2019).

Certain desirable metabolic properties of enterococci attach importance to their use in the production of various foods. The role of enterococci in food-borne diseases is debatable, but they can be regarded as hospital pathogens and threats to public health due to their antimicrobial resistance stemming from their effective ability to transfer genetic material and the emergence of virulence factors. Acquired infections, virulence determinants, and antimicrobial-resistant strains not only occur in hospitals, humans, and veterinary clinics but also soils, waters, insects, plants such as vegetables, and raw and fermented food products due to environmental contamination (Semedo-Lemsaddek et al. 2010, Lawlwy et al. 2012). Moreover, enterococci can decarboxylate amino acids and are known to produce biogenic amines such phenylamine and as tyramine (Nieto-Arribas et al. 2011, Vidal-Carou et al. 2011).

E. faecalis and *E. faecium* are the most commonly isolated species from meat and meat products while *E. gallinarum*, *E. durans*, *E. mundtii*, *E. casseliflavus*, and *E. hirae*, are more rarely isolated (Semedo-Lemsaddek et al. 2010). The presence of such isolates in chicken meats poses a threat to food safety and public health (Aslam et al. 2012).

The vancomycin-resistant enterococcus strains pose serious problems. The identified and characterized vancomycin-resistant phenotypes are *VanA*, *VanB*, *VanD*, *VanE*, and *VanG* (Švec and Devriese 2015). *VanA* and *VanB* are the most common resistance types in clinical Enterococci (Çöleri and Çökmüş 2008). The vancomycin-resistant strains of *E. faecalis* and, to a lesser degree, *E. faecium* are thought to be related to human pathogenesis (Semedo-Lemsaddek et al. 2010).

The aim of this study is to isolate *Enterococcus* spp. from chicken meat samples taken from the Van market in Turkey, and to identify *E. faecalis* and *E. faecium* strains in isolates. In addition, the determination of *VanA* and *VanB* genes, which are known to be the most common causes of resistance in isolates that are phenotypically resistant to vancomycin.

MATERIAL AND METHODS

Bacterial Strains

E. faecalis (ATCC® 51299), *E. faecium* (ATCC® 6057), *E. faecium* (vanA+) and *E. faecalis* (vanB+) that were procured from the Food Hygiene and Technology Department of the Veterinary Faculty of Van Yüzüncü Yıl University were used as the reference strains.

Sample Collection

A total of 100 chicken meat samples comprising breasts and drumsticks were used as the study material. The samples were bought from the sales places under aseptic conditions, brought to the laboratory at +4 ºC, and analyzed immediately.

Isolation of *Enterococcus* spp.

A total of 10 g sample was obtained from the chicken meat samples that were collected under aseptic conditions and brought to the laboratory in a cold chain. Then, was homogenized with 90 ml sterile peptone water for 2 minutes. A total of 0.1 ml homogenate was inoculated into the Slanetz&Bartley Medium (LABM LAB166, UK) using the spread plate method. The petri dishes were firstly incubated at 37 ºC for 4 h and then at 44 ºC for 24-48 h under aerobic conditions. Then, the colonies that were larger than 1-2 mm and with colors ranging from pink-red to brown were identified as *Enterococcus* spp. Five typical colonies that grew on the SB medium were individually inoculated onto the %0.6 Yeast Extract (YE) (LABM, MC001, UK) containing Tryptone Soya Agar (TSA) (LABM, LAB011, UK) and biochemical assays were performed (Anonymous 2015, Švec and Devriese 2015). The isolates that were identified to be *Enterococcus* spp. were confirmed using PCR.

The Conformation of *Enterococcus* spp. and Isolation of *E. faecalis* and *E. faecium*

A commercial kit (GeneAll, ExgeneTM Cell SV, South Korea) was used for DNA extraction from the *Enterococcus* spp. colonies that were isolated from the chicken meat samples. The specific primer pair, namely Ent1 and Ent2, that was developed by Ke et al. (1999) for the tuf gene region was used for the confirmation of the *Enterococcus* spp. isolates. The gene regions that were developed by Jackson et al. (2011) were used for the identification of *E. faecalis* and *E. faecium* using PCR (Table 1). For the preparation of the PCR mixture, 10 µl mastermix (A.B.TTM 2X PCR MasterMix, Turkey), 1.5 µl of each primer, and 5 µl genomic DNA were added and the total volume was brought to 25 µl using PCR water. Table 2 shows the PCR protocol that was followed during the analysis. The amplicons were run on 1.5% agarose gel (jelde (Vivantis, USA+Bioshop, TAE Buffer 50X Liquid concentrate, Canada) and the positive control bands were examined using an imaging device.

Table 1. Primers used in PCR analysis

Microorganism	Oligonükleotid Sequence	bp	Referans
Enterococcus spp.	Ent1-TACTGACAAACCATTCATGATG Ent2-AACTTCGTCACCAACGCGAAC	112	Ke et al. (1999)
E. faecalis	FL1-ACTTATGTGACTAACTTAACC FL2- TAATGGTGAATCTTGGTTTGG	360	Jackson et al. (2011)
E. faecium	FM1-GAAAAAACAATAGAAGAATTAT FM2-TGCTTTTTTGAATTCTTCTTTA	215	
Gene			
*van*A	A1-ATGAATAGAATAAAAGTTGC A2-TCACCCCTTTAACGCTAATA	1032	Saha et al. (2008)
*van*B	B1-GTGACAAACCGGAGGCGAGGA B2-CCGCCATCCTCCTGCAAAAAA	433	Handwerger et al. (1992)

Antibiotic Resistance

Antibiotic Resistance tests were tested by the standard disk diffusion method of Kirbye-Bauer (Bauer et al., 1966) on Mueller Hinton Agar (Oxoid CM0337, UK. Ampicillin (AMP, 10 µg, Liofilchem®, Italy), penicillin (P, 10 U, Liofilchem®, Italy), erythromycin (E, 15 µg, Liofilchem®, Italy), chloramphenicol (C, 30 µg, Liofilchem®, Italy), tetracycline (TE, 30 µg, Liofilchem®, Italy), vancomycin (VA, 30 µg, Liofilchem®, Italy), and gentamicin (CN, 120 µg, Liofilchem®, Italy) were used to determine the antibiotic resistance of the *Enterococcus* isolates. The results were evaluated according to the disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI 2018).

Identification of the *Van*A and *Van*B genes

The presence of the *Van*A and *Van*B resistance genes in the phenotypically and intermediately strains that were identified by applying the disc diffusion method to the confirmed *Enterococcus* spp. was investigated. The specific primer pair that was developed by Handwerger et al. (1992) and Saha et al. (2008) was used for this purpose (Table 2). For the preparation of the PCR mixture, 10 µl mastermix (A.B.T ™ 2X PCR MasterMix, Turkey), 1.5 µl of each primer, and 5 µl genomic DNA were added and the total volume was brought to 25 µl using PCR water. Table 3 shows the PCR protocol that was followed during the analysis.

Table 2. PCR protocol applied in analyzes

Microorganism	Initial Denaturation (ºC/min)	Amplification (Denaturation/Annealing/Extension)	Fınal Extension (ºC/min)	Cycle
Enterococcus spp.	95/10	95 ºC 45 sec/59 ºC 45 sec/72 ºC 45 sec	72/5	35
E. faecalis	95/10	95 ºC 45 sec/55 ºC 60 sec/72 ºC 60 sec	72/7	35
E. faecium				
Gene				
VanA	94/10	94 ºC 45 sec/50 ºC 45 sec/72 ºC 60 sec	72/10	30
VanB	94/10	94 ºC 45 sec/62 ºC 45 sec/72 ºC 60 sec	72/10	30

RESULTS

A total of 27 samples (27%) were determined to be *Enterococcus* spp. positive and a total of 67 *Enterococcus* spp. isolates were obtained from the 27 samples. Among the 67 isolates, 53 (79.10%) were identified to be *E. faecalis* and 14 (20.90%) were identified to be *E. faecium* (Figure 1). Table 3 shows the antibiotic resistance percentages of the 67 *Enterococcus* spp. isolates.

Table 3. Antibiotic resistance percentages of Enterococcus spp. isolates (%)

	AMP (%)			P (%)			E (%)			C (%)			TE (%)			VA (%)			CN (%)		
n	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
***E. faecalis* (53)**	52 (98.11)	-	1 (1.89)	50 (94.33)	-	3 (5.67)	15 (28.30)	16 (30.19)	22 (41.51)	48 (90.57)	3 (5.66)	2 (3.77)	10 (18.87)	5 (9.43)	38 (71.70)	44 (83.01)	2 (3.77)	7 (13.22)	51 (96.23)	-	2 (3.77)
***E. faecium* (14)**	13 (92.86)	-	1 (7.14)	12 (85.71)	-	2 (14.29)	8 (57.14)	1 (7.14)	5 (35.72)	13 (92.86)	-	1 (7.14)	2 (14.29)	2 (14.29)	10 (71.42)	10 (71.43)	1 (7.14)	3 (21.43)	14 (100)	-	-
Total (67)	65 (97.01)	-	2 (2.99)	62 (92.54)	-	5 (7.46)	23 (34.33)	17 (25.37)	27 (40.30)	61 (91.04)	3 (4.48)	3 (4.48)	12 (17.91)	7 (10.45)	48 (71.64)	54 (80.60)	3 (4.48)	10 (14.92)	65 (97.01)	-	2 (2.99)

n: number of positive isolates, AMP: Ampicillin, P: Penicillin, E: Erythromycin, C: Chloramphenicol, TE: Tetracycline, VA: Vancomycin, CN: Gentamicin

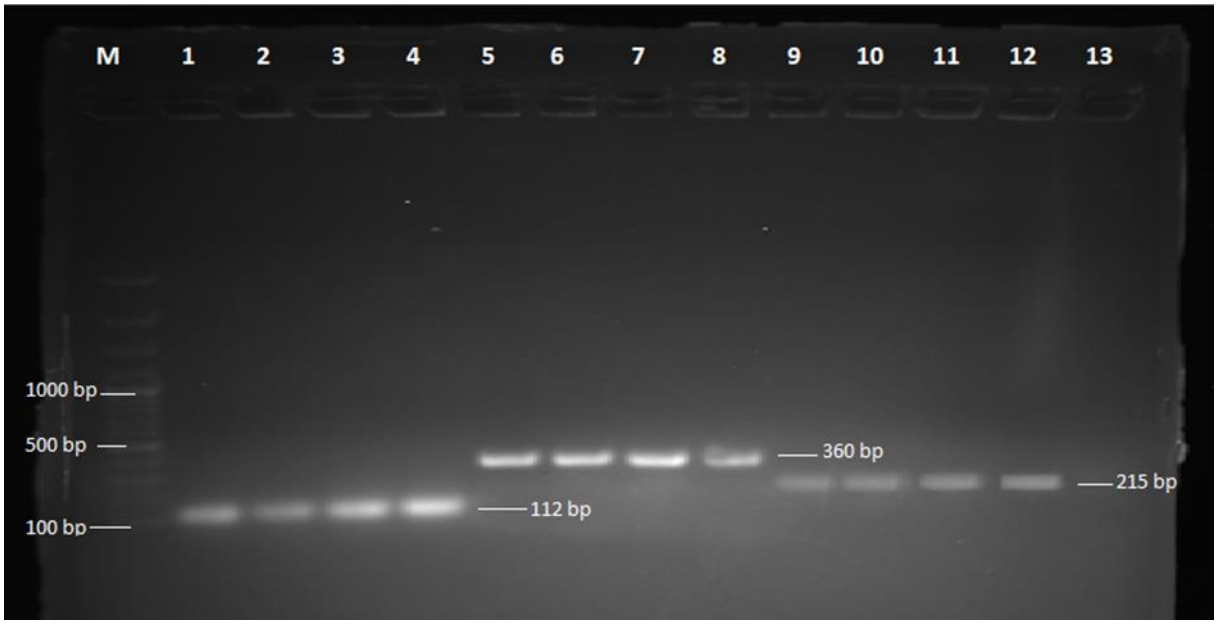

Figure 1: The agarose gel image of the amplicons that were identified in the *Enterococcus* spp. (112 bp), *E. faecalis* (360 bp) and *E. faecium* (215 bp) isolates using PCR (M: 100 bp DNA marker; 1: *E. faecalis* ATCC® 51299; 2-4: *Enterococcus* spp. isolats; 5: *E. faecalis* ATCC® 51299; 6-8: *E. faecalis* isolats; 9: *E. faecium* ATCC® 6057; 10-12: *E. faecium* isolates; 13: Negative control)

The analysis revealed that 48 isolates (71.74%) of the Enterococcus spp. isolates were resistant to two or more antibiotics while 19 isolates (28.36%) of the isolates exhibited resistance to at least one antibiotic (Table 4).

Table 4. Number of E. faecalis and E. faecium isolates resistant to multiple antibiotics

Species (n)	Number of isolates (%)	Multiple antibiotic resistance
E. faecalis (53)	1 (1.89)	C, TE
	3 (5.66)	E, C, TE
	24 (45.28)	E, TE
	2 (3.77)	TE, VA
	1 (1.89)	E, C, TE, VA
	1 (1.89)	A, P, E, TE, VA
	2 (3. 77)	P, E, TE, VA
	3 (5.66)	E, TE, VA
	2 (3.77)	E, CN
E. faecium (14)	4 (28.57)	E, TE
	1 (7.14)	A, P, TE, VA
	1 (7.14)	TE, VA
	1 (7.14)	E, C, TE
	1 (7.14)	P, TE, VA
	1 (7.14)	E, TE, VA

AMP: Ampicillin, P: Penicillin, E: Erythromycin, C: Chloramphenicol, TE: Tetracycline, VA: Vancomycin, CN: Gentamicin

The presence of *VanA* and *VanB* genes in 13 phenotypically vancomycin resistant isolates was investigated by PCR method. According to PCR analysis, *VanA* and *VanB* genes were not detected in any of the isolates.

DISCUSSION

While it is controversial that foodborne enterococci are bacterial pathogens, they can serve as potential virulence and antimicrobial resistance gene reservoirs for host-adapted strains. Studies have revealed that *Enterococcus* spp. contaminated retail meat products to a great degree and the differences in the antimicrobial resistance phenotypes were attributed to the antimicrobials that were used in animal food production environments (Hayes et al. 2003).

The consumption of chicken meat contaminated with enterococci is dangerous for public health, and especially the presence of strains with resistance genes can play a role in the transfer of these genes to consumers (Aslam et al. 2012).

Among the 100 chicken meat samples, 27% were determined to be *Enterococcus* spp. positive, which is close to the value of 28.6% that was reported by Pesavento et al. (2014) and the value of 30% that was reported by Onaran et al. (2019). Yüksel et al. (2013), Kilonzo-Nthenge et al. (2015), Donado-Godoy et al. (2015), Şentürk (2017), Kim et al. (2018), and Sanlibaba et al. (2018) reported higher prevalence values of 43%, 82.2%, 94%, 91.66%, 77.7%, and 79.50%, respectively, while Bayram et al. (2011) and Gousia et al. (2015) reported lower prevalence values of 12.5% and 21.7%, respectively. Enterococci are sensitive to sanitation and can be eliminated when effective cleaning procedures are applied. The constant negligence of cleaning practices will allow the growth of enterococci by causing the formation of a mineral residue that protects organisms from disinfectants (Adams and Moss 2008). The differences in the reported values are attributable to the lack of adherence to hygienic conditions during the production and storage of chicken meats.

E. faecalis and *E. faecium* are known to be the cause of the majority of the human enterococcus infections of hospital and food origin (Cetinkaya et al. 2000; Lawlwy et al. 2012; Lebreton et al. 2013).

In the study, the most prevalent strain was *E. faecalis* with a rate of 79.10% while the rest of the isolates (20.90%) was identified to be *E. faecium*. In agreement with this study, many studies have reported *E. faecium* and *E. faecalis* to be the most identified species (Hayes et al. 2003, Bayram et al. 2011, Kim et al. 2018, Sanlibaba et al. 2018, Molechan et al. 2019, Manson et al. 2019).

Robredo et al. (2000) reported that the dominant species was *E. durans*, followed by *E. faecalis* and *E. faecium*. This difference in our findings is attributable to the differences in the analysis methods as well as to regional differences.

At least 50% of the *E. faecalis* and *E. faecium* strains showed intermediate resistance to ampicillin, penicillin, chloramphenicol, vancomycin, and gentamicin. The antibiotic sensitivity of the *E. faecalis* and *E. faecium* isolates differ depending on geographical conditions. Various studies have been conducted in Turkey (Kasimoglu-Dogru et al. 2010, Onaran et al. 2019; Gökmen and Ektik, 2022), Canada (Aslam et al. 2012), Amerika-Tennessee (Kilonzo-Nthenge et al. 2015), Northwest Greece (Gousia et al. 2015), Colombia (Donado-Godoy et al. 2015), and South Korea (Kim et al. 2018; Kim et al. 2019). In the present study, 40.3% of the isolates were resistant to erythromycin and 71.64% of the isolates exhibited resistance to tetracycline. Molechan et al. (2019) determined that 76% of the chicken meat samples were resistant to erythromycin while all samples were resistant to tetracycline. Kim et al. (2018) reported that most isolates were resistant to erythromycin and tetracycline. In another study, 58% of the *E. faecalis* isolates were determined to be resistant to erythromycin while 71.4% were resistant to tetracycline; 48.6% of the *E. faecium* samples were resistant to erythromycin while 40.5% were resistant to tetracycline (Donado-Godoy et al. 2015). In another study on poultry meat, the majority of the *E. faecalis* and *E. faecium* isolates were determined to be resistant to erythromycin and tetracycline (Gousia et al. 2015). The results of this study agree with those reported in previous studies.

Clinical failures have been reported for fluoroquinolone, erythromycin, tetracycline, and chloramphenicol for the treatment of *Enterococcus* spp. infections, which has been attributed to the widespread resistance of enterococci to erythromycin, clindamycin, and tetracyclines (Korten 2002; Moellering 2005). Moreover, the difference in findings of the studies on antibiotic resistance is attributable to the unconscious and illegal use of antibiotics in addition to geographical differences.

Enterococcus infections have been treated using glycopeptide antibiotics, especially using vancomycin, as they are approved for use in human treatment. However, there has been a drastic upsurge in vancomycin resistance with the widespread clinical use of vancomycin in hospitals (Kirst et al. 1998).

The concern about the increasing number of vancomycin-resistant enterococcus strains have been increasing (Lawlwy et al. 2012). The *Van*A phenotype is mostly found in *E. faecalis* and *E. faecium* and shows highly inducible resistance to vancomycin and teicoplanin while *Van*B shows intermediately inducible resistance to vancomycin (Švec and Devriese 2015). Furthermore, enterococci are known to gain antibiotic resistance through genetic mobile elements such as plasmids, integrongs, and transposons, mutations, chromosomal, and exchange. The species can develop acquired resistance to many antibiotics through their various resistance properties (Hegstad et al. 2010, Hollenbeck and Rice 2012).

As the best-identified resistance genes in enterococci, the presence of the *Van*A and *Van*B genes was examined in the present study. However, the *Van*A and *Van*B genes were not detected in the phenotypically and intermediately vancomycin-resistant strains. Kasimoglu-Doğru et al. (2010) also did not detect the *Van*A and *Van*B genes. In another study, 93.5% of the *E. faecium* isolates that were obtained from different foods and phenotypically resistant to vancomycin had the *Van*A gene while 29% had the *Van*B2,3 gene; the researchers did not detect *Van*A, *Van*B, or *Van*B2,3 in the *E. faecalis* isolates (Gousia et al. 2015). Onaran et al. (2019) identified the *Van*A gene in 16.7% of the *Enterococcus* spp. isolates and the V*an*B gene in 8.3% of the isolates. In addition to the *Van*A and *Van*B genes, which are the best-identified vancomycin resistance genes in enterococci, *Van*C, *Van*D, *Van*E, *Van*G, *Van*L, *Van*M, and *Van*N resistances have also been observed (Arthur and Courvalin 1993, Ahmed and Baptiste 2018). Gökmen and Ektik (2022) found 31.5% *Van*A, 8.2% *Va*nB and 23.3% *Van*C2/C3 resistance genes in Enterococcus spp isolates. This explains the phenotypical resistance to vancomycin that was found in this study. Furthermore, the differences in the findings of the studies are attributable to differences in genetic mobile elements, chromosomal exchange, and mutations.

The presence of *Enterococcus* spp. in the chicken meats indicates the lack of adherence to hygienic conditions. The multiple antibiotic resistance of the isolates will complicate treatments and add to antibiotic resistance. No vancomycin resistance genes were detected in the study. The resistance in different phenotypes and the detection of vancomycin-resistant genes in enterococci that were isolated from foods in different studies pose a threat to public health. To prevent these undesired outcomes, inspections should become firmer, the use of antibiotics should be controlled, and strict policies should be employed.

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RESEARCH ARTICLE

Fattening Performance of Herik Lambs underneath Thermal Stress in Intensive Conditions

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ABSTRACT

This study was carried out to determine the fattening performance of Herik lambs reared under in intensive conditions. Twenty single male lambs, all with an mean body weight of 20.78 kg, were used in the study. Concentrate was given ad libitum and alfalfa was given in the amount of 300 g/lamb/day. The lambs were slaughtered when they reached the live weight of 40 kg. In this study, daily weight gain was generally tended to increase between the initial day and 56th day while it tended to decrease from 56th day to the end of fattening. During the fattening, the daily body weight gain and the feed efficiency were determined as 211 g and 9.668 respectively. Also, the temperature humidity index was determined above to accepted threshold value in ruminants along fattening period. Consequently, daily live weight gain and feed efficiency of Herik lambs were worse than those of Turkish native breeds at the slaughter weight of 40 kg.The unsatisfactory in fattening performance of Herik lambs may be depend on exposed high temperature along fattening period and till now lack of improvement studies on them. Thus, researchs should be done for fattening performance of Herik lambs under different breeding conditions.

Keywords: Climatic conditions, fattening performance, lamb, intensive condition

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Entansif Koşullarda ve Isı Stresi altında Herik Kuzuların Besi Performansı

ÖZ

Bu çalışma, entansif şartlarda yetiştirilen Herik kuzularında besi performansını belirlenmek amacıyla yapılmıştır. Araştırmada ortalama canlı ağırlığı 20.78 kg olan 20 baş tekiz erkek kuzu kullanılmıştır. Kuzular ad libitum konsantre yem ve günde kuzu başına 300 gr kuru yonca otu ile beslenmişlerdir. Kuzular 40 kg canlı ağırlığa ulaştıklarında kesime sevk edilmişlerdir. Bu araştırmada, günlük canlı ağırlık artışının besinin ilk günü ile 56. gün arasında artma eğiliminde olduğu 56. günden besi sonuna kadar ise azalma eğiliminde olduğu tespit edilmiştir. Besi süresince ortalama günlük canlı ağırlık artışı 211 g ve yemden yararlanma oranı 9.668 kg olarak belirlenmiştir. Ayrıca besi dönemi boyunca ısı nem indeksinin, ruminantlar için kabul edilen eşik değerin üzerinde olduğu belirlenmiştir. Sonuç olarak, 40 kg kesim ağırlığındaki Herik kuzularının günlük canlı ağırlık artışı ve yemden yararlanma oranının aynı ağırlıktaki yerli ırk kuzulardan daha düşük olduğu bulunmuştur. Herik kuzularının besi performansının düşük olması, besi dönemi boyunca yüksek sıcaklığa maruz kalmasına ve bugüne kadar üzerinde ıslah çalışmalarının yapılmamış olmasına bağlı olabilir. Bu nedenle, Herik kuzularının farklı yetiştirme koşullarında besi performansı ile ilgili araştırmalar yapılmalıdır.

Anahtar kelimeler: Besi performansı, entansif koşul, iklim koşulları, kuzu

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INTRODUCTION

Meat is an important protein source for human nutrition. Protein is used for growth and development in young people while it is used for the execution of body functions in older people. Livestock animals such as cattle, sheep, goat, pork and broiler chicken are used for meat production. Pork meat is not consumed because of cultural and religious causes in Turkey. Poultry meat is an affordable food source. However, B12 vitamin, iron and zinc in broiler meat are lesser than those of cattle and sheep meat. Cattle and sheep are generally used for red meat production. In addition to being an excellent source of protein, red meat is a nutrient that provides important vitamins and minerals such as B12 vitamin, iron and zinc (Williams 2007).

Sheep production has an important place in the nutrition of household and in come source of rural people in Turkey. In 2020, a total of 4 358 732 heads of sheep have been slaughtered and approximately 95 thousand tons of red meat production which was about 8.81% of the total red meat production in Turkey (FAOSTAT 2022). These results show that, researches should focus more on lamb meat production in Turkey. In this respect, evaluation of local sheep breeds is important in terms of fattening performance characteristics.

Herik sheep is one of the local sheep breeds, raised in the border area between Central Anatolia and Black Sea Region, in Turkey. Herik sheep is thought to have resulted from the irregular crossbreeding of the Akkaraman breed, raised predominantly in Central Anatolia, with the Karayaka breed, raised predominantly in the Black Sea Region (Akçapınar 2000). Herik sheep has a fat-tail, wide at the base narrows towards the end. (Yalçın 1986, Akçapınar 2000). It has a white or brown-black fleece and has black or brown spotted around the eyes and mouth and on the legs. Rams has horizontal spiral and strong horns extending sideways, sheep has poor structure horns. It is reported to be adult live weight 60 and 47 kg; wither height 65 and 61 cm; body length 67 and 62 cm. Also it is reported to be fleece weight 1.8-3.5 kg, lamb born weight 3.3-3.5 kg and the number of lambs per parturition 1.1 (TAGEM 2009).

Climate conditions such as ambient temperature and relative humidity are used for the identification of thermal stress (Donelly 1984, Davis 2003). Relative humidity affects the feeling degree of ambient temperature. The common effect of ambient temperature and relative humidity is expressed in a single value which called "temperature-humidity index (THI)" on production traits in livestock. In measurement of thermal stress, usage of temperature-humidity index is recommended (Armstrong 1994). The threshold value of THI is known as 74 for ruminants (Mader et al. 2006).

Recently years researchers have focused on determining the slaughter and carcass characteristics (Teke et al. 2017, Uğurlu et al. 2017, Teke et al. 2018) and meat quality (Uğurlu et al. 2017) in Herik lambs. However, there has no information about the fattening performance of Herik lambs under the intensive conditions. The current study aimed to investigate the fattenning performance of Herik lambs under the intensive conditions and to compare their fattening performance with that of other indigenous sheep breeds.

MATERIAL AND METHODS

Animals, housing, nutritional and climatic conditions

The present study was approved by the Animal Experiments Ethical Committee of Ondokuz Mayis University (HADYEK 2014/37). The study was conducted at a private farm, where the altitude is approximately 171 m Samsun Province. Preparatory work was performed before the planning of this study and it was determined that the Herik lambs have two types of tails, i.short round fat tail, ii. Long and semi-fat tail. In this study twenty single male lambs, ten with short-round fat tails and ten with long, semi-fat tails, were randomly selected after weaning (90 days), with an approximately initial live weight of 20 kg.

The stocking density at stockyard was 0.7 m^2. In the stockyard, 30 cm for concentrate feeder and 30 cm for roughage feeder per lamb were allocated. All lambs were vaccinated against clostridial disease and they were treated with anthelmintics for parasitic disease (Akçapınar 2000).

Nutrient content of concentrate mixture and alfalfa hay were analyzed (AOAC 2000). As for, metabolic energy was calculated for concentrate mixture (TSE 1991) and alfalfa hay (Kirchgessner and Kellner 1981). Before the fattening period, the amount of diet offered to animals daily was 10% more than the previous day's consumption, the two weeks of dietary adaptation was applied. All lambs were fed with ad libitum concentrate mixture consisting of approximately 2700 kcal/kg metabolic energy and 18% crude protein until lambs reached 30 kg live weight and approximately 2500 kcal/kg metabolic energy and 17% crude protein from 30 kg live weight until to the end of fattening period. Alfalfa hay (300 g lamb/day) and free access to water were also supplied. Lambs were weighed individually on a weekly basis, after fasting for 16 h with free access to water and daily feed consumption was recorded during the fattening period.

Climatic data such as maximum daily temperature, minimum daily temperature and relative humidity was provided by the General Directorate of Turkish State Meteorological Service (MGM, 2022). Temperature-humidity index (THI) was calculated with a formula reported by Davis et al. (2003). THI=(0.8 x maximum

ambient temperature)+[% relative humidity/100 x (mean ambient temperature-14.4)]+46.4.
Descriptive statistics of live weight, average daily weight gain, daily feed intake and feed conversion ratio were determined for fattening performance (SPSS, 1998).

RESULTS

Fattening performance

The results of average daily weight gain, daily concentrate intake and feed conversation ratio are shown in Table 1. In this study, the highest daily weight gain (297 g) was determined between 43-49. days of the fattening period while feed conversation ratio of that period was determined to be 7.138. Daily weight gain followed a fluctuating pattern between the initial and 56th day, however it generally tended to increase.. Also, it was seen that the daily weight gain tended to decrease after 56th day until the end of fattening (Figure 1). During the fattening period, the daily weight gain and the daily average feed consumption were determined as 211 g and 9.668 kg/day respectively.

Table 1. Fattening performance of the Herik lambs

Days	n	BW (kg)	ADWG (g)	DFI (g)	FE
Initial-7.	20	22.73±0.49	279±0.021	1422	5.096
8-14.	20	24.03±0.58	195±0.027	1795	9.205
15-21.	20	24.96±0.59	132±0.016	1958	14.833
22-28.	20	25.98±0.61	146±0.014	1861	12.746
29-35.	20	27.94±0.71	279±0.024	1780	6.378
36-42.	20	29.37±0.67	211±0.023	2170	10.284
43-49.	20	31.45±0.66	297±0.023	2120	7.138
50-56.	20	32.97±0.69	216±0.018	2549	11.800
57-63.	18	33.73±0.55	211±0.022	2227	10.554
64-70.	18	35.05±0.55	198±0.019	2704	13.656
71-77.	18	36.36±0.58	202±0.018	1883	9.321
78-84.	17	37.50±0.53	192±0.023	2160	11.250
85-91.	15	38.52±0.54	202±0.029	2068	10.237
92-98.	14	39.50±0.47	181±0.026	2089	11.541
99-105.	14	40.38±0.54	160±0.032	1820	11.375
Initial-35.	-	-	204±0.011	1763	8.642
Initial-70	-	-	218±0.007	2043	9.371
Initial-105.	-	-	211±0.006	2040	9.668

ADWG: Average Daily Weight Gain, BW: Body weight, DFI: Daily feed intake, FI: Feed efficiency

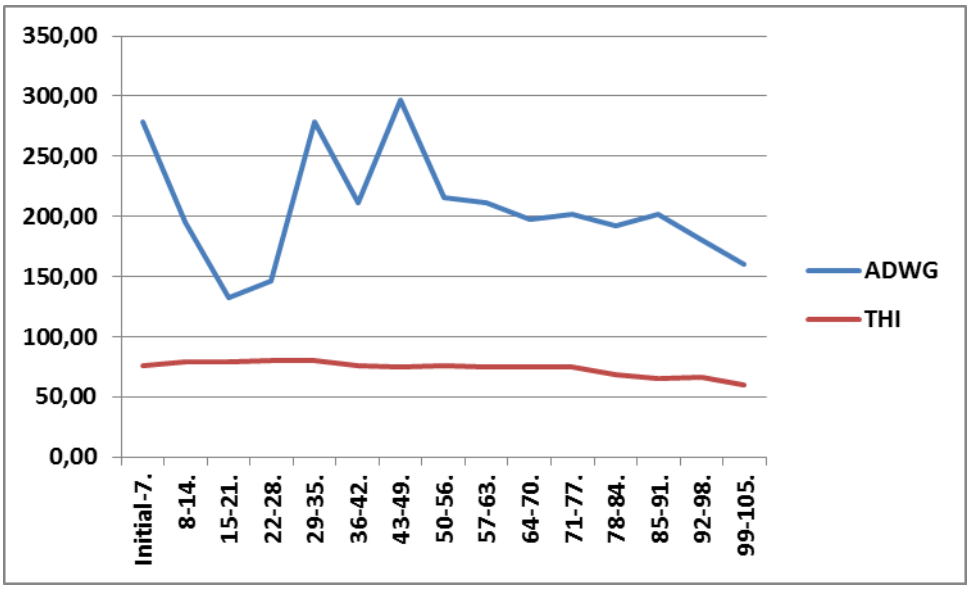

Figure 1: Change of ADWG and THI during the fattening period
ADWG: Average Daily Weight Gain, THI: Temperature Humidity Index

Climatic conditions

The results of climatic conditions, during the fattening period are shown in Table 2. The average ambient temperature and relative humidity at the basin were 22 °C and 76% during the study period respectively. This study was conducted between 16-July-2015 and 30-October- 2015. Thus, the ambient temperature and relative humidity were high during the first 70 days of the fattening period, However, the ambient temperature and relative humidity decreased after the 71st day.

Table 2. Climatic conditions during the fattening period

Days	MADT (°C)	MIDT (°C)	RH (%)	THI
Initial-7.	26.38± 0.18	19.46±0.54	64.26±0.79	75.53±0.33
8-14.	28.45±0.24	21.44±0.33	66.88±2.11	78.79±0.30
15-21.	29.82±0.78	23.10±0.36	66.06±1.23	79.61±0.62
22-28.	29.51±0.27	22.52±0.23	64.04±1.04	79.72±0.21
29-35.	29.31±0.22	23.64±0.49	68.28±1.24	79.93±0.34
36-42.	27.75±0.50	21.54±0.75	62.04±2.19	76.07±0.93
43-49.	26.80±0.21	19.14±0.54	60.21±2.28	75.28±0.45
50-56.	27.30±0.88	19.94±0.35	66.98±2.87	75.78±1.09
57-63.	26.34±0.37	20.48±0.52	72.08±1.61	75.05±0.69
64-70.	26.60±0.45	19.82±0.25	73.27±1.33	75.10±0.58
71-77.	26.26±0.27	19.10±0.20	72.05±0.71	74.69±0.55
78-84.	23.07±0.74	16.82±0.73	70.25±1.66	68.25±1.75
85-91.	20.74±1.19	14.75±1.16	71.10±3.20	65.11±1.60
92-98.	20.77±0.58	14.94± 0.82	78.07±1.77	66.07±1.09
99-105.	19.15±1.90	13.02±0.91	70.22±1.24	60.44±2.19

MADT: Maximum Daily Temperature (ºC), MIDT: Minimum Daily Temparature (ºC),
RH: Relative Humidity (%), THI: Temperature Humidity Index

DISCUSSION

In previous studies, slaughtering and carcass characteristics (Teke et al. 2017, Uğurlu et al. 2017, Teke et al. 2019) and meat quality traits (Uğurlu et al. 2017) of Herik lambs in intensive conditions were published However, there is no information about fattening performance of Herik lambs. Herik sheep is considered to be produced from the irregular crossbreeding of the Akkaraman, a fat-tailed breed, and with the Karayaka, a long and thin-tailed breed (Akçapınar 2000). For this reason, results obtained in the current study were compared and interpreted with previous results of Akkaraman, Karayaka and other native breeds. Fattening performance was determined with the use of average daily weight gain and feed conversion rate. It was reported that slaughter weight of lambs is generally range from 25 to 40 kg based on market conditions (Diaz et al. 2002, Zapletal et al. 2010). In this study, the lambs were slaughtered when they reached alive weight of 40 kg. In this study, average daily weight gain and feed conversion rate were determined to be 211 g and 9.668, respectively for the entire fattening period (105 days) (Table 2). Average daily weight gain and feed efficiency for approximately slaughter weight of 40 kg was reported to be 224 g and 5.77 for Akkaraman (Akmaz and Şahin 2002), 214 g and 8.60 (Balcı and Karakaş 2007), 212 and 7.06 (Olfaz et al. 2005) for Karayaka, 218 g and 6.16 for Awassi (Tekel et al. 2007), 227 g and 4.63, 241 g and 4.96 (Yakan and Ünal, 2010) for Bafra lambs. Daily live weight gain and feed efficiency of Herik lambs were lower than the results of above mentioned native breeds at the slaughter weight of 40 kg. Therefore, fattening performance of Herik lambs were insufficient compared to other native breeds. This situation of Herik lambs may be due to lack of improvement studies on them. Also, daily weight gain followed a fluctuating course from begining of fattening until end of fattening. In parallel with this, the daily ambient temperature and relative humidity and temperature humidity index were generally above to threshold value for thermal stress during fattening period. The threshold temperature humidity index is known as 74 for ruminants (Mader et al., 2006). Therefore, one of the reasons for the lower daily weight gain in the current study may be the temperature humidity index above the threshold value during fattening period.

CONCLUSION

Consequently, our study indicates that the fattening performance of Herik male lambs was poor than that of Turkish native breeds at the same slaughter weight. Thus, it can be considered that fattening performance of Herik lambs was unsatisfactory. However, THI was generally above to threshold value along fattening

period due to this study was conducted during the summer and autumn months. Therefore, insufficient of fattening performance in Herik lambs might be attributed to the combined effects of the high ambient temperature and relative humidity, namely temperature humidity index, Also, researchers should planed for fattening performance characteristics of Herik lambs under different breeding conditions due to scarce information about to fattening performance of Herik lambs.

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RESEARCH ARTICLE

In Vitro Antimicrobial Susceptibility of Staphylococci Isolated from Dogs with Otitis Externa

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ABSTRACT

Otitis is a multifactorial disease and various bacteria play role in its etiology. It is important to determine the etiologic agents and perform antimicrobial susceptibility tests for the effective treatment of the disease. In this study, it was aimed to isolate *Staphylococcus* species from ear samples taken from dogs with otitis externa and to determine their *in vitro* antimicrobial susceptibility to various antimicrobial agents. A total of 82 ear swap samples from 41 dogs, 13 (31.7%) females and 28 (68.3%) males, belonging to 12 different dog breeds, aged between 3 months and 10 years, were used. While a bacterial agent was isolated from 30 (73.17%) dogs, there was no growth in 11 (26.83%). *Staphylococcus* species were isolated from 24 (80%) out of 30 culture positive dogs. From those 24 dogs, *Staphylococcus aureus* from 7 (29.1%), *S. schleiferi* subsp. *schleiferi* from 6 (25%), coagulase negative staphylococcus (CNS) from 5 (20.8%), *S. haemolyticus* from 4 (16.6%) and *S. epidermidis* from 2 (8.3%) were isolated. *In vitro* antimicrobial susceptibility of the isolates obtained in the study was evaluated, and it was determined that all *S. aureus* strains were susceptible to cefoperazone/sulbactam and cephalothin, and 83.3% of *S. schleiferi* subsp. *schleiferi* strains were resistant to tobramycin. Multi-antibiotic resistance was determined in 40% of *Staphylococcus* species, while methicillin resistance was found to be 44%. As a result of the study, i) *S. schleiferi* subsp. *schleiferi* may be the primary bacterial agents of otitis externa cases in dogs, ii) dogs with otitis externa have the potential to transmit both multi-antibiotic resistant and methicillin resistant *Staphylococcus* species to humans, other animals and the environment.

Keywords: Dog, otitis, *Staphylococcus* spp., *in vitro* antibiogram

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Otitis Eksternalı Köpeklerden İzole Edilen Stafilokokların *İn Vitro* Antibiyotik Duyarlılıkları

ÖZ

Multifaktöriyel bir hastalık olan otitisin etiyolojisinde, çeşitli bakteriler de rol oynamaktadır. Hastalığın etkin tedavisinde etiyolojinin bilinmesi ve antimikrobiyal duyarlılık testleri önem arz etmektedir. Bu çalışmada, otitis eksternalı köpeklerden alınan örneklerde stafilokok türlerinin izolasyonu ve izolatların çeşitli antimikrobiyal maddelere *in vitro* duyarlılıklarının saptanması amaçlandı. Yaşları 3 ay ile 10 yıl arasında değişen, 12 farklı köpek ırkına ait 13'ü (%31.7) dişi, 28'i (%68.3) erkek olmak üzere toplam 41 adet köpekten alınan 82 adet kulak svap örneği materyal olarak kullanıldı. Köpeklerin 30 (%73.17) adetinden bakteriyel bir etken izole edilirken, 11 (%26.83) adet köpeğe ait örneklerde ise üreme olmadı. Kültür pozitif 30 adet köpeğin 24 (%80) adetinden stafilokok cinsine ait bakteriler izole edildi. Bu hayvanların 7 (%29.1) adetinden *Staphylococcus aureus*, 6 (%25) adetinden *S. schleiferi* subsp. *schleiferi*, 5 (%20.8) adetinden koagulaz negatif stafilokoklar (KNS), 4 (%16.6) adetinden *S. haemolyticus* ve 2 (%8.3) adetinden ise *S. epidermidis* izole edildi. İzolatların antimikrobiyal duyarlılıkları değerlendirildiğinde, *S. aureus* suşlarının tamamının sefoperazon/sulbaktam ve sefolatine karşı duyarlı olduğu, *S. schleiferi* subsp. *schleiferi* suşlarının ise %83.3'ünün tobramisine dirençli olduğu belirlendi. İzolatlarının %40'ında çoklu antibiyotik dirençliliği belirlenirken, %44'ünde ise metisilin dirençliliği saptandı. Sonuç olarak bu çalışmada, i) *S. schleiferi* subsp. *schleiferi*'nin köpeklerdeki otitis eksterna vakalarının primer bakteriyel etkenlerinden biri olabileceği, ii) otitis eksternalı köpeklerin insanlar, hayvanlar ve çevreye gerek çoklu antibiyotik dirençli gerekse metisilin dirençli stafilokok türlerini bulaştırma potansiyeline sahip olabilecekleri düşünüldü.

Anahtar Kelimeler: Köpek, otitis, *Staphylococcus* spp., *in vitro* antibiyogram

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GİRİŞ

Dış kulak yolunun akut veya kronik yangısı olarak tanımlanan otitis eksterna, köpeklerde sıklıkla karşılaşılan bir hastalıktır. Hastalığın oluşmasında çeşitli mikroorganizmalar, genetik faktörler ve anatomik yapı rol oynamaktadır (August 1988, Murphy 2001, Keskin ve ark. 2010). Multi-faktöriyel bir etiyolojiye sahip olan hastalık, hayvanı olduğu kadar, bazı patojenleri bulaştırması bakımından hayvan sahiplerini, tedavide barındırdığı bazı sorunlar nedeniyle de veteriner hekimlik uygulamalarını olumsuz etkilemektedir (Rosser 2004, Murugan ve ark. 2016).

Köpeklerdeki otitis vakalarının etiyolojisinde bakteriyel etkenler önemli rol oynamaktadır. Bu amaçla, otitis ekternalı köpeklerde bakteriyel etiyolojiyi ve etkenlerin *in vitro* antimikrobiyal duyarlılıklarını belirlemek amacıyla Türkiye ve diğer ülkelerde çeşitli çalışmalar yapılmıştır (August 1988, Keskin ve ark. 1999, Türkyılmaz, 2008, Öztürk ve ark. 2016). Yapılan çalışmalarda, klinik olarak otitis eksterna tanısı konulan köpeklerden alınan örneklerde genellikle en yüksek oranda *Staphylococcus* cinsine ait bakterilerin izole edildiği bildirilmiştir (Murugan ve ark. 2016, Öztürk ve ark. 2016). Bununla birlikte *Streptococcus* spp., *Corynebacterium* spp., *Pseudomonas* spp., *Candida* spp., *Proteus* spp. ve *Enterobacteriacea* familyasında bulunan bazı Gram negatif bakterilerin de izole edildiği rapor edilmiştir. Araştırmalarda, elde edilen izolatların çeşitli antimikrobiyal maddelere karşı direnç profilleri de belirlenmiş ve dirençlilik oranlarının da farklı olduğu gözlenmiştir (Özer ve ark. 1997, Oliveira ve ark. 2006, Murugan ve ark. 2016, Öztürk ve ark. 2016).

İstanbul'da yapılan bir çalışmada, 17 adeti otitis eksternalı, 8 adeti ise klinik olarak sağlıklı görünen köpeklerden alınan örneklerin bakteriyolojik analizinde, otitisli köpeklerden en yüksek oranda *S. aureus* (%41.1), *Proteus* spp. (%41.1) ve *Pseudomonas* spp. (%11.1); sağlıklı görünen hayvanlardan ise koagulaz negatif stafilokokların (KNS) izole edildiği bildirilmiştir. Çalışmada sonuç olarak, köpeklerdeki otitis eksterna vakalarının sağaltımında enrofloksasin, flokonazol ve metilprednizolon kombinasyonunun sistemik uygulanmasının etkili bir tedavi seçeneği olabileceği rapor edilmiştir (Özer ve ark. 1997). Öztürk ve ark. (2016), Burdur'da otitis eksternalı 52 adet köpekten aldıkları toplam 58 adet kulak svap örneğinin 52'sinden (%89.66) kültür pozitif sonuç aldıklarını bildirmişlerdir. Örneklerden sırayla *S. aureus* (%31.25), KNS (%14.06), *Streptococcus* spp. (%12.5), *Corynebacterium* spp. (%6.25), *Proteus* spp. (%4.68), *Pseudomonas aeruginosa* (%3.13) ve *Candida* spp. (%28.13) izole ettiklerini rapor etmişlerdir. Araştırıcılar *S. aureus, Streptococcus* spp., *Corynebacterium* spp. ve KNS izolatlarının tamamının (%100), *Proteus* spp. izolatlarının ise %66.67'sinin amoksisilin/klavulanik asite duyarlı olduğunu; izolatların enrofloksasin, amoksisilin, oksitetrasiklin, gentamisin, trimetoprim/sulfametoksazol ve eritromisine olan duyarlılıklarının ise değişik oranlarda (%0-%100) olduğunu rapor etmişlerdir. *P. aeruginosa* izolatlarının tamamının enrofloksasin ve gentamisine, %50'sinin de oksitetrasikline duyarlı olduğu ifade edilmiştir. Hindistan'da yapılan bir çalışmada otitis eksternalı 81 adet köpekten alınan kulak svap örneklerinin bakteriyolojik analizinde; *Staphylococcus* spp. (%48.6), *Pseudomonas* spp. (%21.5*)*, *Proteus* spp. (%14.95), *Escherichia coli* (%8.41) ve *Klebsiella* spp. (%6.54) izole edildiği bildirilmiştir. Yapılan *in vitro* antibiyogram testinde, stafilokok cinsine ait bakterilerin %94.2'sinin amoksisilin/klavulanik asite, %11.5'inin ise penisilin G'ye duyarlı oldukları bildirilmiştir (Murugan ve ark. 2016). Brezilya'da yapılan bir çalışmada, 62 adet otitisli köpeğe ait örneklerin bakteriyolojik analizinde, toplam 30 (%46.8) hayvandan çeşitli Gram pozitif ve Gram negatif bakterilerin izole edildiği bildirilmiştir. Hayvanlardan en yüksek oranda *S. intermedius* (%43.3), *S. aureus* (%30) ve *Pseudomonas* spp.'nin (%13.3) izole edildiği çalışmada, *S. intermedius* ve *S. aureus*'un penisilin G, ampisilin, eritromisin, klindinamisin, trimetoprim/sulfametoksazol, sefolatin, sefoksitin, imipenem, oksasilin, amikasin, gentamisin, neomisin, tetrasiklin, enrofloksasin, eritromisin, tetrasiklin, kloramfenikol ve vankomisine değişik oranlarda (%7.6-%100) dirençli oldukları bildirilmiştir (Oliveira ve ark. 2006).

Bu çalışmada, klinik olarak otitis eksterna semptomları gösteren köpeklerden alınan kulak svap örneklerinde stafilokok türlerinin varlığı ve çeşitli antibiyotiklere karşı *in vitro* duyarlılıklarının ortaya konulması amaçlandı.

MATERYAL ve METOT

Materyal

Çalışmanın materyalini, Ocak 2021-Haziran 2022 tarihleri arasında Balıkesir Üniversitesi Veteriner Fakültesi Hayvan Hastanesi'ne getirilen köpeklerden alınan svap örnekleri oluşturdu. Köpekler klinik olarak muayene edilip; kulak bölgesinde ağrı, kaşıntı ve akıntı semptomları gösteren 41 adet hayvandan materyal alındı. Farklı ırklara ait, yaşları 3 ay ile 10 yıl arasında değişen köpeklerin 13'ü (%31.7) dişi, 28'i (%68.3) ise erkekti (Tablo 1). Daha önceden çeşitli nedenlerle (üriner sistem enfeksiyonu ve otitis gibi) antibiyotik tedavisi aldığı bildirilen 5 köpekten, son antibiyotik uygulamasını takiben yaklaşık 20 gün sonra örnekler alındı. Svap örnekleri her iki kulaktan ayrı ayrı alınarak (82 adet), mikrobiyolojik analiz için laboratuvara getirildi.

Tablo 1. Örnek alınan köpeklerin ırk, cinsiyet ve yaşları.
Table 1. Breed, gender and age of the dogs sampled.

Irk	Dişi	Erkek	Yaş	Toplam
Kopay	3	7	11 ay-7 yaş*	**10**
Melez	2	6	3 ay-4 yaş*	**8**
Golden Retriever	-	5	9-10 yaş*	**5**
Alman Çoban Köpeği	1	4	2-6.5 yaş*	**5**
Kangal	2	2	1-6 yaş*	**4**
Cane Corso Italiano	3	-	3-4 yaş*	**3**
Terrier	1	-	5.5 yaş	**1**
Cooker Spiniel	-	1	2 yaş	**1**
Dogo Argentin	-	1	10 yaş	**1**
İngiliz Setter	1	-	5 yaş	**1**
Pointer	-	1	1 yaş	**1**
Barak	-	1	5 yaş	**1**
Toplam	**13**	**28**	**3 ay-10 yıl***	**41**

*Hayvanların yaş aralığı

İzolasyon
Svap örnekleri %5-7 defibrine koyun kanlı agara (1.10886, Merck, Darmstadt, Germany) ve MacConkey agara (Oxoid, CM0007, Hampshire, England) ekilerek, 37°C'de ve aerobik ortamda 1-3 gün inkübe edildi. Koloniler makroskopik ve mikroskopik morfolojileri, katalaz, koagulaz, DNase aktiviteleri (BD Difco, Sparks, USA), mannitol salt agarda (LAB, 138736/149, Lancashire, UK) üreme yetenekleri ve çeşitli biyokimyasal testlerle birlikte, hızlı test kitiyle (Api Staph, BioMérieux SA, France) identifiye edildi (Kloos ve Wolfshohl 1982, Quinn ve ark. 2011).

Antimikrobiyal Duyarlılık Testi
Çalışma kapsamında izole edilen stafilokok cinsine ait bakterilerin çeşitli antimikrobiyal maddelere karşı duyarlılıkları, disk difüzyon yöntemine göre yapıldı (Bauer ve ark. 1966). Testte; sefoperazon/sulbaktam (CES, 75/30 µg, Bioanalyse), sefalotin (CF, 30 µg, BBL), klortetrasiklin (CT, 30 µg, Himedia), amoksisilin/klavulanik asit (2/1) (30 µg, Oxoid), tobramisin (NN, 10 µg, BBL), neomisin/basitrasin/tetrasiklin (30 µg/10 IU/30 µg, Mast Diagnostic), kloksasilin (OB, 5 µg, Oxoid), seftiofur (EFT, 30 µg, Oxoid) ve oksasilin (OX, 1 µg, Oxoid) diskleri kullanıldı. Testin değerlendirilmesinde CLSI (2013, 2018) kriterleri dikkate alındı. Testte kontrol olarak, Balıkesir Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı Kültür Koleksiyonu'nda bulunan *S. aureus* ATCC 25923 suşu kullanıldı.

BULGULAR

İzolasyon
İncelenen 41 adet köpeğin 30'undan (%73.17) bakteriyel bir etken izole edilirken, 11'inde (%26.82) ise her hangi bir üreme olmadı. Bakteriler köpeklerin 22'sinden (%73.3) saf kültür, 8'sinden (%26.7) ise karışık kültür olarak izole edildi. Kültür pozitif 30 köpeğin 24'ünden (%80) stafilokok cinsine ait bakteriler, 6 (%20) köpekten ise *Corynebacterium* spp. (n: 3), *Candida* spp. (n: 3), *E. coli* (n: 2), *Streptococcus* spp. (n: 1) ve *Proteus* spp. (n: 1) izole edildi. Stafilokok cinsine ait bakterilerin identifikasyonlarında en yüksek oranda izole edilen türün *S. aureus* (%29.1) olduğu görüldü (Tablo 2).

Tablo 2. Otitis eksternalı köpeklerden izole edilen stafilokok türleri.
Table 2. *Staphylococcus* species isoleted from dogs with otitis externa.

Bakteri	n	%
S. aureus	7	29.1
S. schleiferi subsp. *schleiferi*	6	25
KNS	5	20.8
S. haemolyticus	4	16.6
S. epidermidis	2	8.3
Toplam	**24**	**100**

n: Stafilokok cinsine ait bakterilerin izole edildiği köpek sayısı.

Antimikrobiyal Duyarlılık Testi

Toplam 9 farklı antibiyotiğin test edildiği bu çalışmada, en yüksek oranda duyarlılık sefoperazon/sulbaktam ve sefalotine karşı *S. aureus* izolatlarında, en yüksek dirençlilik ise sefoperazon/sulbaktama karşı *S. haemolyticus* izolatlarında saptandı (Tablo 3). *S. aureus* izolatlarının %42.8'inde, *S. schleiferi* subsp. *schleiferi* izolatlarının %33.3'ünde, KNS'ların %40'ında, *S. haemolyticus* izolatlarının ise %75'inde çoklu antibiyotik dirençliliği saptandı. Metisilin dirençliliği *S. aureus* izolatlarında %42.8, *S. schleiferi* subsp. *schleiferi* ve KNS izolatlarında %50, *S. haemolyticus* suşlarında %100 ve *S. epidermidis* izolatlarında ise %33.3 olarak belirlendi.

Tablo 3. Otitis eksternalı köpeklerden izole edilen stafilokokların i*n vitro* antimikrobiyal duyarlılıkları.
Table 3. *In vitro* antimicrobial susceptibility of staphylococci isolated from dogs with otitis externa.

Antibiyotik	***S. aureus*** (n: 8)		***S. schleiferi* subsp. *schleiferi*** (n: 6)		**KNS** (n: 5)		***S. haemolyticus*** (n: 4)		***S. epidermidis*** (n: 2)	
	S (n)	R (n)	S (n)	R (n)	S (n)	R (n)	S (n)	R (n)	S (n)	R (n)
CES	8	-	4	2	3	2	2	2	1	1
CF	8	-	5	1	4	1	1	3	1	1
CT	7	1	5	1	4	1	2	2	2	-
AMC	7	1	4	2	3	2	2	2	2	-
NN	5	3	2	4	3	2	2	2	2	-
NBT	5	3	1	5	3	2	3	1	1	1
OB	2	6	2	4	3	2	3	1	2	-
AFT	6	2	4	2	4	1	2	2	2	-
OX	5	3	4	2	3	2	2	2	1	1

n: Suş sayısı, S: Duyarlı, R: Dirençli, CES: Sefoperazon/sulbaktam, CF: Sefalotin, CT: Klortetrasiklin, AMC: Amoksisilin/klavulanik asit, NN: Tobramisin, NBT: Neomisin/basitrasin/tetrasiklin, OB: Kloksasilin, EFT: Seftiofur, OX: Oksasilin.

TARTIŞMA

Otitis eksterna, köpeklerde özellikle de pet hayvan kliniklerinde yaygın olarak karşılaşılan önemli bir sağlık sorunu olarak görülmektedir. Multi-faktöriyel bir etiyolojiye sahip olan otitis vakalarından çeşitli bakteriyel etkenler izole edilmektedir (August 1988, Türkyılmaz 2008, Murugan ve ark. 2016, Öztürk ve ark. 2016, Li ve ark. 2021).

Stafilokok cinsine ait bakteriler, canlıların deri ve mukoz membranlarının normal florasında bulunan mikroorganizmalardır (Bradley ve Nizet 2011). Gerçekleştirilen bu çalışmada; farklı ırk, yaş ve cinsiyetteki 41 adet otitis eksternalı köpekten alınan örnekler bakteriyolojik yönden analiz edilmiş ve hayvanlardan sırayla *S. aureus, S. schleiferi* subsp. *schleiferi*, KNS, *S. haemolyticus* ve *S. epidermidis* izole edilmiştir. Bu durum, stafilokok cinsine ait bakterilerin, köpeklerdeki otitis eksterna vakalarının bakteriyel etiyolojisindeki rollerini göstermesi bakımından önem arz etmektedir.

Yaklaşık 62 adet türün tanımlandığı stafilokok cinsinde, en önemli patojenin *S. aureus* olduğu bildirilmektedir (Lowy 1988, Quinn ve ark. 2011, Thomson ve ark. 2022). *S. aureus* insan ve hayvanlarda oldukça önemli enfeksiyonlara neden olmaktadır. Etken; inek, koyun, keçi, at, kedi, köpek ve diğer hayvanlarda mastitis ve dermatitis başta olmak üzere çeşitli irinli enfeksiyonlardan sorumlu tutulmaktadır (Quinn ve ark. 2011, Osada ve ark. 2022). Gerek Türkiye'de (Keskin ve ark. 1999, Borum ve ark. 2014, Öztürk ve ark. 2016) gerekse farklı ülkelerde (Olivera ve ark. 2006, Bourély ve ark. 2019, Li ve ark. 2021), otitis eksternalı köpeklerde yapılan izolasyon çalışmalarında hayvanlardan yüksek oranlarda *S. aureus*'un izole edildiği görülmektedir. Gerçekleştirilen bu çalışmada da, stafilokok yönünden kültür pozitif hayvanlardan en yüksek oranda (%29.1) *S. aureus*'un izole edilmiş olması, konuyla ilgili çalışmaları desteklemektedir (Keskin ve ark. 1999, Olivera ve ark. 2006, Borum ve ark. 2014, Öztürk ve ark. 2016, Bourély ve ark. 2019, Li ve ark. 2021).

S. schleiferi subsp. *schleiferi* ilk kez 1988 yılında insan klinik örneklerinden izole edilmesini takiben (Freney ve ark. 1988), günümüzde insan ve hayvanlardaki çeşitli enfeksiyonlardan sorumlu tutulmaktadır (Lee ve ark. 2019). Yapılan çalışmalarda söz konusu bakterinin köpeklerdeki otitis, deri ve yumuşak doku enfeksiyonlarından değişik oranlarda izole edildiği görülmektedir. Kore'de yapılan bir çalışmada, 38 adeti otitis eksternalı, 42 adeti ise klinik olarak sağlıklı görünen köpeklerin her iki kulağından alınan svap

örneklerinin konvansiyonel bakteriyolojik yöntemlerle yapılan analizlerinde, otitis eksternalı hayvanlardan en yüksek oranda *S. pseudintermedius* ve *S. schleiferi* subsp. *schleiferi* izole edildiği rapor edilmiştir (Lee ve ark. 2019). Gerçekleştirilen bu çalışmada da, stafilokok yönünden kültür pozitif otitis eksternalı köpeklerden yüksek oranda (%25) *S. schleiferi* subsp. *schleiferi*'nin izole edilmesi, söz konusu bakterinin köpeklerdeki otitis vakalarının primer bakteriyel etkenlerinden biri olabileceğini düşündürmektedir. Diğer yandan bir pet hayvanı olarak köpeklerin insanlarla yakın temasta olmaları dikkate alındığında, söz konusu bakterinin halk sağlığı bakımından potansiyel zoonotik bir ajan olabileceği görüşünün (Freney ve ark. 1988) önemli olduğu değerlendirilmiştir.

Antibiyotikler, bakteriyel enfeksiyonların tedavisinde kullanılan terapötik ajanlardır. Hayvanlarda görülen stafilokok enfeksiyonlarının tedavi ve korunmasında çeşitli antibakteriyaller kullanılmaktadır. Antibiyotiklerin rastgele ve bilinçsizce kullanılmasının birçok olumsuz sonucu olabileceği gibi, yüksek veya düşük dozda gereğinden daha uzun ya da daha kısa süre kullanılmaları durumlarında, antibiyotik dirençliği başta olmak üzere, çeşitli olumsuzluklar yaşanmaktadır (Brinkac ve ark. 2017, Okalın ve Aksakal 2020, Li ve ark. 2021). Hayvanlarda gelişen antibiyotik dirençliliği, doğrudan ya da dolaylı olarak insan sağlığını da etkilemektedir (Marshall ve Levy 2011). Bu durum, özellikle kedi ve köpek gibi insanlarla yakın temas halindeki pet hayvanları için özel bir önem arz etmektedir (Brinkac ve ark. 2017, Li ve ark. 2021). Bu çalışmada, Balıkesir ve yöresinde evde beslenen otitis eksternalı köpeklerden izole edilen stafilokokların *in vitro* antibiyotik duyarlılıkları ortaya konuldu. Bu amaçla, toplam 24 adet stafilokok izolatı, 9 farklı antibiyotiğe karşı test edildi. Bulgular birlikte değerlendirildiğinde antibiyotiklere karşı en yüksek (%100) duyarlılık *S. aureus* suşlarında sefoperazon/sulbaktam ve sefolatine karşı, en yüksek (%83.3) dirençlilik ise *S. schleiferi* subsp. *schleiferi* suşlarında tobramisine karşı saptandı. Bakterilerin antibiyotiklere olan dirençliliklerinin gerek cins gerekse tür düzeyinde oldukça yüksek değerlerde (%14.2-%83.3) olduğu görüldü (Tablo 3). Konuyla ilgili diğer çalışmalar incelendiğinde, otitis ekternalı köpeklerden izole edilen stafilokok türlerinin *in vitro* antibiyotik dirençliliklerinin oldukça farklı düzeylerde olduğu görülmektedir (Olivera ve ark. 2006, Borum ve ark. 2014, Murugan ve ark 2016, Öztürk ve ark. 2016). Bu durum, test edilen suşların genetik yapısı, bulunduğu coğrafi bölge, daha önceden hayvanlara antibiyotik verilip verilmemesi ve uygulanan yöntemlerin niteliği gibi birçok faktöre bağlı olarak değişebilmektedir.

Otitisli köpeklerin çoklu antibiyotik dirençli bakterilerin insanlara bulaştırılmasında rol oynayabilecekleri bildirilmektedir (Bourély ve ark., 2018). Bu çalışmada, *S. aureus* suşlarının %37.5'inin, *S. schleiferi* subsp. *schleiferi* suşlarının %33.3'ünün, KNS suşlarının %40'ının ve *S. haemolyticus* suşlarının ise %75'inin çoklu antibiyotik direnci gösterdikleri saptandı. Bu araştırmada, test edilen bakteri sayıları az olmasına rağmen, çoklu antibiyotik direncinin en yüksek olarak *S. haemolyticus*'ta saptanmış olması, söz konusu bakterinin makrolidler, tetrasiklinler, aminoglikozitler, penisilinler ve sefalosporiler gibi birçok antibiyotiğe genetik olarak dirençli olmasıyla ilişkili olabilir (Barros ve ark. 2012, Lee ve ark. 2019).

Stafilokok cinsine ait bakterilerde görülen metisilin direnci, daha çok modifiye penisilin bağlayan proteini (*PBP2a*) kodlayan *mec*A geni ile ilişkili olup; penisilinler, sefalosporinler ve karbapenemler dahil tüm beta-laktam grubu antibiyotiklere karşı direnç sağlamaktadır (Weese ve van Duijkeren 2010). Gerçekleştirilen bu çalışmada, metisilin dirençliliği *S. aureus* suşlarında %37.5, *S. schleiferi* subsp. *schleiferi* suşlarında %33.3, KNS suşlarında %40, *S. haemolyticus* suşlarında %100 ve *S. epidermidis* suşlarında ise %50 olarak belirlendi. Bu bulgu, metisilin dirençli stafilokok türlerinin köpekler aracılığıyla insanlara bulaşma olasılığı bakımından önem arz etmektedir. Bu nedenle, özellikle çocuk ve ileri yaşlı bireylerle yakın temas halinde olan köpeklerin, antibakteriyel tedavi gerektiren hastalıklarında, antibiyogram test sonucuna göre tedaviye alınmaları halk sağlığı bakımından önem arz etmektedir.

Bu çalışmada sonuç olarak, otitis eksternalı köpeklerden en yüksek oranda *S. aureus*'un izole edildiği, stafilokok cinsine ait bakterilerde yüksek oranda çoklu antibiyotik ve metisilin dirençliliğinin olduğu görüldü. Bu nedenle, enfekte köpeklerin insanlara ve çevreye çoklu ve metisilin dirençli stafilokokları bulaştırma potansiyeline sahip oldukları ve bu durumun, klinisyen veteriner hekimler tarafından dikkate alınarak, antibiyogram sonucu belirlendikten sonra uygun doz ve sürede hayvanlara antimikrobiyal tedavi uygulanmasının, 'tek tıp sağlık konsepti' bakımından hem halk sağlığı hem de hayvan sağlığı açısından önem arz ettiği düşünüldü.

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

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

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RESEARCH ARTICLE

Evaluation of Some Blood Gas, Hemogram and Biochemical Parameters in Cats with Hemoplasmosis

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ABSTRACT

The aim of this study was to evaluate some blood gases, hemogram, and biochemical parameters in cats with hemoplasmosis. Ten healthy and fifteen infected cats were enrolled in the study. 7 mL of blood sample was taken once from all cats into tubes with and without anticoagulant. Blood gases, complete blood count, and biochemical analyzes were performed from blood samples. While pH, HCO_3 and BE levels of cats with hemoplasmosis were significantly lower than healthy cats, lactate levels were higher ($p< 0.05$). WBC, Mon, Gra, and RDW levels were found to be significantly higher than in healthy cats, while RBC, Hct, Hb, and PLT levels were found to be lower ($p< 0.05$). AST, T.Bil, D.Bil, P, TG, LDH, TP, and CK levels were significantly higher than healthy, while Alb and Ca levels and A:G ratio were found to be low ($p< 0.05$). As a result, it was determined that metabolic acidosis, hyperlactatemia, anemia, hypertriglyceridemia, hypoalbuminemia, hyperbilirubinemia developed in cats with hemoplasmosis. In addition, it can be concluded that the A:G ratio should be considered in the diagnosis of infected cats and it should be evaluated together with other diagnostic test results.

Keywords: A:G ratio, Biochemical parameters, Blood gas, Complete blood count, Hemoplasmosis

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Hemoplazmozlu Kedilerde Bazı Kan Gaz, Hemogram ve Biyokimyasal Parametrelerin Değerlendirilmesi

ÖZ

Sunulan çalışmanın amacı, hemoplazmozlu kedilerde bazı kan gazı, hemogram ve biyokimyasal parametrelerin değerlendirilmesidir. Çalışmaya on sağlıklı ve on beş hemoplazmozlu kedi dahil edildi. Çalışmaya dahil edilen tüm kedilerden antikoagülanlı ve antikoagülansız tüplere bir kez 7 mL kan alındı. Kan örneklerinden kan gazı, tam kan sayımı ve biyokimyasal analizler yapıldı. Hemoplazmozlu kedilerin pH, HCO_3 ve BE düzeyleri sağlıklı kedilere göre anlamlı olarak düşük iken, laktat düzeyleri yüksekti ($p< 0.05$). WBC, Mon, Gra ve RDW seviyeleri sağlıklı kedilere göre anlamlı olarak yüksek bulunurken, RBC, Hct, Hb ve PLT seviyeleri düşük bulundu ($p< 0.05$). AST, T.Bil, D.Bil, P, TG, LDH, TP ve CK düzeyleri sağlıklı bireylere göre anlamlı olarak yüksek bulunurken, Alb ve Ca düzeyleri ile A:G oranı düşük bulundu ($p< 0.05$). Sonuç olarak hemoplazmozlu kedilerde metabolik asidoz, hiperlaktatemi, anemi, hipertrigliseridemi, hipoalbuminemi, hiperbilirubinemi geliştiği tespit edildi. Ayrıca hemoplazmozlu kedilerin tanısında düşük A:G oranının dikkate alınması ve diğer tanısal test sonuçları ile birlikte değerlendirilmesi gerektiği sonucuna varıldı.

Anahtar Kelimeler: A:G oranı, Biyokimyasal parametreler, Kan gazları, Tam kan sayımı, Hemoplazmoz

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INTRODUCTION

Hemotropic *Mycoplasma spp.* are gram-negative microorganisms that do not have a cell wall, are small (0.3-0.8 μm), are found on the surface of erythrocytes, cause varying degrees of hemolytic anemia in infected hosts, and cannot be cultured in laboratory environments (Sykes 2010). Hemotropic Mycoplasmas infect a wide variety of mammalian species, including humans, and have a worldwide distribution. Domestic and wild cats are one of the animal groups most affected by hemoplasma infections (Sykes and Tasker 2013, Aslan 2016). *M. haemofelis* is the most pathogenic species that cause hemoplasmosis in cats and causes the disease called feline infectious anemia (Sykes 2010).

Infections originating from hemoplasmosis are encountered more frequently in cases of various stress factors such as pregnancy, malnutrition, and lactation or co-infection, in addition to immunosuppression caused by various drugs used or retrovirus infections (Aslan 2016). Although it is known that blood-fed arthropods play an important role in the transmission of the disease, the transmission issue for Mycoplasmas is not fully clarified. It has been reported in some studies that the agent can be transmitted through blood transfusion, vertically, and even through saliva/spittle when cats bite each other (Woods et al. 2005, Willi et al. 2006, Willi et al. 2007).

The severity of clinical findings associated with *M. haemofelis* infection varies according to the period of infection, the virulence of the organism, and the severity of anemia. Lethargy, weakness, decreased appetite, dehydration, weight loss, pallor of the mucous membranes, and intermittent fever are usually determined as clinical findings in cats with hemoplasmosis (Tasker et al. 2018). As a result of hypoxia developing in severely anemic patients, dyspnea, tachypnea, tachycardia, heart murmur, and gallop rhythm may develop (Evans and Gruffydd-Jones 1984, Carney et al. 1993, Saki and Ozer 2011).

Hemoplasmas cause anemia through hemolysis and sequestration. The binding of the organism to erythrocytes directly damages the cell membrane, resulting in a shortening of the erythrocyte lifespan. Although intravascular hemolysis can occur with direct damage to erythrocytes, most hemolysis is thought to be extravascular. Cats that recover from the infection remain chronically infected with hemoplasmas for an indefinite period, which in some cases can last a lifetime. If the cat's carrier state is not successful in clearing the infection, it can also follow antibiotic treatment. Parasitemia is not seen on blood smears during this period and such animals are usually clinically normal, but infection can often be detected by Polymerase Chain Reaction (Tasker 2006).

For the diagnosis of hemoplasmosis cases, many studies have been encountered, which have been evaluated on different laboratory parameters (Kurtdede and Ural 2004, Akkan et al. 2005, Aslan et al. 2010, Sykes and Tasker 2013, Weingart and Kohn 2015). Although the findings of these studies are instructive, a definite conclusion has not been reached yet. This research was aimed to evaluate some blood gas, hemogram, and biochemical parameters in cats diagnosed with hemoplasmosis. It is aimed to contribute to a better understanding of the clinical-pathological changes that occur in hemoplasmosis cases and to expand the diagnostic approaches.

MATERIALS and METHODS

Animals

The animal material of the study consisted of 25 owned cats, aged 1-5 years, of different breeds and gender, 15 diagnosed with hemoplasmosis, and 10 healthy (bred to the clinic for general control without vaccination, antiparasitic application, or any disease). Cats with positive feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV), feline infectious peritonitis (FIP) as a result of the rapid test kits (Asan Easy Test FIV Ab/FeLV Ag, and Asan Feline Corona Virüs (FCoV) Ab, ASANPharm, Korea) and other diseases were not included in the study. An informed consent form was received from the patient owners stating that they had accepted all the interventions to be performed before the applications.

Collecting and analyzing the blood samples

7 mL blood samples were taken once from all cats included in the study from the vena cephalica antebrachium into tubes with and without anticoagulant (1 mL into K_3-EDTA tubes for complete blood count and 1 mL into heparin syringe for blood gases analysis; 5mL for biochemical analysis) tubes using an appropriate IV cannula. Blood gases and complete blood count analyzes were performed within 5 minutes following blood collection. Blood power of hydrogen (pH), partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), oxygen saturation (SO_2), potassium (K), sodium (Na), chlorine (Cl), lactate (Lac), base excess (BE), and bicarbonate (HCO_3) levels were measured in a blood gas device (ABL 90 Flex Blood Gas/Electrolyte Analyser, Model 5700 Radiometer, ABD). White blood cell (WBC), Lymphocyte (Lym), Monocyte (Mon), Granulocyte (Gra), red blood cell (RBC), mean corpuscular volume (MCV), haematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW),

hemoglobin (Hb), and platelet (PLT) levels in blood tubes with K_3-EDTA were determined in the complete blood count device (MS4 CFE 279, Haematology Analyser, France). Blood samples taken into gel tubes were centrifuged at 5000 rpm for 5 minutes and serum samples were extracted. Then blood urea nitrogen (BUN), creatinine (Cr), glucose (Glu), alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), amylase (AMY), cholesterol (Chol), triglyceride (TG), creatine kinase (CK), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT) levels, total bilirubin (T.Bil), direct bilirubin (D.Bil), calcium (Ca), phosphorus (P), magnesium (Mg), total protein (TP), albumin (Alb), and albumin:globulin (A:G) ratio were measured with an autoanalyzer (Biotecnica BT 3000 Plus, Italy).

Diagnosis of Hemoplasmosis

Blood smears were prepared by taking one drop of blood from each of the anticoagulant (K_3-EDTA) blood samples. The smears fixed with methyl alcohol for 3-5 minutes were stained with Giemsa for 45 minutes. After the staining period was over, the smeared samples were washed for a minimum of 1 minute under the tap water to prevent the formation of stain residues and dried by placing them in an upright position on a dry surface. The dried smears were examined microscopically (Leica, Germany) under the light microscope by scanning more than 100 areas for about 5 minutes with a 100x magnification lens by dripping immersion oil. The Haemoplasma bacterias that settled in blood cells (erythrocyte) were determined (Figure 1).

Statistical analysis

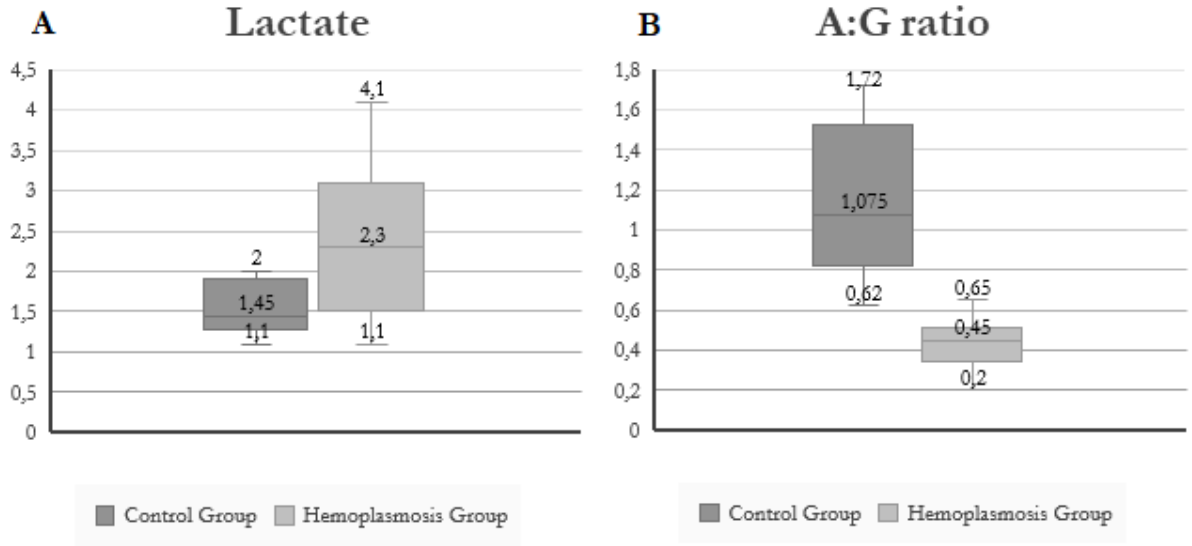
SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical program was used to evaluate the data. One-sample Kolmogorov-Smirnov test was applied to evaluate the normal distribution (parametric or nonparametric) preconditions of the data. The Mann-Whitney U test was used to compare the data with nonparametric distribution between groups and presented as median (min/max). The values of $p< 0.05$, $p< 0.01$, and $p< 0.001$ were accepted for the significance level of the tests.

RESULTS

Venous blood gases parameters of cats with hemoplasmosis and healthy cats are presented in Table 1. While blood gas pH, BE, and HCO_3 levels of cats with hemoplasmosis were significantly lower than healthy cats, lactate concentration (Graphic 1A) were higher ($p< 0.05$). Complete blood count results of cats with hemoplasmosis and healthy cats are presented in Table 2. WBC, Mon, Gra, and RDW levels of cats with hemoplasmosis were found to be significantly higher than healthy cats ($p< 0.05$). RBC, Hct, Hb, and PLT levels were found to be significantly lower ($p< 0.05$).

The results of serum biochemical analysis of cats with hemoplasmosis and healthy cats are presented in Table 3. AST, T.Bil, D.Bil, P, TG, LDH, TP, and CK levels were found to be significantly higher in cats with hemoplasmosis compared to healthy cats ($p< 0.05$). Alb and Ca levels from biochemical parameters and the A:G ratio (Graphic 1B) were found to be low ($p<0.05$).

Şekil 1. Sağlıklı ve hemoplazmozlu kedilerin laktat konsantrasyonu (A) ve A:G oranı (B).
Graphic 1. Lactate concentation (A) and A:G ratio (B) of healthy and hemoplasmosis cats.



Tablo 1. Sağlıklı ve hemoplasmoz ile enfekte kedilerin venöz kan gaz sonuçları (median (min/max).
Table 1. Venous blood gases results of healthy and hemoplasmosis-infected cats (median (min/max).

Parameters median (min/max)	**Control Group (n=10)**	**Hemoplasmosis Group (n=15)**	**P value**
pH	7.38 (7.33/7.41)	7.33 (7.22/7.40)	**0.016**
pCO_2 (mmHg)	34.7 (29.50/40.20)	31.7 (14.90/41.80)	0.080
pO_2 (mmHg)	41.6 (32.60/46.10)	37.2 (26.30/57.80)	0.892
SO_2 (mmHg)	61.15 (44.50/71.10)	54.2 (24.20/83.70)	0.567
K (mmol/L)	4.2 (3.60/5.30)	3.7 (2.80/4.60)	0.643
Na (mmol/L)	156.5 (151/158.00)	156 (152.00/168.00)	0.807
Cl (mmol/L)	121.5 (118/125.00)	123 (116.00/137.00)	0.216
Lac (mmol/L)	1.45 (1.10/2.00)	2.3 (1.10/4.10)	**0.026**
BE (mmol/L)	-4.8 (-8.30/-0.60)	-9 (-16.30/2.40)	**0.004**
HCO_3 (mmol/L)	20 (17.90/22.90)	17.1 (11.30/26.50)	**0.004**

pH: Power of hydrogen, pCO_2: partial pressure of carbon dioxide, pO_2: partial pressure of oxygen, SO_2: oxygen saturation, K: potassium, Na: sodium, Cl: chlorine, Lac: lactate BE: base excess, HCO_3: bicarbonate

Tablo 2. Sağlıklı ve hemoplasmoz ile enfekte kedilerin tam kan sayımı sonuçları (median (min/max).
Table 2. Complete blood count results of healthy and hemoplasmosis-infected cats (median (min/max).

Parameters median (min/max)	Control Group (n=10)	Hemoplasmosis Group (n=15)	P value
WBC (x 10^9 cells/L)	10.7 (5.80/18.60)	39.9 (10.93/125.10)	**0.000**
Lym (x 10^9 cells/L)	4.92 (2.23/33.80)	11.08 (2.01/17.76)	0.071
Mon (x 10^9 cells/L)	0.94 (0.61/1.64)	2.26 (0.48/6.13)	**0.016**
Gra (x 10^9 cells/L)	5.28 (2.58/11.27)	30.38 (3.28/101.21)	**0.000**
RBC (x 10^3 cells/mL)	10.5 (7.26/12.30)	5.5 (2.20/8.78)	**0.000**
MCV (fL)	49.4 (45.80/53.20)	43.4 (26.80/70.20)	0.397
Hct (%)	46.5 (35.40/52.50)	20.7 (12.90/38.10)	**0.000**
MCH (pg)	12 (7.40/15.70)	13.6 (0.60/17.30)	0.723
MCHC (g/dL)	23.6 (15.10/34.00)	28.7 (1.20/37.20)	0.285
RDW (%)	11.4 (10.30/17.70)	12.1 (10.80/19.60)	**0.031**
Hb (g/dL)	12 (8.40/16.60)	5.6 (0.20/14.20)	**0.000**
PLT (x 10^9 cells/L)	137 (97.00/186.00)	78 (10.00/102.00)	**0.000**

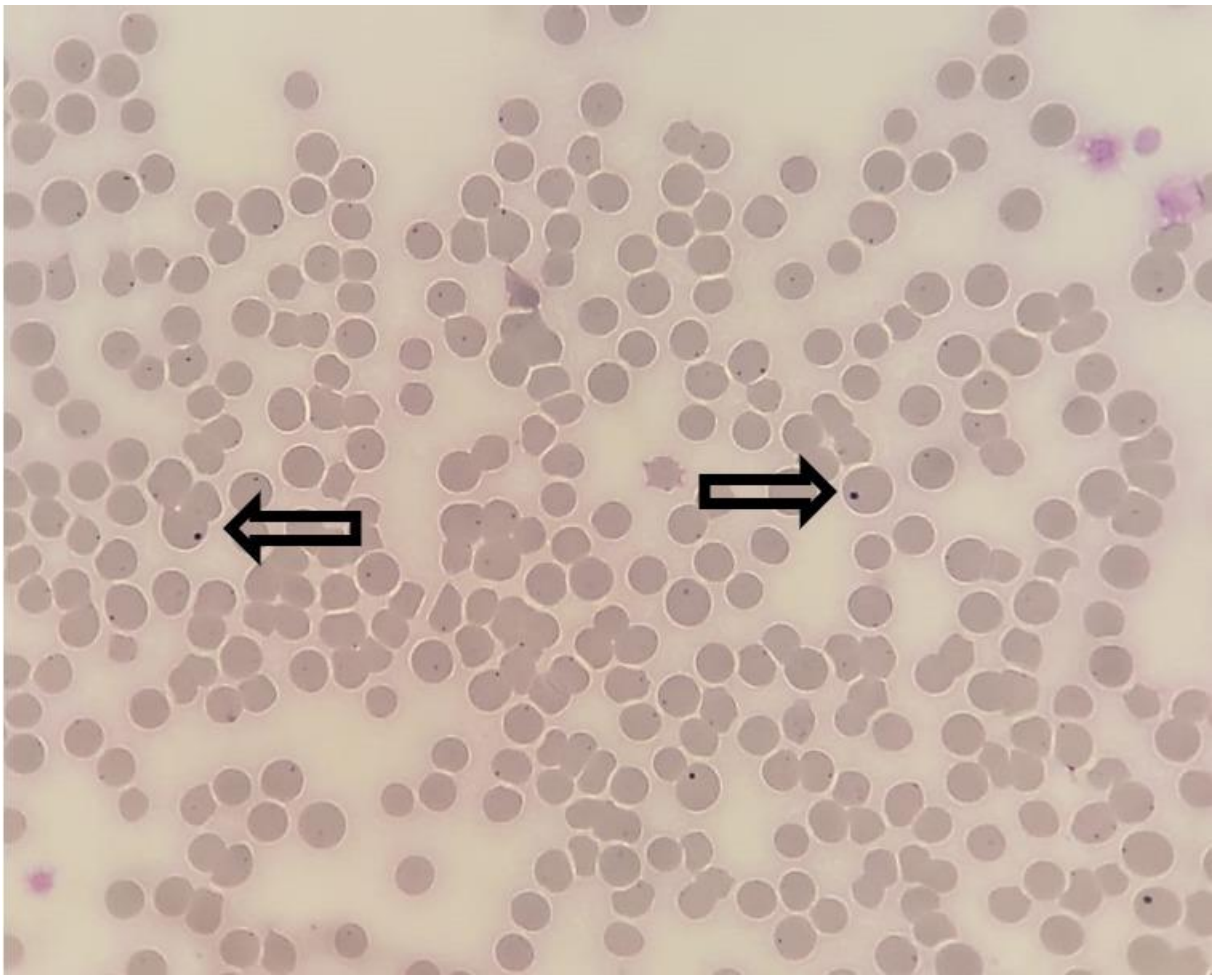
WBC: white blood cell, Lym: lymphocyte, Mon: monocyte, Gra: Granulocyte, RBC: red blood cell, MCV: mean corpuscular volume Hct: haematocrit, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, Hb: Hemoglobin, PLT: platelet

Tablo 3. Sağlıklı ve hemoplasmoz ile enfekte kedilerin biyokimyasal analiz sonuçları (median (min/max).
Table 3. Serum biochemical analysis results of healthy and hemoplasmosis-infected cats (median (min/max).

Parameters median (min/max)	Control Group (n=10)	Hemoplasmosis Group (n=15)	P value
BUN (mg/dL)	18.90 (4.69/23.58)	27.18 (8.70/90.17)	0.055
Cr (mg/dL)	1.27 (0.75/1.50)	1.2 (0.39/5.10)	0.935
Glu (mg/dL)	106.02 (12.10/186.86)	108.51 (14.00/167.00)	0.978
ALT (U/L)	45.60 (24.00/81.29)	62 (21.09/279.99)	0.103
AST (U/L)	21.33 (15.33/87.00)	80.92 (17.48/134.00)	**0.001**
ALP (U/L)	41.73 (24.62/113.00)	33.72 (2.86/156.08)	0.285
AMY (U/L)	1443.98 (904.00/2453.10)	1551.83 (204.77/2898.05)	0.495
Chol (mg/dL)	193.47 (82.00/279.46)	173.76 (86.87/439.42)	0.367
TG (mg/dL)	43.84 (24.00/148.16)	73.41 (44.75/488.00)	**0.012**
CK (U/L)	169.59 (61.97/324.07)	320.56 (149.55/1544.00)	**0.004**
LDH (U/L)	121.69 (84.00/267.40)	437.89 (111.49/2127.13)	**0.000**
GGT (U/L)	3.02 (1.43/3.36)	3.75 (1.00/10.47)	0.160
T.Bil (mg/dL)	0.38 (0.10/1.35)	1.62 (0.18/10.10)	**0.001**
D.Bil (mg/dL)	0.26 (0.10/1.35)	1.18 (0.15/3.40)	**0.001**

Ca (mg/dL)	10.67 (6.12/12.75)	7.98 (6.10/14.10)	**0.026**
P (mg/dL)	4.48 (3.46/8.69)	6.26 (2.55/12.39)	**0.014**
Mg (mg/dL)	1.83 (1.55/2.07)	1.71 (1.10/3.60)	0.165
TP (g/dL)	7.19 (5.90/7.92)	8.6 (4.38/10.90)	**0.004**
Alb (g/dL)	3.63 (2.80/4.12)	2.56 (1.40/3.30)	**0.000**
A:G ratio	1.07 (0.62/1.72)	0.45 (0.20/0.65)	**0.000**

BUN: blood urea nitrogen, Cr: creatinine, Glu: glucose, ALT: alanine transaminase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, AMY: amylase, Chol: cholesterol, TG: triglyceride, CK: creatine kinase, LDH: lactate dehydrogenase, GGT: gamma-glutamyl transferase, T.Bil: total bilirubin, D.Bil: direct bilirubin, Ca: calcium, P: phosphorus, Mg: magnesium, TP: total protein, Alb: albumin, A:G ratio: albumin globulin ratio



Resim 1. Giemsa ile boyanmış kan frotisinde eritrositlerde *Mycoplasma spp.*'nin görünümü. Siyah oklar, kırmızı kan hücrelerinin yüzeyinde bulunan etkenleri göstermektedir (x100).
Figure 1. Appearance of *Mycoplasma spp.* in the erythrocytes on a Giemsa-stained blood smear. The black arrows indicate the organisms positioned on the surface of the red blood cells (x100).

DISCUSSION

In the present study, blood gas, complete blood count, and biochemical analysis parameters were evaluated in cats with hemoplasmosis, and it was determined that metabolic acidosis, anemia, and changes in biochemical parameters occurred in cats with hemoplasmosis. It was evaluated that the A:G ratio should be considered in the diagnosis of hemoplasmosis.

To the authors' knowledge, no study has been found evaluating blood gases in cats with hemoplasmosis. In the present study, blood pH, BE, and HCO_3 levels of cats with hemoplasmosis were significantly lower than those of healthy cats, while lactate concentration were found to be higher ($p< 0.05$). The findings show that metabolic acidosis and hyperlactatemia develop in cats with hemoplasmosis. Studies in anemic patients infected with Plasmodium species have reported that systemic hypoxia due to anemia may lead to an increase in anaerobic glycolysis, higher lactate production, and lactic acidosis (English et al. 1997, Dabadghao et al. 2015). It has also been reported that malaria-infected erythrocytes produce up to 100 times more lactate than uninfected erythrocytes (Vander Jagt et al. 1990, Mehta et al. 2005). In our study, it was evaluated that hyperlactatemia occurring in cats with hemoplasmosis may be associated with decreased tissue perfusion due to anemia and increased lactate production from infected red blood cells.

Although it has been reported that the WBC and granulocyte levels of cats with hemoplasmosis may be normal, high, or low, it is stated that there is an increase in monocyte levels (Kurtdede and Ural 2004, Akkan et al. 2005, Aslan et al. 2010, Sykes 2010, Evans and Gruffydd-Jones 1984, Carney et al. 1993, Saki and Ozer 2011). Erythrophagocytosis by monocytes or macrophages may be observed if blood films are scanned at low magnification (Messick and Harvey 2012). In the present study, the increase in total leukocyte count, granulocytosis, and monocytosis in cats with hemoplasmosis was interpreted as an indicator of active inflammation due

to infection. The most common abnormality in whole blood analysis of cats with hemoplasmosis is regenerative anemia. Regenerative anemia is characterized by the presence of an adequate reticulocyte response for the current degree of anemia (Tasker 2006). Non-regenerative anemia can also be seen in cases where sufficient time has not passed yet for a regenerative response. In addition, the release of uninfected red blood cells from the spleen can cause a rapid increase in packed cell volume seen in some infected cats (Sykes and Tasker 2013). In a study on Van cats (Akkan et al. 2005), normochromic anemia was determined in cats with haemobartonellosis, and it was reported that Hct and Hb levels were low in a *Mycoplasma spp.* positive Persian cat (Senthil et al. 2014). Alan et al. (2022) found that the platelet count (PLT), platelet indices plateletcrit (PCT), RBC, Hct, and Hb levels were lower in infected cats than in healthy animals, and platelet volume (MPV) was higher. In the present study, it was determined that while the RBC, Hct, Hb, and PLT levels of cats with hemoplasmosis were significantly lower, RDW levels were significantly higher than healthy cats. It was determined that regenerative anemia developed in cats with hemoplasmosis, which resulted in similar results to the studies conducted in our research (Kurtdede and Ural 2004, Tasker 2006, Alan et al. 2022).

It has been reported that the main biochemical changes in cats with hemoplasmosis are a moderate increase in ALT and AST enzyme activities due to hepatic hypoxia resulting from anemia and an increase in bilirubin concentrations due to extravascular and intravascular hemolysis (Tasker 2006, Fathi et al. 2009, Saqib et al. 2016, Weingart and Kohn 2015). It has been reported that the development of hepatic lipidosis together with anorexia may contribute to the increase in liver enzyme activities (Harvey 1998). Akkan et al. (2005) reported that there was no significant difference in ALT, AST, and bilirubin levels in their study of Van cats, contrary to the researchers mentioned above. Hypertriglyceridemia has been described in various diseases with hemophagocytosis (Visser et al. 2013). In humans, *P. falciparum* infection is characterized by hypertriglyceridemia and hypocholesterolemia, and hypocholesterolemia has been associated with cholesterol depletion by the malaria parasite, whereas hypertriglyceridemia has been associated with hemolysis of infected red blood cells (Bouyou-Akotet et al. 2014). In the present study, serum AST enzyme activity, bilirubin, and triglyceride concentrations were found to be higher in cats with hemoplasmosis compared to the control group. It was evaluated that the high bilirubin and triglyceride concentrations were caused by intravascular and extravascular hemolysis in these patients, while the increase in AST activity was interpreted to be due to hepatic lipidosis resulting from anemia-induced hepatic hypoxia and anorexia. (Harvey 1998, Fathi et al. 2009, Weingart and Kohn 2015, Saqib et al. 2016)

CK and LDH are intracellular enzymes widely used in the detection of tissue damage (Kristjansson et al. 2016). CK is an enzyme that catalyzes the ATP-dependent phosphorylation of creatine and is important for energy buffering in tissues with variable energy demands, particularly skeletal and cardiac muscle (Wallimann et al. 1992). LDH is a widely distributed enzyme in the cells of various living systems where it is involved in carbohydrate metabolism that catalyzes the interconversion of lactate and pyruvate with NAD^+. LDH levels in blood serum are increased in various hematological and neoplastic disorders as well as heart, liver, skeletal muscle, and kidney diseases (Klein et al. 2020). LDH together with AST have higher activity in erythrocytes compared to plasma, and its levels increase in vivo or in vitro hemolysis. AST, ALT, LDH, and CK levels may increase in hemolysis cases (Terlizzi 2012). While elevated CK and LDH levels in cattle with babesiosis are associated with anoxia and muscle damage as a result of perfusion disorder (Wright et al. 1981), it has been reported that hemolysis is effective in addition to muscle damage in plasmodium-infected humans (Garba and Ubom 2005). Akkan et al. (2005) reported that there was no significant difference in CK enzyme activity in cats with hemoplasmosis compared to healthy cats. In the present study, LDH and CK levels were found to be higher in cats with hemoplasmosis compared to healthy cats. It was evaluated that high LDH and CK levels in our study may be associated with hemolysis rather than muscle damage (Garba and Ubom 2005, Terlizzi 2012).

When the mineral profile was evaluated in the present study, the phosphorus levels of the cats with hemoplasmosis were higher than the healthy cats, while the calcium levels were found to be lower. While high phosphorus levels in cats with hemoplasmosis were considered to be associated with intravascular hemolysis, low calcium levels were interpreted as a result of decreased albumin concentrations and decreased calcium binding to albumin in infected cats (Martin et al. 2015, Sharp et al. 2009).

Changes in albumin, total protein, and A:G ratio in infectious diseases are generally associated with the inflammatory response that develops during the progress of the disease. Hyperglobulinemia and hypoalbuminemia have been identified in studies in cats with hemoplasmosis (Kurtdede and Ural 2004, Weingart and Kohn 2015). In the present study, it was determined that while TP levels of cats with Hemoplasmosis were significantly higher compared to healthy cats, Alb and A:G ratios were lower. Although the findings were consistent with the findings of other investigators, it was remarkable that the A:G ratio was <0.6 in infected cats, except for one case. Hyperglobulinemia and low A:G ratio have been reported to be an important diagnostic

parameter in cats with FIP (Paltrinieri et al. 2002, Addie et al. 2009, Pedersen et al. 2009, Riemer et al. 2016). A:G ratio less than 0.6 has been reported to be an important diagnostic parameter in patients infected with FIP (Hirschberger et al. 1995). The low A:G ratio in cats with hemobartonellosis in our study is similar to the studies in cats with FIP (Paltrinieri et al. 2002, Addie et al. 2009, Pedersen et al. 2009, Riemer et al. 2016). Therefore, we think that hemoplasmosis should be considered in addition to FIP in cats with a low A:G ratio.

CONCLUSION

In this study, the small number of animals and the lack of molecular confirmation of hemoplasmosis were important limiting factors. Despite limitations, our findings show that there are significant changes in cats with hemoplasmosis, such as metabolic acidosis, hyperlactatemia, anemia, hypertriglyceridemia, hypoalbuminemia, and hyperbilirubinemia. In addition, it was concluded that low A:G ratio should be considered in the diagnosis of cats with hemoplasmosis and should be evaluated together with other diagnostic test results.

Conflict of Interest: The authors declare that there is no actual, potential or perceived conflict of interest for this article.

Authorship Contributions: Mİ:%30, MKD:%20, SSİ: %20, CC:%15, MCK:%15

Ethical Approval: This study was approved by Selcuk University, Institutional Ethics Committee of the Faculty of Veterinary Medicine (2022/45).

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RESEARCH ARTICLE

Determination of *In vitro* Antioxidant Activities and Macro and Micro Elements Level in Different Extracts of *Cynara Scolymus* L. leaf

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ABSTRACT

Globe Artichoke (*Cynara scolymus* L.) belonging to the family of Astericaea, has antioxidant, hepatoprotective and hypoglycemic activities, its leaves have traditionally been used for diuretic and choleretic purposes. Therefore the main goal of this study is to determine the total phenolic content of artichoke leaf and its extracts with methanol, ethyl acetate and n- hexane, some *in vitro* antioxidant activities, selected macro (Na, K, Ca, Mg, P) and micro (Zn, Cu, Mn, Cr, Se, I) elements' levels. Accordingly the total phenolic content values for the methanol, ethyl acetate and N-hexane extracts found 5,375 mg, 0,917 mg, 0,167 mg Gallic acid (GAE)/g respectively. Methanol extract showed highest DPPH free radical scavenging activity (87,73%), ethyl acetate extract possessed the highest Superoxide radical scavenging activity (SRSA) (49,02 %) whereas N-hexane extract contained high level metal chelating ability (289,32µM Fe). In terms of macro and micro elements (except I and Cr levels), the highest concentrations are recorded in its leaves, which are considered as a natural mineral source. Accordingly, it is evaluated that artichoke leaves provide a potential natural sources of K and Zn, while methanol and N-hexane extracts are good sources of P and Zn.

Keywords: Antioxidant activity, artichoke, *Cynara scolymus* L., leafextracts, minerals

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Cynara scolymus L. Yaprağının Farklı Ekstraktlarda *In vitro* Aktioksidan Aktivitelerinin ve Makro ve Mikro Element Seviyelerinin Belirlenmesi

ÖZ

Papatyagiller familyasına ait olan Küre enginarın (*Cynara scolymus* L.) antioksidan, hepatoprotektif ve hipoglisemik etkilere sahiptir, yaprakları geleneksel olarak idrar söktürücü ve koleretik amaçlarla kullanılmaktadır. Bu nedenle bu çalışmanın temel amacı enginar yaprağının ve yaprağın metanollü, etil asetatlı ve n-hekzanlı ekstraktlarının toplam fenolik içeriğini bazı *in vitro* antioksidan aktiviteleri, seçilmiş makro (Na, K, Ca, Mg, P) ve mikro (Zn, Cu, Mn, Cr, Se, I) element düzeylerini, belirlemektir. Toplam fenolik içerik değerlerine göre metanol, etil asetatlı ve n-hekzan ekstraktları sırasıyla 5,375 mg, 0,917 mg, 0,167 mg Gallik asit (GAE)/g bulunmuştur. Metanol ekstraktı en yüksek DPPH serbest radikal süpürme aktivitesi (%87,73) gösterirken, etil asetat ekstraktı en yüksek süperoksit radikal süpürme aktivitesine (%49,02) sahip iken n-hekzan ekstraktı yüksek seviye metal şelatlama kapasitesi (289,32 µM Fe) içermektedir. Makro ve mikro elementler açısından (I ve Cr seviyeleri hariç) en yüksek konsantrasyonlar doğal mineral kaynağı olarak kabul edilen yapraklarında kaydedilmiştir. Buna göre enginar yapraklarının potansiyel bir doğal K ve Zn kaynağı sağladığı, metanol ve n-hekzan ekstraktlarının ise iyi P ve Zn kaynağı olduğu değerlendirilmektedir.

Anahtar Kelimeler: Antioksidan aktivite, *Cynara scolymus L.*, enginar, mineraller, yaprak ekstraktları

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INTRODUCTION

Artichoke (*Cynara scolymus* L.) is a plant belonging to the Asteraceae family that grows naturally in Mediterranean region countries and is a cultured variety of wild artichoke (*Cynara cardunculus* L.). Artichoke, whose head, flower and leaves can be eaten, is traditionally used in hepatobiliary diseases, indigestion (dyspepsia), obesity and hyperlipidemia. *In vitro* and *in vivo* studies have demonstrated that artichoke and its leaves have antimicrobial (Vamanu et al. 2011), antioxidant (Ben Salem et al. 2017a, Biel et al. 2020), hypocholesterolemic (Mocelin et al. 2016, Öcal et al. 2019), hypoglycemic (Ben Salem et al. 2017b), anticancer (Nadova et al. 2008, Sokkar et al. 2020), antifungal (Ben Salem at al.2017b), hepatoprotective (Colak et al. 2016) and choleretic Kraft (1997) effects. Artichoke head and leaves mainly contain phenolic compounds such as chologenic acid and cynarin, flavonoids such as luteolin and apigenin, polyphenolic compounds, carotenoids, inulin, fiber, vitamin C, E and minerals(Ben Salem et al. 2015, Biel et al. 2020, Salekzamani et al. 2019). Artichoke, which is a natural source of antioxidants in this respect, has been used in human and animal nutrition since ancient times, and at the same time, artichoke and leaf extracts are used in foods both as flavoring and to prolong the shelf life of foods by preventing lipid and protein oxidation(Biel et al. 2020). It is known that reactive oxygen species (ROS) such as O_2^-, $^*OH$, H_2O_2 which are released as aresult of ongoing metabolic reactions in the organism, induce/lead to oxidative stress that causes endotoxic shock, neurogenerative disorders and cancer (Ben Salem et al. 2015, Yazar et al. 2004).It has been shown in many studies that besides the cellular antioxidant mechanism, flavanoids, phenolic compounds as catechins, anthocynanins and the other minerals and vitamins that are naturally found in the structure of many plants such as artichoke, support the antioxidant defense system against oxidative stress (Avci et al. 2021, Ben Salem et al. 2017a, Biel et al. 2020, Erdogan et al. 2020). The presence of micro elements such as Mn, Cu, Zn and Se in plants as cofactors in the structure of enzymes such as superoxide dismutase and glutathione peroxidase in the antioxidant defense system (Sobirova and Murodova 2021, Tokalioglu 2012) is also important for the prevention of oxidative stress.

With the current increase in the use and consumption of raw plant materials, it is very important to monitor contaminants present in plants and set maximum standards for their concentrations in order to ensure safe use (Biel et al. 2020). Because of the bitter taste of artichoke leaves, extracts and tinctures, prepared with water ,are used rather than infusions, so quantitative determination of antioxidant activity and mineral concentrations in medicinal plants extracts which are prepared in different solvent are important in terms of determining their dosages and pharmacological effectiveness in the treatment of various diseases. Therefore, in this study, the total phenolic substances, *in vitro* antioxidant activities (DPPH radical scavenging activities, Superoxide radical scavenging activity, and metal chelating ability) and macro and micro elements levels determined.

MATERIALS AND METHODS

Plant Material

Leaves of artichoke (*Cynara scolymus* L.) were bought from an organic market in Ankara province, Turkey in May 2021. The voucher specimen of the plant was authenticated by Prof. Dr. EsraAkkol from Gazi University, Department of Pharmacognosy, Faculty of Pharmacy, Ankara, Turkey and the specimen was deposited in the Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Total Phenolic Concentration (TPC)

The total phenolic substances of artichoke leaf extracts were measured using Folin-Ciocalteu's phenol reagent (FCR) according to procedure of Singleton and Rossi (1965). The principle of this method is based on the formation of a blue colored compound as a result of the phenolic substances reducing FCR.

The Gallic acid was used as standard solution. 0.5 mL of test extracts solution was mixed with 0.5 mL FCR and incubated for 3 minutes. Then, 2% Na_2CO_3 was added and was stored at room temperature for 2 h. After incubated, absorbance of reaction mixture was measured at 760 nm against blank as distilled water. The result was expressed as mg of Gallic acid equivalents (GAE)/g of extract by using an equation that was obtained from standard Gallic acid graph. All the experiment was conducted in three replicates.

Determination of DPPH Radical Scavenging Activity

The DPPH removal activity of artichoke leaf extracts was determined by using the method of Blois (1958). The method is based on the principle of removing DPPH, which is a stable free radical and has a dark purple color. When the DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow. Briefly, DPPH (2 mL, 0,1 mM) was added to 0.5 mL artichoke leaf extracts. Each mixture was kept in the dark for 30 min and the absorbance was measured at 517 nm against a blank ethanol. The solution without any extract and with DPPH and ethanol was used as control. The experiment was replicated in three independent assays. Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula:

DPPH radical scavenging activity (%)=(A blank - A sample/ A blank) × 100

Determination of Superoxide Radical Scavenging Activity

The superoxide radical removal activity is based on the principle that the superoxide radical produced by the NADH (nicotinamide adenine dinucleotide)/PMS (phenazinemethosulfate)/O_2complex reduces nitro blue tetrazolium (NBT) from yellow to purple-colored formazone (Nishimiki et al. 1972). The decrease of the absorbance values indicates consumption of superoxide radical anions.

According to method, 0.5 mL NBT (156μM) and NADH (468 μM) in the sodium phosphate buffer (20 mM, pH 7.4) were added to different concentrations of extract solutions (0.5 mL, 100-250-500-1000 µg/mL) in phosphate buffer. The reaction initiated by adding PMS (50µL, 60 µM) to the mixture and incubated at room temperature for 5 minutes. Then, the absorbance was measured at 560 nm against the corresponding distilled water as control. Inhibition of superoxide radical in percentage was calculated by the formula:

Inhibition % = (A control - A sample/ A control) × 100

Determination of Metal Binding Activity

Iron (II) ions of chelating activity of artichoke leaf extracts was performed according to the Carter (1971) and Dinis et al. (1994).

The principle of this method is based on the inhibition of the formationof ferrous iron-ferrozine complex of chelating agents in the test tube. The decreasing of red colour was determined by the decrease absorbance of the ferrous iron-ferrozine complex at 562 nm.

1 mL sample, 3.7 mL deionized water and 100 µL $FeCl_2$ solutions (2 mM, dissolved in distilled water) were then mixed thoroughly and incubated for 30 min. Then ferrozine (5 mM, 0.1 mL) was added to the mixture. After 10 min, absorbances of the test tubes and EDTA as standarts (50-250 µg/mL)were measured spectrophotometrically at 562 nm. The blank contains only distilled water except that $FeCl_2$ and ferrozine. The metal binding activity was calculated with this formula:

Metal binding (µM Fe) =[(A sample-A blank)/(A standart-A blank)]x 100

Mineral Composition

The elemental composition of mineral substances of the extracts have been analyzed using the ICP-MS device (inductively coupled plasma mass spectrometer) Perkin Elmer NexION 300. The ICP-MS operating conditions are shown in Table 1.

Table 1. ICP-MS Instrumental conditions

Auxiliary gasflow rate (L/min)	1.4
Plasma (Ar) gas flow rate (L/min)	18
Analog HV (V)	-1975
Pulse HV (V)	950
RF power output (W)	1600
Sample uptake rate (µL/min)	300
Dwell time (sec)	50
Sample run time (min/sample)	1,5
Nember of replicates	3

Dry plant powdered sample and its extracts were digested by microwave digestion system. 0.25 g sample extract was taken in a PTFE teflon vessel. After adding 5 ml of conc HNO_3 and 5 ml distilled water, the vessel was kept standing for a while at room temperature. Decomposition of the samples was carried out in a microwave system (CEM MARS, Italy, A five-step programme (see Table 2) was applied to the samples. There were no undissolved parts in vessels.

Table 2. Parameters for microwave digestion

Step	Effect,W	Time,Min
Step 1	250	1
Step 2	0	1
Step 3	250	5
Step 4	400	5
Step 5	650	5

After the digestion process the solution was filtered with a membrane filter (pore size 0,45 μm) which was subjected as the analysis solution to determine the total concentrations of diverse elements in globe artichoke leaf and its extracts by ICP-MS. The resulting solutions were quantitatively transferred into a volumetric flask and made up to 25 mL with doubled distilled water then used for direct injection into the spray chamber of device or diluted with doubled distilled water again. The internal standard elements (Ge, In and Re) were added for correcting matrix effect. Calibration was performed by external standards where the method of standard additions was used.

Statistical analysis

The data obtained in the research were analyzed with the SPSS 22.0 for Windows program. The statistical differences among groups were analysed through One way ANOVA. Least significant difference test (LSD) was used as a posthoc test. Statistical significance of all data was determined as $P < 0.05$.

RESULTS

Antioxidant activity results of artichoke leaves extracts in methanol, ethyl acetate and N-hexane solvents are showed in Table 3.

In this study, the total phenolic content values for the methanol, ethyl acetate and N-hexane extracts found 5,375 mg, 0,917 mg, 0,167 mg Gallic acid (GAE)/g respectively (Fig1).

Methanol extract showed highest DPPH free radical scavenging activity (87,73%) (Fig 2, ethyl acetate extract possessed the highest Superoxide radical scavenging activity (SRSA) (49,02 %) (Fig 3) whereas ethyl acetate extract contained high level metal chelating ability (289,32μM Fe) (Fig 4).

Table 3. Total phenolic substances concentration and invitro antioxidant activities of the extracts obtained from artichoke leaves.

Extracts	**Total phenolic substances (mgGAE/g extract)**	**DPPH radical removal activity (%)**	**Superoxide radical removal activity (%)**	**Metal binding activity(μM Fe)**
Methanol	5,735	87,73	44,15	133,01
Ethyl acetate	0,917	44,15	49,02	151,46
N-hexane	0,167	14,53	30,22	289,32

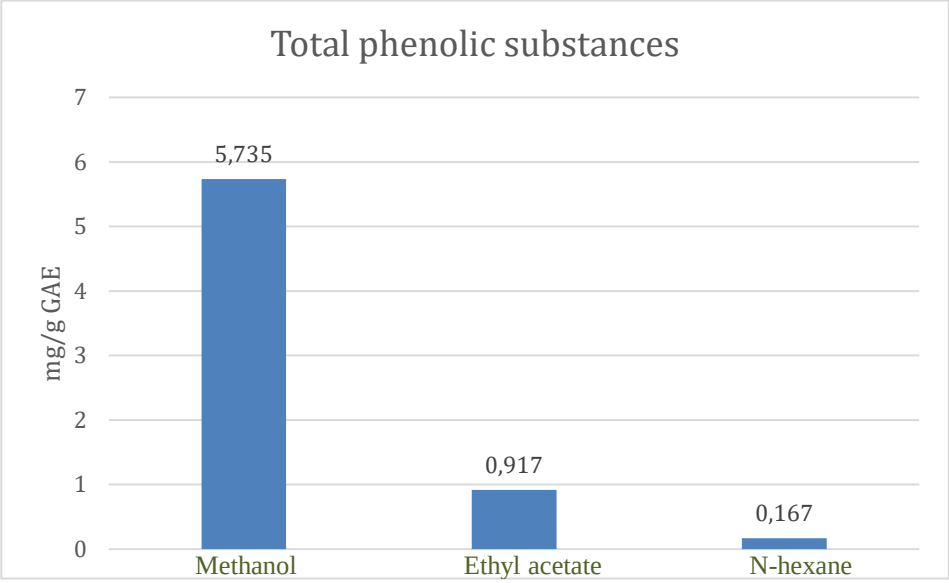

Figure 1: Total phenolic substances concentration (mg GAE/g) of the extracts obtained from artichoke leaves. Result represents means of triplicates of different concentrations analyzed.

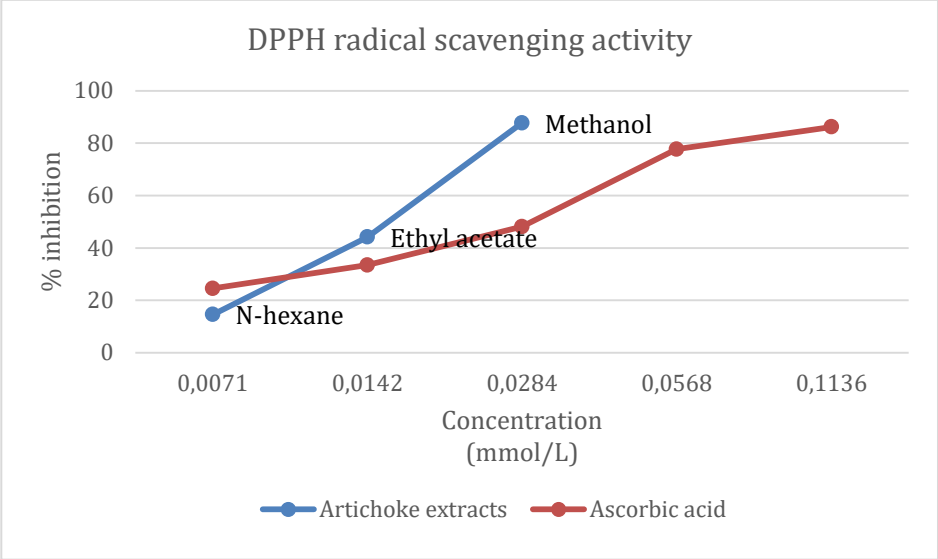

Figure 2: DPPH radical scavenging activities of the extracts of artichoke leaves and ascorbic acid. Result represents means of triplicates of different concentrations analyzed.

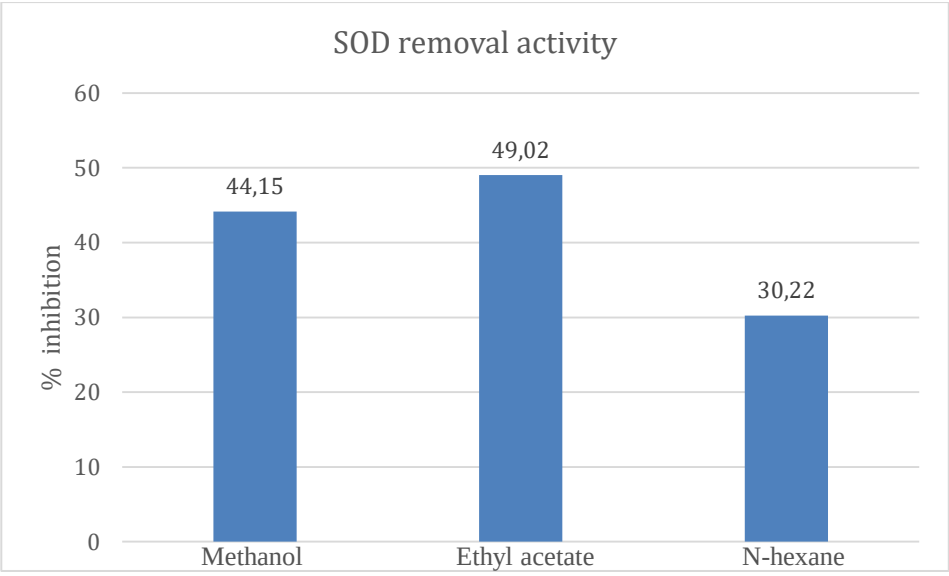

Figure 3: Superoxide radical removal activity (inhibition %) of the extracts obtained from artichoke leaves. Result represents means of triplicates of different concentrations analyzed.

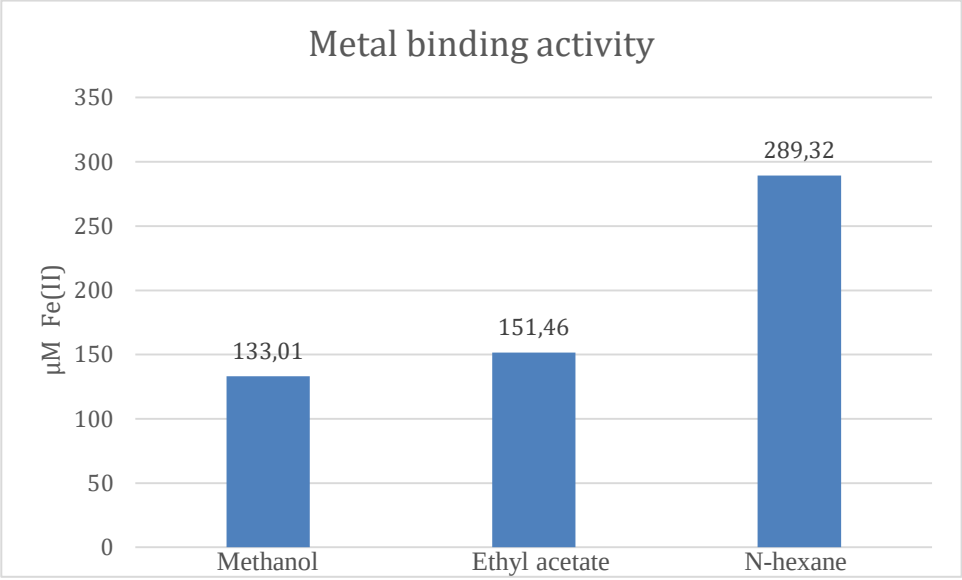

Figure 4: Metal binding activity of iron (II) ions(µM) of the extracts obtained from artichoke leaves. Result represents means of triplicates of different concentrations analyzed.

The macro element levels are statistically higher in the artichoke leaf compared to all other extracts, with the highest K and the lowest Na levels.
Among the extracts, it is seen that the methanol extract's macro minerals have the lowest level compared to the other extracts and P concentration (127.03±2.98 mg/g) is highest among macro minerals. As Mg is highest in ethyl acetate extract, Na, K and Ca levels are highest compared to other extracts. While P is found at the highest level in the N-hexane extract, it is determined as the extract containing the most macro minerals (Table 4).

Table 4. Levels of minerals in powdered leaf and organic solvents.

Macro elements(mg/100g)	**Artichoke Leaf X̄±S**	**Methanol X̄±S**	**Ethyl acetate X̄±S**	**N-hexane X̄±S**	**P**
Na (Sodium)	84.58±3.09^{a}	3.73±0.11^{d}	14.00±0.20^{b}	9.83±0.14^{c}	***
K (Potassium)	238.95±5.84^{a}	4.91±0.20^{d}	36.09±0.20^{b}	15.24±025^{c}	***
Ca (Calcium)	125.00±2.58^{a}	16.24±0.83^{d}	57.91±0.61^{b}	23.44±0.32^{c}	***
Mg (Magnesium)	175.68±18.60^{a}	33.58±0.44^{c}	99.17±1.36^{b}	91.88±0.77^{b}	***
P (Phosphor)	189.14±3.67^{a}	127.03±2.98^{b}	47.3±0.88^{c}	186.86±0.96^{a}	***
Micro elements (mg/100g)					
Fe (Iron)	5.25±0.45^{a}	1.57±0.91^{c}	Not detected	2.22±0.41^{b}	***
Zn (Zinc)	5.71±0.23^{a}	1.78±0.68^{c}	0.44±0.18^{d}	3.66±0.46^{b}	***
Cu (Copper)	1.52±0.30^{a}	0.92±0.32^{b}	0.72±0.32^{c}	0.28±0.08^{d}	***
Mn (Mangan)	2.18± 0.36^{a}	0.19±0.03^{d}	0.70±0.20^{c}	2.11±0.13^{b}	***
Cr (Chromium)	0.12±0.03^{a}	0.05±0.01^{c}	0.08± 0.00^{b}	0.11±0.08^{a}	***
Se (Selenium)	0.12±0.03^{c}	0.02±0.00^{b}	0.01±0.02^{ab}	0.006±0.00^{a}	*
I (Iodine)	0.04±0.00^{d}	0.03±0.01^{c}	0.06±0.00^{b}	0.21±0.01^{a}	*

X̄±S: mean ±std deviation, a,b,c,d: Different letters in the same row represent the statistically significant difference between the mean values ($p< 0,05$). *: $p < 0,05$ ***: $p < 0,001$

When all groups were examined in terms of microelements the microelements in the artichoke leaf (except I and Cr levels) were statistically at the highest level compared to all other extracts.
Accordingly, Zn and Fe levels were the highest in artichoke leaves and the lowest I was found. Fe and Zn were found to be highest in methanol extract, while ethyl acetate extract contained high levels of Cu and Mn, but it was determined as the extract containing the lowest level of micro minerals. While Fe, Zn and Mn levels were determined the highest in the N-hexane extract, it was also seen that it contained the micro minerals at the highest level compared to the other extracts (Table 2).

DISCUSSION

The interest in artichoke, a natural antioxidant, as well as its extracts is due to their versatile therapeutic effect (Biel et al. 2020). Oxidative stress is characterized by insufficient enzymatic (SOD, CAT, GPx and GSH) and non-enzymatic (Vitamin E, Vitamin C, carotenoids, flavonoids, polyphenols and others) antioxidant capacity due to excessive increase in reactive oxygen species (ROS), so shifting the balance towards oxidants (Salekzamani et al. 2019). While increasing oxidative stress has been reported to play a role in many disease such as aging, diabetes mellitus, hypertension, cardiovascular diseases, cancer, Parkinson's and Alzheimer's diseases (Valko et al. 2007), plants with strong antioxidant activity such as artichoke contain polyphenols, vitamins and minerals have a role in reducing the risk of disease that develop due to oxidative stress by inhibiting ROS production and reducing radicals (Salekzamani et al. 2019). Indeed Demir and Agaoglu (2021) have reported in many studies caffeoylquinic acid derivatives, luteolin and apigenin glucosides found in *C. scolymus* L. showed strong antioxidant properties. Phenolic compounds in plants are natural sources that show their antioxidant activities by binding free radicals or chelating with metals in proportion to the number and structure of –OH groups in the phenol ring (Falowo et al. 2014).

In addition, phenolic compounds have the ability to delay, slow or prevent oxidation at low concentrations and remain in a stable form when converted to free radicals (Kalogianni et al. 2020).

In studies carried out, Wand et al. (2003) report the total phenolic content (TPC) in Green Globe leaves as 8760-9561 mg CAE (Chlorogenic acid equivalents)/100 g DM and Salata and Gruszecki (2010) report 3168 mg caffeic acid equivalent/100 g DM. In our study the total phenolic content values, based on standard of Gallic acid, In the methanol, ethyl acetate and N-hexane extracts found 5,375 mg, 0,917 mg, 0,167 mg Gallic acid (GAE)/g, respectively. So, the reason why a value higher than the one reported by Biel et al. (2020) as 2795 mg CAE/100 g DM was found in the methanol extract of the leaf and may be due to the different standards used in the tests. Although not found enough studies on the total phenolic content of ethyl acetate and N-hexane extracts, it is seen that the highest phenolic content is in the methanol extract.

Considering the versatile mechanisms of antioxidant compounds, the need for various types of measurements to determine their activities has arisen (Biel et al. 2020). DPPH, widely used in the determination of the free radical scavenging activities of antioxidants, is a stable free radical (DPPH•) (Chen et al. 2007, Wojdylo et al. 2007), and when this purple-colored substance interacts with antioxidants, it transforms into a yellow-colored reduced form of DPPH (DPPH-H). Therefore, the lower the measured absorbance, the higher the free radical scavenging activity of the antioxidant (Chen et al. 2007).

Accordingly, DPPH radical removal activity being the highest in methanol extract (87.73% inhibition) in this study can be explained by the fact that the total amount of phenolic substances in this extract is higher than the others. These results are also compatible with the observation that the antioxidant properties of plant extracts originate in polyphenols, which donate hydrogen atoms or electrons, have the ability to neutralize free radicals (such as DPPH) (Sahoo et al. 2013). Thus, it is obvious that this activity is weakened due to the reduction in total phenolic content in ethyl acetate and N-hexane extracts. Biel et al. (2020) reported this activity in artichoke methanol extract at approximately 44% inhibition according to the trolox standard and the difference in the results of this study may be due to the evaluation based on the ascorbic acid standard.

When the solvents used in the extracts in the study are evaluated in terms of their polarity, the most non-polar solvent to the least is listed as n-hexane>ethyl acetate>methanol. Accordingly, it has been reported that the solvents used in the extraction affect the radical scavenging activity and this activity is higher in polar extracts (Hayouni et al. 2007,Ozcan et al. 2007). In their study, Erdogan et al. (2020) reported that the methanolic extract of olive leaf (*Olea europaea L.*) is more effective in terms of total phenolic substance content, the ferric thiocyanate method in the linoleic acid system, and reducing capacity with antioxidant activity compared to other extracts (ethyl acetate and n-hexane). As a matter of fact, Miliauskas et al. (2004) reported that the DPPH radical scavenging activity among plant extracts is the most effective in methanol extract compared to other extracts (acetone and ethyl acetate), while Shon et al. (2003) reported that hot water and methanol extracts are better than butanol, ethyl acetate, and chloroform extracts. Avci et al. (2021), on the other hand, stated that the different results found *in vitro* antioxidant activities in black cumin (*Nigella sativa* L.) essential oil obtained by water distillation may be due to the differences in the solvent and extraction methods used. Accordingly, apart from these observations, different species of the same plant, different agricultural practices, geographical conditions (daylight, climate), harvest time, and storage conditions are the factors affecting the phenolic content of plants (Heimler et al. 2007) —the reason for the differences in our results can be explained by considering these factors.

SOD, which forms the first line of defense against the harmful effects of superoxide radicals released in different parts of the cell, is one of the metalloenzymes that converts the superoxide radical into H_2O_2 and O_2 (Halliwell et al. 2000). Generally,

SOD has three different isoenzymes in higher plants, namely Mn-SOD, Fe-SOD, and CuZn-SOD Fridovlich (1986), which are found in different organelles of the cell such as mitochondria, peroxisome, and chloroplasts (Palma et al 2006). In this study, the superoxide radical removal activity was listed as ethyl acetate> methanol> N-hexane, and no other study was found on the subject. In a study using similar solvents, Erdogan et al. (2020) reported that the superoxide radical removal activity in olive leaf extracts was determined as ethyl acetate> methanol> N-hexane, which is similar in terms of extracts in the study.

In this study, the metal binding activity was the highest in the N-hexane extract (289.32 μM Fe), and the others were listed as ethyl acetate>methanol — there was not enough study on this subject as well. In studies on lipid peroxidation, Fe is used as an ion catalyst. Fe, a transition metal, acts as a catalyzer of lipid peroxidation due to its biological importance and ability to react directly with oxygen or with lipid peroxides promoting the reaction to form species that can initiate peroxidative events (Dinis et al. 1994).

The antioxidant defense system is generally based on both dietary vitamins and minerals and the presence of essential amino acids required for the synthesis of antioxidant proteins, such as glutathione and albumin (Evans and Halliwell, 2001). It is known that minerals in the structure of tissues are involved in the regulation of acid-base balance and osmotic pressure, the functioning of hormones and enzymes, transcription, and energy metabolism (Connie 2011). As a matter of fact, Ca and K are essential elements for plants, and Mg is a chlorophyll component in plants as well as a cofactor of enzymes in reactions where ATP is used (Evans and Halliwell 2001, Silvaet al. 2016). In this study, the macro element levels were listed as K > P > Mg > Ca > Na in the artichoke leaf, and the highest K (238.95 mg/100g DM) and the lowest Na (845.8 mg/100g DM) levels were detected. At the same time, it is noteworthy that the macro element levels are higher in the artichoke leaf than in all other extracts, and the Na mineral is at the lowest (37.3-845.8 mg/100g DM) level in all the extracts. In this study, the macro minerals in the methanol extract were lined at the level of P > Mg > Ca > K > Na, and the high P level was found as considerably lower than the values of 890-980 mg P/100g DM in the methanol extract of the leaf and 414 mg P/100g DM in the artichoke leaf (127.0 mg/100g DM) as reported by (Biel et al. 2020, Colla et al. 2013) respectively. Although there is no study comparing different extracts in terms of minerals, Biel et al. (2020) reported the macro element levels in the methanol extract of artichoke leaves in order of K > P > Ca > Mg > Na, and this high level in K (506.3 mg/100g DM) is due to the fact that it is the most absorbed mineral during the growth cycle of the plant.This observation was found to be consistent with the finding of this study that the most abundant mineral in the artichoke leaf was K (238.9 mg/100g DM), which is much higher than the K found in the methanol extract (4.9 mg/100g DM).

The macro minerals were found as Mg > Ca > P > K > Na in the ethyl acetate extract, and as P > Mg > Ca > K > Na in the N-hexane extract. Although there is no comparable study for these two extracts, in this study, Ca (125.0-16.24 mg/100g DM) and Mg (175.6-33.5 mg/100g DM) valuesfound in artichoke leaves and all its extracts were lower than the values reported by Biel et al. (2020) and Orlovskaya et al. (2007) in leaf methanol extract and leaf. According to the results of this study, while artichoke leaf is a source of K, methanol and n-hexane extracts can be good sources of P. In a study on various medicinal plants (*Cynara scolymus* L., Harpagophytumprocumbens D.C., Maytenusilifolia (March) ex Reiss.), including artichokes, Ca, K, Mg, and Na levels were found to be high, according to which such plants can be a natural source in the treatment of diseases related to the deficiency of these elements (Tannus et al. 2021).

Trace elements not only play an important role in the formation of active chemical ingredients in medicinal plants but also are responsible for their medicinal and toxic effects (Tokalioglu 2012). While trace elements act as cofactors of enzymes for their conversion to products in enzymatic reactions, some of them are responsible for taking or giving electrons in redox reactions which are important in the production and use of metabolic energy (Al-Fartusie and Mohssan 2017). Zn, which is a cofactor of more than 200 enzymes in the central nervous system, immune, skeletal and reproductive systems, is included in the structure of SOD (CuZnSOD) together with Cu. Cu is also required for ceruloplasmin, which transfers iron to transferrin, and deficiencies in Cu affect the activity of SOD more than Zn (Roohani et al. 2013).While Cu acts as a cofactor in the structure of many enzymes such as cytochrome c oxidase, tyrosinase, and dopamine beta-hydroxylase, contrarily, excess Cu causes peroxidative damage in membrane lipids. Indeed, in the presence of reducing agents such as superoxide radical, ascorbic acid, and glutation, Cu^{+2} is reduced to Cu^{+1}, which can catalyze the formation of hydroxyl radicals from hydrogen peroxide, and these radicals cause the oxidation of biological molecules (Gaetke and Chow 2003). Zn prevents the peroxidation of membrane lipids and has a stabilizing effect on membranes, possibly by replacing bound transition metal ions (Evans and Halliwell 2001). In this study, when microelements were examined in terms of all groups, the highest level of microelements was determined in artichoke leaf compared to all other extracts (except I and Cr levels). When the microelements in the artichoke leaf were evaluated, the order of Zn > Fe >Mn> Cu > Cr =Se > I was seen, with the highest level being Zn (5.71 mg/100g DM) and the lowest level I (0.004 mg/100g DM). Colla et al. (2013) reported the Zn

level (2.07-2.35 mg/100 g DM) in artichoke leaves, and in this study, Zn levels were found higher than in the relevant report.

Biel et al. (2020) reported the micro-minerals in the methanol extract of the leaf in the order of Zn > Fe > Cr >Mn, and the Zn and Fe levels are 2.10 mg/100g DM and 1.6 mg/100g DM. In the same study, it was stated that Cd, Pb, and Ni levels could not be determined. In this study, the ordering of micro minerals in Methanol as Zn > Fe > Cu >Mn> Cr > Se > I was found to be consistent with the reports, but the Zn level (1.78 mg/100g DM) was found to be lower than the previous reports yet consistent with the Fe level. Zn > Fe >Mn> Cu > Cr > I > Se sequence was seen in the N-hexane extract, and the Zn level was found higher at 3.66 mg /100g DM. In the study, while Fe remained below the detection level in the ethyl acetate extract, the minerals were listed as Cu >Mn> Zn >Cr > I > Se. When evaluated in terms of extracts, the ethyl acetate was the extract containing the lowest level of micro minerals and Se was found at the lowest level in all the extracts (Table 2).

The Mn level was found to be between 2.18-0.19 mg/100g DM in all groups, and Mn, which is the cofactor of the enzymes involved in the biosynthesis of fatty acids and cholesterol, is an essential element in the structure of mitochondrial Mn-SOD (Evans and Halliwell, 2001). While this element helps regulate oxidative phosphorylation, mucopolysaccharide and cholesterol metabolism, and the urea cycle, the excessive amounts are toxic to the organism and cause neurological effects (Rehnberg et al. 1982). Cr is the most important factor that increases insulin activity in the organism and enables the use of glucose by activating the phosphoglucomutase enzyme. The literature suggests that its use is especially beneficial against type-2 diabetes and insulin resistance. And in its deficiency, glucose tolerance decreases, which can lead to cardiovascular diseases and diabetes (Hua et al 2012, Tokalioglu 2012). In this study, Cr levels were found to be between 0.12-0.05 mg/100gDM in all groups and were higher than the reports of Biel et al. (2020) in leaf methanol extract and Tannus et al. (2021) in artichoke.

Se, which presents itself in the structure of glutathione peroxidase in the organism, also acts as an antioxidant against different abiotic stresses in plants, including salinity, drought, overtemperature, and toxic metals/metalloids stresses (Hasanuzzaman et al.· 2020). In this study, Se levels were found to be between 0.12-0.006 mg/100g DM in all groups, and it was found to be lower than the 0.37 mg/100g DM level reported by Tannus et al. (2021) in artichokes. Also, I levels were found to be between 0.21-0.03 mg/100g DM in all groups, and no comparable study was found for artichoke.

CONCLUSION

The results of this study show that the herbal extraction methods and the solvents affect the total phenolic substance content, antioxidant activity, and mineral levels. While methanol and ethyl acetate extracts stand out in terms of antioxidant activity and total phenolic substances, it is obvious that artichoke leaf and N-hexane extract are more valuable in terms of mineral source. Accordingly, the conclusion is that the leaf can be a good source of K and Zn, while methanol and N-hexane extracts can be a good source of P and Zn.

Ethics Committee Information: [1] This study is not subject to HADYEK's permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

[2] The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

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RESEARCH ARTICLE

Effects of Rumen Protected Lysine and Methionine Supplementation on Some Blood Metabolic Parameters in Prepubertal Holstein Heifers

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ABSTRACT

The aim of this study was to investigate the effects of supplemental rumen protected lysine and methionine on some blood metabolic parameters in prepubertal Holstein heifers. Forty, healthy, 9-month-old heifers were divided into two groups as control (C) and treatment (T). C heifers were fed a standard diet which has been prepared according to NRC (2001), whereas T heifers were fed a lysine (7.1% of MP) and methionine (2.4% of MP) enriched (Lysigem and Methipearl, Kemin Ind., Belgium) diet. Blood samples were taken from all heifers at the beginning of the study, on the day of artificial insemination and on the day of pregnancy detection. All blood samples were analyzed for glucose (GLU), beta-hydroxybutyric acid (BHBA), non-esterified fatty acids (NEFA), total cholesterol (TCHOL), triglycerides, total protein (TP) and blood urea nitrogen (BUN). It was determined that the levels of GLU, NEFA and BHBA, which are blood metabolism parameters, changed statistically ($p<0.001$) in the periods when prepubertal, insemination and pregnancy were detected, while the levels of TKOL, TRIG and BUN did not change significantly. Moreover, increasing lysine and methionine content of the prepubertal diet caused an increase in serum TCHOL concentration ($p<0.005$) whereas decreased serum TP concentration ($p<0.05$)

Keywords: Heifer, Holstein, prepubertal, serum metabolites

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Holştayn Irkı Düvelerde Pubertas Öncesinde Rasyona Rumen Korumalı Lizin Ve Metiyonin İlavesinin Bazı Kan Metabolizma Parametreleri Üzerine Etkileri

ÖZ

Bu çalışmada Holştayn ırkı düvelerde yaşamın 9. ayından başlanarak gebeliğin belirlendiği zamana kadar rasyona rumen korumalı lizin ve metiyonin ilavesinin bazı kan metabolizma parametreleri üzerine etkileri incelenmiştir. Bu amaçla 40 adet sağlıklı ve 9 aylık yaşı doldurmuş (9-10 ay arası) Holştayn ırkı düveler rastgele örnekleme metodu ile K (Kontrol) ve U (Uygulama) olmak üzere 2 gruba ayrılmıştır. Kontrol grubundaki düveler NRC (2001)'e göre hazırlanmış standart bir rasyonla, uygulama grubundaki düveler ise rumen korumalı amino asitler kullanılarak (Lysigem ve Methipearl, Kemin Ind., Belgium) lizin (MP'nin %7.1'i) ve metiyonin (MP'in %2.4'ü) düzeyleri artırılmış bir rasyonla ad-libitum olarak beslenmiştir. Tüm düvelerden çalışma başlangıcında, tohumlama zamanında ve gebeliğin tespit edildiği gün vena coccygea yolu ile kan numunesi alınmıştır. Alınan kan numuneleri ilgili kitler kullanılarak glukoz (GLU), betahidroksibütirat (BHBA), esterleşmemiş yağ asitleri (NEFA), total kolesterol (TK), trigliserit (TG), total protein (TP) ve kan üre nitrojeni (BUN) analizleri yapılmıştır. Çalışmada kan metabolizma parametrelerinden GLU, NEFA ve BHBA düzeylerinin prepubertas, tohumlama ve gebelik tespit edilen dönemlerde istatiksel olarak anlamlı şekilde değiştiği ($p<0.0001$), TK, TP ve BUN düzeylerinin ise anlamlı şekilde değişmediği ($p>0.5$) tespit edilmiştir. Ayrıca rumen korumalı lizin ve metiyonin kullanılarak rasyonun metabolik lizin ve metiyonin düzeyinin artırılmasının tohumlama zamanında kanda TK seviyesini artırdığı ($p<0.005$), TP seviyesini ise düşürdüğü belirlenmiştir ($p<0.05$).

Anahtar Kelimeler: Düve, Holştayn, prepubertal, serum metabolitleri

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GİRİŞ

Günümüzde genetik ilerlemeler ve yıllardır süregelen ıslah çalışmaları sayesinde özellikle sütçü karakterdeki düvelerin büyüme ve gelişimleri çok hızlı şekillenmektedir. Bu sayede düvelerin sürüye ekonomik olarak kazanç sağlamadıkları süreç kısa sürmekte ve doğumla birlikte onlardan en hızlı şekilde gelir elde edilmeye başlanmaktadır. Ancak bu hızlı büyüme ve gelişme bütün yönleri ile avantajlı değildir. Özellikle pubertas öncesi büyüme hızı ile ilk tohumlama yaşı, ilk doğum yapma yaşı ve ilk laktasyondaki süt verimi arasında negatif bir ilişki vardır (Swanson 1960, Gardner ve ark. 1977, Little ve Kay 1979, Foldager ve Serjsen 1987). Yani bir başka deyişle, büyüme hızını artırmak düvelerden elde edilen hayat boyu verimliliği olumsuz yönde etkilemektedir (Van Amburgh ve ark. 1998).

Holştayn ırkı sütçü düvelerin ilk doğum yaşı ve canlı ağırlığının ideal koşullarda olması yaşam boyu verimliliği etkilediği için süt işletmeleri açısından hem verim hem de karlılık açısından çok önemlidir (Brickell ve ark. 2009). Keown ve Everett (1986)'nın bildirdiğine göre düvelerde ideal ilk doğum yaşı 24 ay, canlı ağırlığı ise 544-567 kg'dır. İdeal ilk doğum yaşını yakalayabilmek için düveler 13 aylık yaştan itibaren seksüel olgunluğa gelerek tohumlanma programına alınmalı ve 15 aylık yaşta gebe kalmaları sağlanmalıdır (Place ve ark. 1998). Büyüme hızı direkt olarak günlük canlı ağırlık artışı (GCAA) ile ilişkilidir. Buna göre özellikle seksüel olgunluk çağına yaklaşılırken yetersiz beslenme nedeniyle düvelerde pubertasın başlaması gecikmekte ayrıca iskelet sisteminin gelişimi de olumsuz yönde etkilenmektedir. Ayrıca prepubertas dönemde yetersiz beslenen düvelerde güç doğum riski de artmakta ve böylelikle hem buzağının yaşama şansı düşmekte hem de düvenin yaşam boyu döl verimi ve sağlık parametreleri olumsuz yönde etkilenmektedir (Ettema ve Santos 2004).

Sütçü düvelerde pubertasa ulaşma yaşının hedeflendiği gibi 12-13 ay olmasında GCAA'nın rolü elbette ki çok büyüktür. Ancak son yıllarda metabolik durumun da pubertasın başlamasında etkili olduğunu bildiren çalışmaların sayısı günden güne artmaktadır (Anderson ve ark. 2015, Funston ve ark. 2012, Perry ve ark. 2012).

Büyüme ile ilişki içerisinde birçok metabolik ve endokrin parametre bulunmaktadır (Brickell ve ark. 2009). Kanda sirküle eden bu metabolitlerden glikoz ve üre gibi enerji ve protein metabolizması hakkında bilgi veren parametreler aynı zamanda büyüme ve gelişme adına da fikir sahibi olunmasını sağlar (Smith ve ark., 2002). Kan üre seviyesi özellikle rasyondaki protein/enerji oranından etkilenir (Hossein-Zadeh ve Ardalan, 2011). Kan glikoz seviyesi hayvanlarda enerji metabolizmasının önemli indikatörlerinden birisi olup yetersiz sindirilebilir karbonhidratça beslenme, yetersiz yem tüketimi ve günün belirli saatlerinde yem bulamama ve buna bağlı aç kalma durumlarında kan glikoz seviyesi düşer (Chelikani ve ark. 2004).

Pubertas öncesinde kan glikoz seviyesinin düşük olmasının (Greenwood ve ark. 2002, Smith ve ark. 2002, Terre ve ark. 2006) da yüksek olmasının (Taylor ve ark. 2004, Swali ve ark. 2008) da zararlı olduğunu bildiren çeşitli çalışmalar mevcuttur. Buna göre düvelerde pubertas öncesi ve sonrası kan glikoz seviyesinin hangi düzeylerde olması gerektiğini tam anlamıyla ortaya koymak için hala çalışmalara ve yeni bilgilere ihtiyaç duyulmaktadır (Brickell ve ark. 2009). Total kolesterol, steroid hormonların yapısına giren bir molekül olduğu için kolesterolün kandaki seviyesi döl verimi ile ilgili parametreler ile yakından ilişkilidir (Anderson ve ark. 2015). Başta progesteron hormonu ve östrojen olmak üzere eşey hormonlarının üretilebilmesi için süt sığırlarında kanda kolesterol seviyesi kritik eşik olan 70 mg/dl'nin altına inmemesi istenir. Kan kolesterol seviyesi ile pubertastaki sütçü düvelerin fertilite parametrelerinin ilişkili olduğunu bildiren çalışmalar mevcuttur (Talavera ve ark. 1985).

NEFA (Esterleşmemiş Yağ Asitleri, Non Esterified Fatty Acids) vücutta depo olarak bulunan yağ doku (Adipoz doku) mobilize olurken kana geçtiği form olup Beta-hidroksibütirik Asit (BHBA) ile sığırlarda negatif enerji dengesinin en önemli indikatörlerini oluştururlar (Ospina ve ark. 2010). NEFA'lar kan yolu ile metabolize edileceği yer olan karaciğere ulaşır, karaciğerde bir kısmı okside olur, bir kısmı tekrar esterleştirilerek trigliseritlere dönüştürülerek ya VLDL (Çok Düşük Dansiteli Lipid, Very Low Density Lipid) şeklinde kana tekrar gönderilir ya da karaciğer dokusunda birikir (Drackley ve ark. 2001). BHBA (Beta-hidroksibütirik Asit) ise NEFA'nın karaciğerde karnitin palmitoltranferaz-1 aktivitesi ile ortaya çıkan oksidasyon ürünlerinden birisidir. Dolayısıyla kandaki BHBA düzeyi ile NEFA seviyesi arasında çok yakın bir İlişki vardır (Anderson ve ark. 2015). Ayrıca BHBA kandaki baskın keton cismi olduğu için özellikle negatif enerji dengesinin hâkim olduğu dönemlerde ketozisin izlenmesi açısından da önemlidir. Dolaysıyla tüm bu kan parametreleri ile vücuttaki enerji-protein metabolizmasının durumu arasında yakın bir ilişki olup, metabolik faaliyetler bu parametrelerin analizi ile takip edilebilir.

Lizin ve metiyonin tüm hayvanlarda olduğu gibi ruminantlar için de esansiyel amino asitler arasında değerlendirilir. Bu iki amino asit karaciğerde l-karnitin biyosentezindeki reaksiyonlara katıldığından dolayı enerji metabolizmasını ilgilendiren metabolik parametreleri etkilemektedir (Civelek ve ark. 2013). Ayrıca metiyonin karaciğerde metil vericisi olarak görev alarak ruminantlarda enerji ve protein metabolizmasına ekstra bir şekilde katkıda bulunur (Pinotti ve ark., 2002). Bu bilgileri destekler şekilde Kröber ve ark. (2009) süt sığırlarının rasyonlarına eklenen rumen korumalı lizin ve metiyoninin kanda GLU, NEFA, asetoasetik asit ve kolesterol seviyelerinde metabolizma için olumlu olarak değerlendirilebilecek değişimlere sebep olduğun

bildirmektedir. Ancak enerji metabolizması ve kandaki metabolik parametreler ile bu kadar yakın ilişki içerisinde olan bu iki amino asitin rasyona eklenmesinin pubertas öncesi sütçü düvelerde metabolik parametrelere olan etkisini detaylı bir şekilde ortaya koyan bir çalışmaya rastlanmamıştır.
Yapılan bu çalışmada da Holştayn ırkı sütçü düvelerin pubertas öncesi rasyonlarında lizin ve metiyonin düzeyinin artırılmasının farklı dönemlerde kandaki glukoz, beta-hidroksibütirat, esterleşmemiş yağ asitleri, total kolesterol, trigliserit, total protein ve kan üre nitrojeni gibi önemli metabolik parametreler üzerine etkisinin belirlenmesi hedeflenmiştir.

MATERYAL ve METOT

Bu çalışma Çanakkale ilinin Gökçalı Köyü'nde faaliyet gösteren Kaanlar Damızlık Süt Sığırı İşletmesi'nde yürütülmüştür. Bu çalışmada Holştayn ırkı düvelerde, yaşamın 9. ayından itibaren gebeliğin belirlendiği zamana kadar rasyona rumen korumalı lizin ve metiyonin ilavesinin bazı kan metabolizma parametreleri üzerine etkileri incelenmiştir. Bu amaçla 40 adet sağlıklı ve 9 aylık yaşı doldurmuş Holştayn ırkı düve rastgele örnekleme metodu ile Kontrol (K) ve Uygulama (U) olmak üzere 2 gruba ayrılmıştır. Kontrol grubundaki düveler çalışma boyunca "Süt Sığırlarının Besin Madde İhtiyacı" (NRC, 2001) adlı referans kaynakta tavsiye edilen enerji, protein, vitamin içeren bir rasyonla ad-libitum olarak beslenmiştir. Uygulama grubundaki düveler ise besin madde düzeyi benzer ancak lizin düzeyi metabolik proteinin %7.1'i lizin, %2.4'ü metiyonin olacak şekilde rumen korumalı amino asitler (LysiGEM ve MetiPEARL, Kemin Industries) eklenmiş bir rasyonla beslenmiştir. Her iki grubun rasyonları ile ilgili ayrıntılı bilgiler Tablo 1., 2. ve 3'te ayrıntılı bir şekilde gösterilmiştir.
Çalışmanın başlangıcında tüm hammaddelerden alınan örneklere, çalışma boyunca ise haftada bir defa alınan TMR örneklerine kuru madde (KM), ham protein (HP), ham yağ (HY), ham kül (AOAC, 1990), NDF ve ADF (Van Soest ve ark.,1981) analizleri yapılmıştır. TMR formülasyonları haftalık olarak bu analizler doğrultusunda kalibre edilmiştir. Ayrıca ad-libitum beslemeyi sağlayabilmek adına TMR formülasyonları günlük olarak her sabah 10.00'da hayvanların ihtiyacının %125'i olacak şekilde hazırlanmış olup, artan yemler taze yem dökülmeden önce yine günlük olarak toplanmıştır. Artan yemler günlük olarak tartılarak yem tüketimi takip edilmiştir.
Çalışma başından itibaren işletmedeki veteriner hekimin günlük kontrolü altında olan düveler 14 aylık yaşa ulaşmalarından sonra takibe alınarak doğal kızgınlık gösterenler suni tohumlama yöntemi ile tohumlanmıştır. Tohumlamayı takip eden 25. günde yine işletmedeki sorumlu veteriner hekim tarafından ultrasonografik yöntemle gebelik muayenesi yapılarak gebe hayvanlar kayıt altına alınmış ve çalışma onlar için sonlandırılmıştır. Tohumlama sonrası gebe kalmayan hayvanlarda ise kızgınlık takibine devam edilmiş ve bir sonraki kızgınlıkta yine aynı işlemler tekrarlanmıştır. Çalışma boyunca hangi düvenin hayatının kaçıncı gününde gebe kaldığı da yine kayıt altına alınmıştır.
Tüm düvelerden çalışma başlangıcında, tohumlama zamanında ve gebeliğin tespit edildiği gün vena coccygea yolu ile kan numunesi alınmıştır. Alınan kan numuneleri oda sıcaklığında 10.000 r.p.m.'de 10 dakika boyunca santrifüj edilerek serumları elde edilmiş, serum numuneleri ise analizin gerçekleştiği güne kadar -21 ^{0}C'de dondurulmuştur. Tüm serum örneklerine işletmenin laboratuvarında bulunan Cobass C111 marka ve model otomatik analizör yardımı ile ve ilgili kitler kullanılarak glukoz (GLU), betahidroksibütirat (BHBA), esterleşmemiş yağ asitleri (NEFA), total kolesterol (TK), trigliserit (TG), total protein (TP) ve kan üre nitrojeni (BUN) analizleri yapılmıştır.
Serum glukoz, NEFA, BHBA, total kolesterol, total protein ve BUN düzeylerine ilişkin istatiksel analizlerde grup, dönem ve grup x dönem etkileşimlerinin ana etki kaynağı olarak yer aldığı tekrarlamalı ölçümlerde varyans analizi kullanılmış olup, BHBA düzeyine ait veriler üzerinde gerçekleştirilen homojenite testi bulguları doğrultusunda veriler logaritmik transformasyona tabi tutulmuşlardır. Grup ortalamaları arasındaki farklılıkların tespitinde Tukey Çoklu karşılaştırma testinden yararlanılmıştır. Tüm istatistik analizlerde SAS, versiyon 9 (1999) paket programından yararlanılmıştır.

Tablo 1. Çalışmada kullanılan TMR bileşimi
Table 1. Feedstuff content of TMR

Hammaddeler	% Kuru Madde
Mısır Silajı	23.93
Yonca Kuru Otu	23.84
Buğday Samanı	20.47
Konsantre Yem Karması	31.76

Tablo 2. Konsantre yem karması bileşimi ve besin madde kompozisyonu
Table 2. Feedstuff content and nutrient composition of the Grain Mix

Hammaddeler (%)	Kontrol	Uygulama
Buğday Kepeği (İnce öğ.)	50.0	50.0
Mısır, Öğütülmüş	27.0	27.0
DDGS, (Mısır)	15.0	15.0
Maya Artıkları	5.0	3.5
Mermer Tozu	2.0	2.0
Vitamin-Mineral Karması	1.0	1.0
LysiGEM (KEMIN IND.)	0.0	1.1
Metipearl (KEMIN IND.)	0.0	0.4

Besin Madde Kompozisyonu (%)	Kontrol	Uygulama
Ham Protein	15.32	15.46
Metabolik Enerji (Mcal/kg)	2.67	2.69
Ham Selüloz	7.91	7.86
Nişasta	31.41	31.38
NFC	39.36	38.74
Kalsiyum	1.02	1.01
Fosfor	0.89	0.88

Tablo 3. Çalışmada kullanılan TMR besin madde kompozisyonu
Table 3. Nutrient Composition of TMR

Besin maddeleri	Kontrol	Uygulama
Ham Protein (% Kuru Madde)	11.48	11.49
Metabolik Enerji (Mcal/Gün)	17.47	17.65
NDF (% Kuru Madde)	48.24	48.19
ADF (% Kuru Madde)	30.35	30.28
NSC (% Kuru Madde)	21.41	21.53
Kalsiyum	0.79	0.79
Fosfor	0.46	0.46
Lizin %MP*	5.35	7.21
Metiyonin %MP*	2.11	2.68

NDF: Nötral Deterjan Lif; ADF; Asit Deterjan Lif; NSC; Yapısal Olmayan Karbonhidratlar
* Çalışmadaki rasyonların lizin ve metiyonin dizeyleri CNCPS (Cornell Net Carbohydrate and Protein System) versiyon; 6.55 işletim sistemine sahip NDS (Nutritional Dynamic System) adlı rasyon programı yardımıyla hesaplandı.

BULGULAR

Yapılan bu çalışmada prepubertal dönemdeki (9-13 aylık yaşta) Holştayn ırkı düvelere lizin ve metiyonince zenginleştirilmiş rasyon ile beslenmesi düvelerde kandaki ana metabolizma parametreleri olan GLU, NEFA, BHBA, TK, TP ve BUN seviyelerine olan etkisi incelenmiştir. Çalışmada pubertas öncesi ve sonrasında metabolizma ve verimlilik açısından en kritik dönemler olan pubertastan 1-2 ay önce (çalışmanın başında), tohumlama zamanında ve gebeliğin tespit edildiği günde tüm hayvanlara kanda metabolizma analizi yapılmıştır. Elde edilen verilere yapılan istatistik analizi neticesinde her iki grupta da kanda GLU, NEFA ve BHBA seviyelerinin zamana bağlı olarak değişim gösterdiği ($p<0.05$) TK, TP ve BUN değerlerinin ise zamana bağlı değişim göstermediği belirlenmiştir (Tablo 4).

Tablo 4. Gruplarda ölçülen serum parametreleri üzerine dikkate alınan etki kaynaklarının istatistik önem seviyeleri
Table 4. Statistical importance level of source of influence on serum parameters in groups

Etki kaynağı	Grup	Dönem	Grup x dönem
Glukoz	0.2888	<.0001	0.1238
NEFA	0.9629	<.0001	0.7466
BHBA	0.2457	<.0001	0.2341
Total kolesterol	0.6224	0.1607	0.0040
Total protein	0.2693	0.8635	0.0381
BUN	0.4148	0.2628	0.3734

Buna göre kan parametrelerinin zamana bağlı değişimi tek tek incelenecek olunursa, glukoz seviyesinin tohumlama zamanında düştüğü ancak gebelik tespit edildiği gün tekrar yükseldiği, NEFA ve BHBA seviyesinin ise tohumlama zamanında yükseldiği ancak gebelik tespitinde düştüğü saptanmıştır (Tablo 5). Zamana bağlı TK, TP ve BUN seviyelerinin ise her ne kadar rakamsal farklılıklar gösterse de istatistiksel olarak anlamlı bir değişiklik göstermediği belirlenmiştir. Bu kan parametrelerinin gruplar arası farklılıkları incelendiğinde ise glukoz, NEFA, BHBA ve BUN değerleri açısından gruplar arasında istatistiki açıdan anlamlı bir değişiklik olmadığı ancak TK ve TP açısından anlamlı bir fark ($p<0.05$) olduğu belirlenmiştir (Tablo 6). Buna göre tohumlama zamanında kanda TK seviyesinin kontrol grubunda önemli düzeyde düşük düzeyde olduğu ($p<0.05$), TP seviyesinin ise yine tohumlama zamanında uygulama grubunda önemli düzeyde düşük olduğu ($p<0.05$) tespit edilmiştir. Bu iki parametre açısından kan örneği alınan diğer dönemler olan pubertas öncesi ve gebelik tespit edildiği günlerde gruplar arasında fark tespit edilmemiştir. İki parametrede (TK ve TP) de elde edilen farklılığın sadece tohumlama zamanında olması, Holştayn ırkı sütçü düvelerin pubertas öncesi beslenmesinde rasyonlarındaki metiyonin ve lizin düzeyini artırmanın hayvanlarda pubertas sonrası tohumlama döneminde yağ ve protein metabolizmasında birtakım etkileri olabileceği izlenimi doğurmaktadır.

Tablo 5. Gruplarda ölçülen serum parametrelerinin döneme ve gruplara göre değişimi
Table 5. Alteration of serum parameters according to period and groups.

Dönem	GLU (mg/dl)	NEFA (mmol/l)	BHBA* (mmol/l)	TK mg/dl)	TP mg/dl)	BUN mg/dl)
Deneme başı	56.01	0.29	0.52	68.60	6.92	9.53
Tohum. Zam.	50.51	0.45	0.58	63.02	6.95	9.80
Gebelik	54.97	0.36	0.52	63.69	6.98	10.24
SHO*	0.666	0.018	0.003	2.225	0.087	0.003
P	<.0001	<.0001	<.0001	0.1607	0.8635	0.2628
Grup						
Kontrol	53.42	0.368	0.54	65.74	7.05	10.00
Uygulama	54.24	0.367	0.55	64.67	6.92	9.71
SHO*	0.544	0.015	0.003	1.816	0.081	0.249
P	0.2888	0.9629	0.2457	0.6224	0.2693	0.4148

GLU; glukoz, NEFA; Esterleşmemiş Yağ Asitleri, BHBA; Betahidroksibütirik Asit, Total Kolesterol; TK, Total Protein; BUN, Kan Üre Nitrojen; SHO; Standart Hata Ortalaması; P: İstatistiki Önem Düzeyi
*BHBA verileri normal dağılım göstermediğinden log 10 tabanında transforme edildi. Tablodaki değerler transforme değerlerdir.

Tablo 6. Gruplarda ölçülen serum parametreleri grup x dönem interaksiyonun etkisi
Table.6. Effect of group x period interaction on serum metabolites

Parametre	Dönem	KONTROL	UYGULAMA
(TK)	Deneme başı	72.17[a]	65.02[abc]
	Tohumlama zamanı	57.40[bc]	68.64[a]
	Gebelik	67.65[ac]	59.73[c] SHO: 3.146, P: 0.004
(TP)	Deneme başı	6.92[ab]	7.10[a]
	Tohumlama zamanı	7.16[a]	6.74[b]
	Gebelik	7.06[ab]	6.91[ab] SHO: 0.124, P: 0.0381

SHO: Standart Hata Ortalaması; P: İstatistiki Önem Düzeyi
*Harfler dikey olarak grup içi zamana bağlı değişimi ifade etmektedir.

TARTIŞMA

Yapılan bu çalışmada prepubertal dönemdeki Holştayn ırkı düvelere lizin ve metiyonince zenginleştirilmiş rasyon ile beslenmesi düvelerde kan glukoz, NEFA, BHBA, BUN seviyelerini değiştirmemiş, ancak total kolesterol seviyesini artırmış, total protein seviyesini ise düşürmüştür.

Kandaki NEFA, BHBA ve glukoz değerleri, hayvanların enerji metabolizma durumları ile ilgili bilgi veren en değerli parametrelerdir. Şiddetli negatif enerji dengesindeki sığırlarda kan NEFA ve BHBA seviyesi yükselmekte (Anderson ve ark. 2015), glukoz seviyesi ise düşmektedir (Bauman ve Curie 1980). Ayrıca araştırmacılar şiddetli düzeyde bir negatif enerji dengesinden bahsedilebilmesi için kanda NEFA seviyesinin 0.8 mmol/l'nin üzerinde (Roberts ve ark. 2012), BHBA seviyesinin 1 mmol/l'nin üzerinde (Duffield 2000), glukoz seviyesinin ise 40 mg/dl'nin altında (Gordon, 2013) olması gerektiğini bildirmektedirler. Ancak yapılan bu çalışmada gerek uygulama grubu gerekse kontrol grubundaki hayvanlarda kan alınan hiçbir dönemde (pre-postpubertal) bu üç parametre de bahsedilen seviyelere gelmemiştir. Sadece tohumlama zamanında glukoz seviyesi 50 mg/dl seviyesine yaklaşmıştır. Dolayısıyla yapılan bu çalışmada hayvanlar şiddetli bir negatif enerji dengesine maruz kalmamışlardır. İlaveten, kanda GLU, NEFA ve BHBA seviyeleri her iki grupta da zamana bağlı bir değişim göstermiş olup GLU seviyesi tohumlama zamanında düşmüş ($p<0.0001$), aksine NEFA ve BHBA seviyeleri ise yükselmiştir ($p<0.0001$). Bu bulgular negatif enerji dengesini ifade eder (Gross ve ark., 2011).

Tohumlama döneminde gelişen negatif enerji dengesinin ana sebebi hayvanda meydana gelen hormonal değişim olabilir. Çünkü tek mideli hayvanlarda olduğu gibi (Butera ve Beikirch, 1989) geviş getiren hayvanlarda da kanda östrojen hormonunun artışı ile yem tüketimi arasında negatif bir korelasyon olduğu bildirilmektedir (Forbes, 1986; Grummer ve ark., 1990). Ayrıca kızgınlıktaki sığırların yemlikte daha az süre durduğunu (Hurnik ve ark 1975) daha az yem tükettiğini (Reith ve ark. 2014) ve yetersiz kuru madde aldığını (Diskin ve Sreenan 2000) bildiren çalışmalar da bulunmaktadır. Dolayısıyla tohumlama döneminde kanda artan östrojen hormonunun etkisi ile yem tüketimi düşerek çalışmadaki hayvanların hafif düzeyde negatif enerji dengesinin etkisi altına girmelerine neden olmuş olabilir. Ancak asıl sebebin daha detaylı bir şekilde açıklanabilmesi için gerek pubertas öncesi ve sonrası dönemde düvelerde enerji metabolizmasını daha detaylı ele alan çalışmalara gerekse metabolik parametrelerin çeşitlendirilerek benzer çalışmalar yapılmasına ihtiyaç duyulmaktadır

Çalışmada gruplar arasında fark görülen parametreler ise kanda TK ve TP'dir. Kanda TK düzeyi uygulama grubunda tohumlama döneminde daha yüksek bulunmuştur ($P<0.05$). Kanda kolesterol seviyesi ile lizin ve metiyonin arasındaki bağlantıyı kapsamlı bir şekilde açıklayan araştırmalardan birisi de Bouyeh ve Gevorgyan (2011)'a aittir. Araştırmacılar broiler piliçlerde NRC (1994)'te bildirilen seviyelerden daha yüksek düzeyde lizin ve metiyonin içeren bir rasyonla besleme durumunda kanda kolesterol seviyesinde önemli bir artış olduğunu tespit etmişlerdir. Bunun en önemli sebeplerinden birisi olarak da bu iki amino asitin L-karnitinin ön maddesi olarak kullanılmasını ve buna bağlı olarak L-karnitin sentezindeki artışı

göstermişlerdir. Araştırmacılara göre L-karnitin sentezinin artışı ile kaslarda ve karaciğerde karnitin konsantrasyonu artar, buna bağlı olarak karnitin asetiltransferaz aktivitesi yükselir ve asetil-CoA'nın mitokondiriden sitozole geçişi artar. Asetil-CoA ise kolesterol sentezinde, kolesteroldeki tüm karbon atomlarının ana kaynağını oluşturur. Böylece karaciğerde kolesterol sentezi artar ve kandaki seviyesi yükselir. Araştırmacılar bunu destekler şekilde yüksek oranda lizin ve metiyonin ile besledikleri piliçlerin kanlarında trigliserit seviyesinin de azaldığını belirtmişlerdir. Dolayısıyla karaciğerde trigliseritlerden kolesterol sentezleme reaksiyonunun artmış olabilmesi söz konusudur. Benzer bir şekilde Giroux ve ark. (1999) da yüksek düzeyde lizin ve metiyonin ile beslenen tavşanlarda kan kolesterol seviyesinin yükseldiğini bildirmişlerdir. Bu çalışmalarla benzer şekilde yapılan bu çalışmada pubertas dönemindeki sütçü sığırlarda rasyonun metiyonin ve lizince zenginleştirilmesi ile kanda kolesterol seviyenin yükselmesi önemli bir bulgudur. Çünkü steroid hormonların yapısına giren bir molekül olduğu için kolesterolün kandaki seviyesi döl verimi ile ilgili parametreler ile yakından ilişkilidir (Anderson ve ark. 2015). Başta progesteron hormonu ve östrojen olmak üzere eşey hormonlarının üretilebilmesi için süt sığırlarında kanda kolesterol seviyesi kritik eşik olan 70 mg/dl'nin altına inmemesi istenir (Talavera ve ark. 1985). Bu hipotezi kuvvetlendirecek şekilde (Anderson ve ark. 2015) yaptıkları bir çalışmaya göre Holştayn ırkı sütçü düvelerin rasyonunda enerji düzeyi sabit tutulmak kaydıyla DDGS (Kurutulmuş, Damıtılmış Tahıl Küspesi, Dried Distilled Grain Soluble) eklenerek yağ oranı artırıldığında kan kolesterol seviyesi yükselmiştir. Araştırmacıların bildirdiğine göre kanda kolesterol seviyesinin yükselmesi hem 260 günlük yaştan daha erken dönemde (sayısal olarak, $p>0.05$) hem de 300 kg canlı ağırlığın altındaki düvelerde (istatiksel olarak, $p<0.05$) ovaryumlarda siklik aktivitenin başlamasına katkıda bulunmakta, pubertasa erişme çağını da istatistiki olarak düşürmektedir.

Pubertasa ulaşma çağı öncesinde yüksek oranda lizin ve metiyonin içeren bir rasyonla beslenen düvelerde kanda seviyesi değişen diğer bir parametre de TP'dir. Uygulama grubundaki düvelerde tohumlama döneminde kanda TP seviyesi yükselmiştir ($p<0.05$). Kan total proteinin rasyona bağlı en önemli kaynakları rumen fermentasyonundan korunarak ince bağırsağa ulaşan protein ve rumende mikrobiyal fermentasyonla amonyağa kadar parçalanan azot kaynaklarının kullanılması ile sentezlenen mikrobiyal proteindir. Ancak mikrobiyal protein sentezi sırasında rasyondaki azotun bir kısmı rumen duvarından emilmek suretiyle mikrobiyal protein sentezinde kullanılamamakta olduğu için rasyondaki protein düzeyi ile kandaki TP düzeyi arasında direkt olarak bir ilişki kurabilmek imkansızdır (Tomlinson ve ark 1997). Rumende mikrobiyal protein sentezinden kaçarak rumen duvarından emilen ve karaciğere gelen azot kaynakları burada üreye dönüştürülür ve kan dolaşımına gönderilir. Dolayısıyla rasyondaki proteinin bir yararlanımı ve kan TP üzerine etkisi ele alınırken kandaki BUN seviyesi de mutlak suretle dikkate alınmalıdır. Kanda TP düzeyinin düşük olmasının bir diğer nedeni de anabolizma reaksiyonları olabilir. Büyüme ve gelişme çağındaki hayvanların kan total protein düzeyi aynı türün erişkin bireylerine göre daha düşük bulunmaktadır (Doornenbal ve ark. 1988). Bunun temel sebebi olarak kas yapımındaki artış gösterilmektedir. Yapılan bu çalışmada tohumlama zamanında her iki grupta benzer kan BUN seviyesi olmasına karşın uygulama grubunda TP seviyesinin düşük olması, uygulama grubundaki hayvanlarda daha fazla kas dokusu üretilmesine bağlı olabilir. Daha net bir açıklama yapılabilmesi için gruplardaki hayvanların günlük canlı ağırlık artışı verilerine ihtiyaç duyulmaktadır.

SONUÇ

Bu çalışmada prepubertastan itibaren Holştayn ırkı sütçü karakterdeki düvelerin rasyonlarının lizin ve metiyonince zenginleştirilmesinin kandaki majör enerji parametreleri olan NEFA, BHBA ve glukoz üzerine önemli bir etkisinin bulunmadığı, ancak önemli bir döl verimi parametresi olan kolesterol seviyesini artırdığı, total protein seviyesini ise azalttığı belirlenmiştir. BUN seviyesi sabit olduğu için total protein seviyesindeki azalma anabolik reaksiyonların arttığı izlenimi oluşturmuştur. Ayrıca çalışmanın önemli sonuçlarından birisi de sütçü düvelerin pubertas çağına ulaştıklarında hafif düzeyde de olsa negatif enerji dengesinin etkisi altına girdikleri, gebe kaldıklarında bu etkinin ortadan kalkması durumudur. Buna sebep olan etkenlerin daha detaylı bir şekilde ortaya koyulabilmesi, tohumlama çağındaki hayvanlarda kan kolesterol seviyesinin yükselip protein seviyesinin düşmesinin hayvanlarda sağlık ve döl verimi parametreleri üzerine ne şekilde ve düzeyde etki edeceğini belirleyebilmek adına daha kapsamlı çalışmaların yapılmasına ihtiyaç duyulmaktadır.

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

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

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RESEARCH ARTICLE

Consumers' Perception of Animal Welfare in the West Aegean Region, Türkiye

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ABSTRACT

This research was conducted to examine consumers' perceptions of the West Aegean region regarding animal welfare. The Animal Welfare Perception Scale consisted of 5 extents and a total of 52 items, including housing, feeding, personnel, health, and other conditions. The participants comprised 415 consumers over 18 years of age in İzmir and Aydın City centers and districts. The animal welfare perception scale was applied to consumers who decided to participate in the study face-to-face. It was founded that the West Aegean region's consumers perceived animal welfare positively and associated the animal welfare concept with animal health, ethical values, natural food, and food safety and quality. These findings revealed that the consumers' knowledge, opinion and awareness were in parallel with the basic welfare needs of the animals. However, the consumers had insufficient knowledge about animal welfare and the effects of animal breeding methods practiced in intensive production systems on farm animal welfare. One-third of consumers declared they wanted to buy products produced under animal welfare standards and were willing to pay more. The perception of animal welfare was influenced by consumers' gender, educational background, companion animal ownership, food-label reading behaviour, and willingness to pay. It was concluded that the West Aegean region's consumers perceive animal welfare as very important, and the proportions of consumers who demand welfare-friendly products and are willing to pay more could be increased with increased knowledge of animal welfare.

Key Words: Animal welfare, Consumer, Perception, Turkiye, West Aegean Region

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Batı Ege Bölgesi'ndeki Tüketicilerin Hayvan Refahı Algısı

ÖZ

Bu araştırma Batı Ege bölgesindeki tüketicilerin hayvan refahına ilişkin algısının incelenmesi amacıyla yapılmıştır. Hayvan Refahı Algı Ölçeği barındırma, besleme, personel, hayvan sağlığı ve diğer şartları içeren 5 boyut ve toplam 52 adet maddeden oluşmuştur. Araştırmanın evrenini İzmir ve Aydın İl Merkezleri ile bağlı ilçelerde bulunan ve 18 yaş üzerindeki toplam 415 tüketici oluşturmuştur. Araştırmaya katılmayı kabul eden tüketicilere Hayvan Refahı Algı Ölçeği yüz yüze uygulanmıştır. Batı Ege bölgesi tüketicilerinin hayvan refahını pozitif algıladıkları ve hayvan refahı kavramını hayvan sağlığı, etik değerler, doğal gıda ve gıda güvenliği ve kalitesi ile ilişkilendirdikleri belirlenmiştir. Bu bulgular tüketicilerin bilgi, düşünce ve farkındalık durumlarının hayvanların temel refah gereksinimleri ile paralellik gösterdiğini ortaya koymuştur. Bununla birlikte, tüketicilerin hayvan refahı ve yoğun üretim sistemlerinde uygulanan hayvan ıslahı yöntemlerinin çiftlik hayvanlarının refahına etkisi konularındaki bilgisinin yetersiz olduğu görülmüştür. Tüketicilerin üçte birisi hayvan refahı standartları altında üretilen ürünleri satın almak istediğini ve daha fazla ödemeye gönüllü olduğunu beyan etmiştir. Hayvan refahı algısı, tüketicilerin cinsiyeti, eğitim seviyesi, evcil hayvan sahipliği, gıda etiketi okuma davranışı ve ödeme istekliliği ile etkilenmiştir. Batı Ege bölgesi tüketicilerinin hayvan refahını çok önemli olarak algıladıkları ve hayvan refahı bilgisi arttıkça refah-dostu ürünlere talep yapan ve yüksek ödemeye gönüllü olan tüketicilerin oranının artabileceği sonucuna varılmıştır.

Anahtar kelimeler: Algı, Batı Ege bölgesi, Hayvan refahı, Tüketici, Türkiye

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INTRODUCTION

Food safety is a high topic on the global policy agenda, and the impact of animal welfare on the food chain is increasing. The most important reasons for this trend are that intensive production systems cause high stress on animals (Blokhuis et al. 2008, Oltenacu and Broom 2010), healthier and quality foods are obtained from farm animals raised under high welfare standards, and increased consumer concern about healthy food (Blokhuis et al. 2008).

As a general concept, animal welfare is the ability of an animal to adapt to its environment without being forced (Broom 1991). According to other definition of animal welfare provided by the World Organization for Animal Health (OIE), an animal is in a good state of welfare if it is healthy, comfortable, well-fed, safe, able to express innate behaviours, and does not experience negative feelings such as pain, fear, and distress (Vapnek and Chapman 2010). Animal welfare reflects the animal's physical and mental health and well-being (Alonso et al. 2020). In this context, the World Organization for Animal Health (OIE) has been conducting policies that take into account the critical relationship between animal health and animal welfare since 2003. This actions has encouraged risk managers and legislators in the food chain to integrate animal health, animal welfare and food safety a legal framework in an integrative way (Blokhuis et al. 2008, Fernandes et al. 2021). Nowadays, efforts to develop sustainable animal production strategies and environment-friendly and animal-friendly production policies are rising. Food producers and public authorities adopt the application of high animal welfare standards in order to reduce the loss of confidence in intensive production systems and eliminate consumer concerns, and establish a traceability policy to withdraw related products from the market when necessary (Henry et al. 2018, Garnett et al. 2013, Pettitt 2001). In this context, legal infrastructure and sectoral practices related to animal welfare assurance schemes and animal-friendly food certifications in the food industry are being completed rapidly (Pettitt 2001). To prevent consumer concerns from causing a consumption crisis, food manufacturers began to take the management initiative in the food chain to ensure high standards that could be proven through auditing and thus gain a commercial advantage (Blokhuis et al. 2008, Pettitt 2001).

In the last 30 years, global health risks such as BSE, A (H7N9), Salmonella, and the horsemeat scandal have drawn attention to food quality and safety and have caused loss of confidence of consumers in the food supply (Pettitt 2001). Many consumers, who attach importance to various quality parameters of food, are increasingly giving importance to animal welfare quality parameters. They believe that animal-friendly food products are healthier, better quality, tastier, cleaner, traditional, and eco-friendly (Alonso et al., 2020). This approach reveals that consumers are also interested in the welfare of animals. They want to ensure that the food products satisfy all the criteria covered by good animal welfare (Blokhuis et al. 2008, Miranda-de la Lama et al. 2019). To measure and assess the welfare of animals on the farm level and to manage potential risks to meet societal concerns and market demands, efforts are ongoing to develop practical welfare improvement strategies and reliable on-farm monitoring systems. It is crucial to determine what information consumers want about final products because there is a need to establish an intensive dialogue with all segments of society on animal welfare issues and develop effective communication strategies to communicate animal welfare standards to the public (Blokhuis et al. 2003). However, it is also seen that consumers' knowledge of animal welfare is relatively low, their concerns are not evenly distributed across all livestock species, and there is no clear consistency in their willingness to pay more for higher animal welfare (Alonso et al. 2020). In these circumstances, information on consumers' opinions on animal welfare is needed so that consumers' concerns about farm animal welfare do not drive them not to purchase products produced in intensive production systems and so that farmers and the food chain's actors can construct informed decisions. Many studies examining the opinions, concerns, preferences, and perceptions of consumers regarding animal welfare were conducted in the EU (Nocella et al. 2010), Japan (Derstappen and Christoph-Schulz 2022, Kitano et al. 2022), USA (McKendree et al. 2014), Poland, Italy, and South Korea (Derstappen and Christoph-Schulz 2022) and China (Carnovale et al. 2021). In Turkiye, conducting negotiations with the EU for full membership, EU legislation related to animal welfare at the farm level and during transport has already been transposed. Still, there is an urgent need to analyze consumer approaches that will set light on developing strategic policies regarding animal welfare in the food sector. There are very few studies on the opinions, perceptions, and attitudes of Turkish farmers and consumers regarding animal welfare (Çelik and Bozkurt 2016, Kılıç and Bozkurt 2020, Sarıal and Bozkurt 2020). However, it was reported that consumer perception could be affected by demographics and the residential region of consumers (Dimitri and Dettmann, 2012).

Studies examining the effect of geographic regions on animal welfare perception are pretty limited. Results regarding the consumers' animal welfare perception living in the West Aegean region (Izmir, Aydın, Muğla), one of the regions with the highest population and animal food consumption could provide valuable information on sustainable animal production models to be developed in Türkiye. This research was conducted to

examine consumer perceptions about animal welfare in the West Aegean region of Turkiye.

MATERIAL AND METHOD

The research data were collected using the field survey method. For this purpose, a questionnaire consisting of two parts was used. In the first part of the questionnaire, there were questions that demonstrated the consumers' demographic and individual characteristics that may affect their animal welfare perceptions. In this part, questions were asked to the consumers regarding the status of having a child and companion animal ownership, monthly income, occupation, residential area, animal welfare knowledge, the habit of food-label reading, and frequency of animal food consumption. They were subsequently asked how much more they are willing to pay to purchase food produced under high animal welfare standards. The second part of the questionnaire involved the Animal Welfare Perception Scale on factors affecting animal welfare developed by Kılıç and Bozkurt (2014).

The Animal Welfare Perception Scale consists of 5 extents and 52 items in total: housing conditions (11 items), feeding conditions (6 items), personnel conditions (7 items), health conditions (15 items), and other conditions (13 items). Consumers marked their opinions on the effect of each of these extents on animal welfare by choosing among the options (1: No impact at all, 2: Slight impacts, 3: Moderate impacts, 4: Strong impacts, 5: Extreme impacts) prepared with the 5-point Likert system. The research population consisted of consumers over the age of 18 in İzmir and Aydın city centers and their districts. After the content and purpose of the research were explained, face to face, the animal welfare perception scale was applied to the consumers who agreed to participate in the research. In the study, sampling was used due to limitations such as time and cost. The following formula was used, employing the stratified sampling method to represent groups of different ages, education, marital status, and gender. The formula (N>10.000) suggested by Sekaran (2003), for quantitative research and infinite universes was used to calculate the minimum sample size.

$n = S2. Z\alpha 2 / d2$

In the formula;

S (standard deviation) = 1,

$Z\alpha 2$= 1.96 (corresponding theoretical value for significance level $\alpha = 0.05$)

D= 0.1 (effect size) were used as parameters, and the minimum sample size was calculated as 384. Considering that some questionnaires may be excluded due to incomplete, inaccurate, or low reliability, 550 questionnaires were printed, 415 were evaluated as reliable, and statistical analyses were made on 415 questionnaires in the research. The Animal Ethics Committee of Afyon Kocatepe University approved this study with decision number AKUHADYEK-87-18.

Statistical Analysis

The demographic characteristics were presented with frequency, and the scale items were described with frequency percentage distributions, arithmetic mean, and standard deviation values. In addition, Cronbach's alpha coefficients for the perception scale and its sub-extents were calculated for the reliability analysis of the characteristics. After that, t-tests for two groups (independent samples) and One-way-ANOVA were used for more than two groups to compare consumers' perceptions toward animal welfare according to individual characteristics. The data obtained from the consumers were analyzed with the SPSS 21st version package program. 0.05 was taken for the significance level (Ural and Kılıç 2013).

RESULTS

Descriptive statistics of consumers' perceptions regarding the impact of housing conditions on animal welfare are given in Table 1. Consumers exhibited the lowest participation in items "Barn emergency planning for animals to provision against disasters" ($\bar{X}$=3.80), "Barn lighting" ($\bar{X}$=3.96), and "Barn floor surfaces' characteristics" ($\bar{X}$=3.99) related to the impact of housing conditions on animal welfare. The highest participations of consumers for this sub-extent were in items "Barn temperature" ($\bar{X}$=4.50), "Barn ventilation system and indoor air quality"($\bar{X}$=4.44), "Barn humidity" ($\bar{X}$=4.44), and "Barn cleaning" ($\bar{X}$=4.41).

The results on consumers' perception of the impact of feeding conditions on animal welfare are given in Table 2. The consumers participated at the lowest rates for the items "Early weaning of young animals" ($\bar{X}$=4.03), "The physical conditions provided to the animals during feeding" ($\bar{X}$=4.13), and "The characteristics of the vegetation in the pasture" ($\bar{X}$=4.13). The items with the highest participation rate were respectively, "The size of the pasture where the animals are grazed" ($\bar{X}$=4.38) and "The feed characteristics that animals are fed" ($\bar{X}$=4.32).

The descriptive statistics on consumers' perception of the impact of personnel conditions on animal welfare are shown in Table 3. The highest participation rates of the consumers were related to the items "The behaviours of animal carers or farmers toward animals" ($\bar{X}$=4.38) and "Personnel's happiness and job satisfaction" ($\bar{X}$=4.21), and the lowest participation rates were determined for the item " Gender of the personnel" ($\bar{X}$=2.71) and "Education level of the animal carers or farmers" ($\bar{X}$=3.35).

The obtained results on the effects of health conditions on animal welfare are provided in Table 4. The participants scored the highest on the items "Pain and suffering of animals due to their illness" ($\bar{X}$=4.42), "Stress and exhaustion in the animals" ($\bar{X}$

=4.40), "The happiness of the animals" ($\bar{X}$=4.38), "Slaughtering conditions in the abattoir" ($\bar{X}$=4.37), and "Violence against animals" ($\bar{X}$=4.34). Their lowest scores were for the items "Cutting ears, tails, claws, nails, beaks, wings and fingers of animals" ($\bar{X}$ =3.68), "Castration (for cat, dog, horse, bull, etc.)" ($\bar{X}$ =3.68) and "Culling (killing) sick animals)" ($\bar{X}$=3.81).

The descriptive statistics on the effects of other conditions on animal welfare are delivered in Table 5. The highest consumer participation rates were for the items "Climatic conditions" ($\bar{X}$=4.41), "Touching animals (stroking, hugging)" ($\bar{X}$=4.31), "Conditions that frighten animals" ($\bar{X}$=4.23), and "The state of animal feels safe" ($\bar{X}$=4.20). In contrast, the items "Abandoning animals on the streets (cats, dogs, etc.)"($\bar{X}$=3.73) and "Giving names to animals" ($\bar{X}$ =3.95) received the lowest scores from the consumers.

The Cronbach's Alpha coefficient for the animal welfare perception scale was determined as 0.988. The Cronbach's Alpha coefficients of the housing, feeding, personnel, health, and other conditions sub-extents of the scale were 0.954; 0.955; 0.853; 0.966 and 0.957 respectively (Table 6). The findings on the effects of consumers' demographic and individual characteristics on their animal welfare perception are shown in Table 7. The consumers' perception of animal welfare was affected ($p<0.05$) by gender, educational background, companion animal ownership, food-label reading behaviour, and willingness to pay more for animal-friendly foods. However, age, marital status, number of children, occupation, monthly income, residential area, animal welfare knowledge, and frequency of animal food consumption did not significantly affect consumers' animal welfare perception.

DISCUSSION

According to the consumers who participated in the survey, the climatic conditions (temperature, humidity, air quality), equipment, farm cleanliness, and housing density (crowded housing of the animals) affect the welfare of the animals. In light of these findings, it was seen that consumers have a perception that housing conditions affect animal welfare. According to them, the comfort inside the farm (cleanliness, quality air, comfort-enhancing equipment, adequate resting area) had the most potential to affect the welfare of the animals. This view is accurate because housing comfort straight affects animal welfare (Grandin 2017, Kaplan et al. 2018).The consumers thought that the floor characteristics of the animal barn, the lighting inside the barn, the noise, or the measures to be taken to protect the barn against natural disasters would affect the animal welfare relatively less. However, unsuitable or wet floors may cause animals to slide, inappropriate lighting may cause developmental delay and reproductive problems, or natural disasters may cause the death of animals (Waiblinger 2009).

Consumers in the study thought that animal welfare was most affected by nutritional conditions (quality of feed, water and equipment, grazing capacity, and early weaning). In other words, consumers were aware that good animal feeding would support the health and well-being of the animals and positively affect the quantity and quality of animal products. This result was not surprising because the relationship between feeding and health, fitness, and positive emotions involves a fundamental biological dialectic. Broom (2010) reported that low welfare decreases animal health and vitality. Napolitano et al. (2008) reported that lambs weaned at an early age and separated from their mothers experience nutritional deficiencies, adversely affecting their development. Various functions of these lambs are significantly damaged. In addition, consumers agreed with the judgment that grazing animals on large pastures would improve animal welfare. However, only pasture-based feeding may not meet the daily nutritional needs of especially high-yielding farm animals (Knaus 2016). These findings showed that consumers think traditional and natural animal husbandry conditions increase animal welfare more than intensive animal production systems (mainly carried out in closed barns). They may have thought that keeping animals in captivity would negatively affect their health and emotions. The reason for their high participation in the judgment regarding the grazing impact may be based on their view that the quality of the air and rest in the barn are poor and adversely affects animal welfare. These findings show that consumers perceive that animals grazing on large pastures will be happier and healthier if fed with natural and quality plants, breathe fresh air, and are not restricted. Spooner et al. (2014) reported that the citizens in their research mostly associate animal welfare with a positive emotional state and access to natural living conditions. Also, Clark et al. (2016) reported that people think that naturalness and humane treatment of animals is central to good animal welfare. Similar results are also seen in consumer perception and attitude towards organic products where pasture grazing is mandatory. Miele (2010) stated that it is widely believed that organic production systems provide positive animal welfare. Sutherland et al. (2013) stated that one of the most important reasons for the worldwide demand for food products grown under organic principles is the high level of welfare provided to farm animals in this method of production. Soroka and Wojciechowska-Solis (2019) also determined that Polish consumers think organic foods contain fewer harmful substances and are healthier.

Table 1. Descriptive statistics related to housing condition extent of the animal welfare perception scale

Items	Effect level (%)					$\bar{X}$	SD
	1	2	3	4	5		
The barn dimensions and the living area allocated per animal in the barn (sufficient or crowded, etc.)	5.3	5.5	12.5	9.4	67.3	4.28	1.19
Barn cleaning	5.3	5.5	3.6	14.2	71.4	4.41	1.13
Barn ventilation system and indoor air quality	5.3	5.5	3.6	11.1	74.5	4.44	1.14
Barn temperature	5.3	5.5	3.6	5.3	80.3	4.50	1.14
Barn humidity	5.3	5.5	3.6	11.3	74.3	4.44	1.14
Barn equipment	5.3	5.5	3.6	28.0	57.6	4.27	1.11
Barn lighting	5.5	8.9	14.2	26.5	44.9	3.96	1.20
Barn isolation	5.5	5.3	17.8	23.1	48.3	4.03	1.17
The noise level in the barn	5.5	8.9	14.2	22.7	48.7	4.00	1.22
Barn floor surfaces' characteristics	8.9	8.4	14.7	11.1	56.9	3.99	1.36
Barn emergency planning for animals to provision against disasters(warning system, alarm, evacuation, etc.)	8.9	8.4	21.9	15.2	45.6	3.80	1.33

Table 2. Descriptive statistics related to feeding condition extent of the animal welfare perception scale

Items	Effect level (%)					$\bar{X}$	SD
	1	2	3	4	5		
The feed characteristics that animals are fed	5.5	5.3	12.5	4.8	71.9	4.32	1.21
The drinking water characteristics that animals are given	5.5	5.3	3.6	25.8	59.8	4.29	1.12
The physical conditions provided to the animals during feeding	8.9	5.5	8.9	16.6	60.1	4.13	1.30
The size of the pasture where the animals are grazed	5.5	5.3	3.6	16.6	69.0	4.38	1.14
The characteristics of the vegetation in the pasture	5.3	5.5	12.5	24.1	52.6	4.13	1.16
Early weaning of young animals	5.5	5.3	16.1	27.0	46.1	4.03	1.16

Table 3. Descriptive statistics related to personnel condition extent of the animal welfare perception scale

Items	Effect level (%)					$\bar{X}$	SD
	1	2	3	4	5		
The behaviours of animal carers or farmers toward animals	5.5	5.3	0.0	24.1	65.1	4.38	1.11
Education levels of the animal carers or farmers	17.1	13.0	18.3	20.7	30.9	3.35	1.46
Animal welfare knowledge of the animal carers or farmers	14.5	5.3	12.3	29.2	38.7	3.73	1.40
Animal breeding experience of the animal carers or farmers	8.9	5.5	11.8	22.2	51.6	4.02	1.29
Gender of the personnel	43.1	5.5	12.5	15.2	23.7	2.71	1.67
Personnel's motivation level (living and working conditions, salaries, insurances, etc.)	8.9	5.5	11.8	16.9	56.9	4.07	1.31
Personnel's happiness and job satisfaction	5.3	5.5	16.1	9.2	63.9	4.21	1.21

Table 4. Descriptive statistics related to animal health condition extent of the animal welfare perception scale

Items	Effect level (%)					$\bar{X}$	SD
	1	2	3	4	5		
Providing regular veterinary care to animals	5.5	8.9	11.8	14.2	59.6	4.13	1.25
Types and ways of the treatments applied to sick animals	5.5	8.9	8.9	14.2	62.5	4.19	1.24
The minerals and vitamins are given to animals on a veterinarian's advice	5.5	5.3	15.4	7.7	66.1	4.23	1.21
Pain and suffering of animals due to their illness	5.5	5.3	3.6	12.5	73.1	4.42	1.14
The happiness of the animals	5.5	5.3	7.2	9.2	72.8	4.38	1.17
Stress and exhaustion in the animals	5.5	5.3	3.6	14.2	71.4	4.40	1.14
Cleaning of the animals	5.5	5.3	16.1	24.3	48.8	4.05	1.17
Culling (killing) sick animals	14.2	5.5	8.9	27.5	43.9	3.81	1.41
Castration (for cat, dog, horse, bull, etc.)	10.6	8.9	19.3	24.1	37.1	3.68	1.33
Cutting ears, tails, claws, nails, beaks, wings, and fingers of animals	10.6	8.9	14.9	32.5	33.1	3.68	1.30
Modifications such as claw pulling in cats forced molting in laying hens, and dehorning of calves	5.5	11.8	12.5	16.4	53.8	4.01	1.28
Fighting, wrestling, or racing involving animals(dog racing, cock and dog fighting, etc.)	5.5	5.3	15.4	25.5	48.3	4.06	1.16
Violence against animals	5.5	5.3	6.7	14.2	68.3	4.34	1.16
Slaughtering conditions in the abattoir	3.6	5.5	8.9	14.5	67.5	4.37	1.09
Handling animals with power tools (such as electric pads to walk cattle)	5.5	5.3	16.1	10.6	62.5	4.19	1.21

Table 5. Descriptive statistics related to other conditions extent of the animal welfare perception scale

Items	Effect level (%)					$\bar{X}$	SD
	1	2	3	4	5		
Climatic conditions	5.5	5.3	6.5	8.2	74.5	4.41	1.17
Conditions that frighten animals	5.5	5.3	12.5	13.5	63.2	4.23	1.19
Applications to increase animal productivity (hormone, genetic selection, etc.)	5.5	8.9	8.9	14.5	62.2	4.19	1.24
Conditions on the animal reproductive process (artificial inseminations, embryo transfer, etc.)	5.3	5.5	14.9	28.4	45.9	4.04	1.14
Conditions that adversely affect the relationship of animals with their offspring	5.3	5.5	12.5	31.6	45.1	4.06	1.13
Technical equipment for raising animals on the farm	5.3	11.8	12.8	17.6	52.5	4.00	1.27
The state of animals feels safe	5.3	5.5	7.2	27.7	54.3	4.20	1.13
Business terms and strategies such as crisis and risk management	8.9	5.5	16.1	13.0	56.5	4.02	1.32
Accepting animals as individual	8.2	5.5	10.8	22.2	53.3	4.07	1.27
Giving names to animals	9.2	11.8	8.9	15.2	54.9	3.95	1.39
Touching animals (stroking, hugging)	5.3	3.6	9.2	18.8	63.1	4.31	1.12
Conditions during the transport of animals from one place to another	5.5	5.3	12.5	34.9	41.8	4.02	1.12
Abandoning animals on the streets (cats, dogs, etc.)	7.2	14.5	16.9	21.0	40.4	3.73	1.32

Table 6. Cronbach's Alpha coefficients, mean and standard deviations of the animal welfare perception scale and its sub- extents

Scale and sub-extents	n	Cronbach's Alpha	$\bar{X}$	SD
Perception scale	415	0.988	4.097	0.965
Sub-extents				
Housing condition	415	0.954	4.191	0.989
Feeding condition	415	0.955	4.213	1.069
Personnel condition	415	0.853	3.780	0.990
Animal health condition	415	0.966	4.131	1.003
Other conditions	415	0.957	4.094	0.991

Table 7. The effects of consumers' demographic and individual characteristics on their animal welfare perceptions

Variable	Groups	n	$\overline{X}$	SEM	P
Gender	Women	218	4.195	0.063	0.029*
	Men	197	3.988	0.070	
Age	25 and younger	108	4.087	0.101	0.816-
	26-32	134	4.160	0.078	
	33-40	89	4.043	0.104	
	41 and older	84	4.065	0.103	
Marital status	Single	137	4.100	0.085	0.957-
	Married	278	4.095	0.057	
Educational background	Primary education	79	3.844 b	0.122	0.033*
	Secondary school	58	4.125 ab	0.119	
	Higher education	278	4.163 a	0.056	
Companion animal ownership	No	311	4.081b	0.055	0.044*
	One animal	84	4.031b	0.114	
	More than one animal	20	4.615a	0.088	
Number of children	No	116	4.021	0.096	0.491-
	One child	145	4.164	0.079	
	More than one child	154	4.090	0.074	
Occupation	Public employee	48	4.232	0.109	0.395-
	Private sector employee	177	4.010	0.080	
	Merchants	83	4.108	0.097	
	Farmer	107	4.170	0.091	
Monthly income (TL)	3000 and less	158	3.981	0.084	0.101-
	3001-5000	130	4.112	0.083	
	5001 and more	127	4.226	0.075	
Residential area	Province	192	4.024	0.074	0.360-
	District	166	4.162	0.072	
	Town and village	57	4.152	0.112	
Animal welfare knowledge	Well know	89	3.969	0.109	0.325-
	Know litte	249	4.117	0.062	
	Do not know	77	4.180	0.099	
Food-label reading	Yes	58	3.804b	0.150	0.043*
	Sometimes	109	4.120a	0.092	
	Always	248	4.155a	0.058	
Frequency of animal food consumption	Sometimes	41	4.055	0.167	0.605-
	Generally	170	4.154	0.073	
	Always	204	4.058	0.067	
Willingness to pay more	No	155	3.927b	0.084	0.030*
	I pay 15% more	109	4.182ab	0.092	
	I pay 30% more	92	4.139ab	0.097	
	I pay 50% more	59	4.318a	0.097	

*:p<0.05, -: Non-significant a, b: The means within the same columns with different letters differ significantly (p<0.05).

According to the results on the personnel conditions of this study, the consumers thought that the behaviour of the personnel who have direct contact with the animals and are responsible for animal care affected animal welfare. This consumer approach was compatible with the proven relationship between the quality of human-animal interaction and animal welfare (Bozkurt et al. 2013, Napolitano et al. 2019). Kılıç and Bozkurt (2013) determined that there is a significant positive relationship between the perception of animal welfare of the farmers responsible for the care and management of sheep and the welfare standards they provide for their sheep. Nonetheless, consumers participating in this study were less likely to agree that other individual characteristics of personnel (such as gender, education level, knowledge of animal welfare, motivation, and job satisfaction) affect the quality of

animal behaviour and may affect the level of animal welfare. These findings may be because consumers need more information about how animals are handled in intensive production systems and the scope of human-animal interactions. Consumers thought with strong common sense that stroking animals by touching them (such as hugging) will increase animal welfare, but in intensive livestock systems, animal-human contacts are minimal. Similar results were reported by Kılıç et al. (2013).

Consumers who have been surveyed thought that animal health significantly impacted animal welfare. As Webb et al. (2019) reported, animal happiness, which reflects how an animal feels and is predominantly associated with an excess of positive emotional states, is associated with the affective extent of animal welfare (Webb et al., 2019). Consumers also believed that negative (pain, fear, stress, frustration, violence) and positive (happiness) feeling states would significantly affect animal welfare. As expected, these results revealed that consumers could establish the relationship between a healthy animal and a healthy product and between a negative feeling state and poor well-being. However, it was noted that they showed a high level of participation in the need for positive animal feelings, especially for high animal welfare. Well-being and positive emotions are requirements for high animal welfare (Broom and Corke 2002, Sutherland et al. 2013). The participants thought that the practices (modifications such as castration, tail clipping, and beak trimming) that are routinely performed in livestock farming and can cause acute and chronic pain and suffering in animals would have a relatively less impact on animal welfare. However, they thought that some processes and breeding practices (hormones, genetic selections, etc.) applied to increase animal yields would affect the animals' health more. This seemingly contradictory situation in consumer perception suggested that they were familiar with high-yielding animals (with genetic selection) to maintain the food supply and that they might have accepted this situation to some extent. Godfray and Garnett (2014) also identified similar contradictions regarding the sustainability of food production systems. In this research, it was seen that consumers generally associate the concept of health with positive or negative emotions of animals more intensely in animal health issues. Also, Spooner et al. (2014) reported that although the people participating in their study agreed that it is essential to maintain good health and biological functioning in animals, they primarily participate in the benefits of natural life.

In general, surveyed consumers associate animal welfare with animal health (good nutrition, housing, and resting comfort), ethical values related to animal handling (animal suffering, animal happiness), and safe and natural food (hormone use). These consumers were mainly respectful of animal nature (early weaning and the importance of the mother-child relationship) and the emotions of animals in the food chain (pain, suffering, fear, happiness, inhumane treatment methods, and stance against animal violence). They cared about the biological functionality of animals (good nutrition) and their needs, such as comfortable rest, freedom and comfort, and good treatment from birth to slaughter. These findings revealed that consumers' knowledge, opinions, and awareness are in parallel with the basic animal welfare needs of the animals. However, conflicting scores indicate that they do not know the basic concepts of animal welfare well enough. Respondents agreed that slaughterhouse conditions would affect animal welfare but placed less emphasis on the judgment that using electric pads would reduce animal welfare. They believed that the personnel's behaviour towards the animal, job satisfaction, or happiness would significantly affect the animal welfare. However, they identified less emphasis on other socio-demographic factors that impacted the personnel's behaviour. They highly agreed that touching or cuddling animals would improve animals' well-being but placed less emphasis on getting to know the animal as an individual (as opposed to the damage done by mass animal handling in the production chain). Similarly, according to participants' scores, consumers believed that genetic modifications for higher yields or hormone use on animals would affect animal welfare. On the other hand, they showed less sensitivity to the effects of painful practices involving nearly all farm animals (ear and tail clipping, neutering, dehorning) on animal welfare.

Consumers agreed that violence against animals would reduce animal welfare but placed less emphasis on the impact of violent animal fights. Similarly, they evaluated the weak relationship between leaving animals on the street and animal welfare. This may be related to the fact that consumers do not find the streets very unsafe for animals or that 75% of the participants do not have a companion animal. Spooner et al. (2014) reported in the study they conducted with non-animal producers in Canada that the participants did not have enough knowledge about modern and intensive production systems, and they requested information about animal welfare, which they consider an ethical basis. Miele (2010) found that in France and the Netherlands, consumers' and citizens' knowledge of farm animal welfare and animal husbandry practices is fragmented, ambivalent, and intertwined with negative emotions. They found that the majority of respondents in Italy had little knowledge of animal welfare and production systems. Topuzoglu et al. (2007) reported that consumers approve at a relatively low rate of attitudes requiring food information. In addition, these researchers pointed out a lack of knowledge regarding consumers choosing the right products for healthy nutrition. Already, only 21.45% of the participants in this study stated that they knew animal welfare well. Sarıal

Kubilay and Bozkurt (2020) reported similar results for companion animal owners. Broom (2010) reported that poor welfare reduces animal health and well-being, and the loss of quality in products derived from sick and afflicted animals is unacceptable for many people. Clark et al. (2016) noted that approaches toward modern and intensive farming methods for consumers are primarily negative. The fact that most of the participants in this study lived in big cities (46.27% in provinces) may have caused them to have minimal observations and experience in livestock management. Only 25.78% of the participants are farmers, supporting this interpretation. It is also seen that consumers' perception of animal welfare is associated with high food safety and quality. There was high agreement that factors such as high meat, milk, and egg yields and the use of hormones that affect the safety and quality of food, regular veterinary care, and animal fitness will affect animal welfare. They were acutely aware of the benefits of treating sick animals or providing regular veterinary care. In addition, 90% of these consumers consumed animal food and associated animal welfare with healthy food and wildlife.

As it was widely evidenced in the literature, female consumers in the West West Aegean region had a higher perception of animal welfare than males (Kılıç and Bozkurt 2013, García-Gudiño et al. 2021). As the level of education increased, participants' animal welfare perception also increased. This result shows that consumers who learn about animal breeding practices are more aware of how animals are treated in actual commercial conditions (Estévez-Moreno et al. 2021). Companion animal ownership has gradually influenced consumers' perceptions of animal welfare. Because there is an increase in the perception of animal welfare, especially among consumers who own more than one companion animal, this situation is not surprising. Sarıal Kubilay and Bozkurt (2020) reported similar results. They stated that companion animal owners believed that animal welfare was most affected by housing, feeding, and sanitation conditions and least by slaughtering, sacrificing, or naming animals. A linear relationship was found between the West Aegean region consumers' willingness to pay more for animal-friendly foods and their perceptions of animal welfare. It was also seen that the Food-label reading behaviours of the same participants were also positively related to their perceptions of animal welfare. These findings showed that the consumption behaviours of the participants were affected by the animal welfare standards in the food chain.

CONCLUSION

As a result, it was determined that the West Aegean region consumers perceive animal welfare as necessary and associate it with animal health, ethical values, natural food, and food safety and quality. These findings revealed that consumers' knowledge, thoughts, and awareness are in parallel with the basic needs of animal welfare. However, it has been determined that the level of knowledge of consumers on animal welfare is weak, and their knowledge about how breeding practices in intensive animal production systems affect animal welfare losses was also poor. In addition, the perception of animal welfare was influenced by consumers' characteristics such as gender, educational background, companion animal ownership, food-label reading behaviour, and willingness to pay. In light of these findings, it was concluded that the West Aegean region consumers have a high perception of animal welfare, and if their information needs are met, consumers' demand for animal welfare-friendly products may increase even more.

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RESEARCH ARTICLE

Crimean Congo Hemorrhagic Fever Virus-Specific Antibody Detection in Equids

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ABSTRACT

Crimean-Congo hemorrhagic fever (CCHF) virus infection is a tick-borne zoonotic disease that is endemic in a large region of the world and is a potentially fatal disease, especially threatening human health. One of the main indicators that the disease is endemic in a region is the presence of antibodies specific to CCHFV in animal populations. Many animal species may carry CCHFV asymptomatically and hence participate to the disease's transmission cycle. Serological studies have shown that equids are important to the survival of CCHFV in nature. Nevertheless, need for more studies on this subject in Türkiye. For this study, blood samples were taken from 11 horses and 86 donkeys of different sex and variable ages in the provinces of Afyonkarahisar and Burdur. The presence of CCHFV-specific antibodies in the blood serum of these animals, which are held by the breeders for a variety of purposes, was studied. The double-antigen ELISA test method, which is a fast and reliable method, was used for the detection of specific antibodies. As a result, a total of 51.54% (50/97) seropositivity was detected. Findings indicate that equidae may play an important role as a reservoir in the epidemiology of CCHFV.

Keywords: Crimean Congo Hemorrhagic Fever, Donkey, ELISA, Equide, Seroprevalence

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Tek Tırnaklı Hayvanlarda Kırım Kongo Kanamalı Ateşi Virusu'na Spesifik Antikorların Tespiti

ÖZ

Kırım Kongo kanamalı ateşi (KKKA) virus enfeksiyonu, dünyanın geniş bir bölgesinde endemik olan ve özellikle insan sağlığını tehdit eden, potansiyel olarak ölümcül bir hastalık olan kene kaynaklı zoonotik bir hastalıktır. Hastalığın bir bölgede endemik olduğunun ana göstergelerinden biri, hayvan popülasyonlarında KKKAV'ye özgü antikorların varlığıdır. Birçok hayvan türü KKKAV'yi asemptomatik olarak taşıyabilir ve bu nedenle hastalığın bulaşma döngüsüne katılabilir. Serolojik çalışmalar, doğada KKKAV' nin hayatta kalması için tek tırnaklıların önemli olduğunu göstermiştir. Ancak Türkiye'de bu konuda daha fazla çalışmaya ihtiyaç vardır. Araştırma için Afyonkarahisar ve Burdur illerinde çeşitli cinsiyet ve yaştaki 11 at ve 86 eşekten kan örnekleri alındı. Yetiştiriciler tarafından çeşitli amaçlarla tutulan bu hayvanların kan serumlarında KKKV'ye özgü antikorların varlığı araştırıldı. Spesifik antikorların tespiti için hızlı ve güvenilir bir yöntem olan çift antijenli ELISA test yöntemi kullanıldı. Sonuç olarak toplamda %51.54 (50/97) seropozitiflik tespit edildi. Bulgular, tek tırnaklı hayvanların KKKA'nın epidemiyolojisinde rezervuar olarak önemli bir rol oynayabileceğini göstermektedir.

Anahtar Kelimeler: Kırım Kongo Kanamalı Ateşi, Eşek, ELISA, Equide, Seroprevalans

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INTRODUCTION

Crimean-Congo hemorrhagic fever virus (CCHFV) is an enveloped, segmented, and negative-sense single-stranded RNA virus in the *Orthonairovirus* genus in the *Nairoviridae* family within the order *Bunyavirales* (Lombe et al. 2021, Sana et al. 2022). The CCHFV is a tick-borne virus that was named for the geographical places where it was first characterized in the Crimea (1944) and recognized in the Congo (1969) (Bente et al. 2013). CCHFV is the causative agent of *Crimean-Congo hemorrhagic fever* (CCHF) in humans. (Blanco-Penedo et al. 2021). CCHF, is a zoonotic arboviral disease that poses a great threat to public health therefore according to the World Health Organization (WHO), CCHFV is the most predominant causative agent of viral hemorrhagic fever outbreaks worldwide (Sorvillo et al. 2020). Humans and a wide range of other animal species are at risk of contracting CCHFV (Ali et al. 2019, Ali et al. 2021, Kaba 2022). It has been reported that CCHFV can be transmitted to humans through a tick bite, contact with blood, body fluids, or tissues of a viremic animal or human (Bente et al. 2013). In addition, it has been stated that infection can occur in humans through contact with raw or undercooked meat after slaughter and through the consumption of contaminated meat (Fazlalipour et al. 2016, Mostafavi et al. 2017). In humans, acute CCHF is characterized by fever, chills, muscular discomfort, headache, nausea and vomiting, stomach ache, and joint pain. Ecchymosis and bleeding from the mucous membranes of the nose and vagina can worsen the condition in extreme situations. The fatality rate from CCHF ranges between 9% and 50% on average (Hawman and Feldmann 2018). Although it is life-threatening for humans, it generally does not cause illness in animals that carry it asymptomatically. However, domestic and wild animals play a crucial role in the circulation of the virus into new areas by carrying transovarially infected mature ticks (Spengler et al. 2016).

It has been reported that CCHF has spread to a wide geography as far as Africa, Eastern Europe, the Middle East, and Central and South Asia (Sana et al. 2022). In Türkiye, CCHF cases have also emerged in western regions as well as in Central Anatolia and the Black Sea region in recent years (Elaldı 2004, Simsek et al. 2018). More than 30 different species of ticks from various genera carry the virus in places where CCHF is endemic (Shahid et al. 2021). The CCHFV is maintained as a reservoir and spreads by the bites of *Ixodid* (hard) ticks, particularly those belonging to the genus *Hyalomma*. Tick species have been reported in the Western Mediterranean regions of Afyonkarahisar and Burdur in Türkiye (Bakırcı 2009, Eser 2012). Among these identified species, there are also tick species that are effective in the transmission of CCHFV (Gargili et al. 2017).

In previous studies, it has been reported that antibodies against CCHF have been detected in many domestic and wild animals, including cattle, goats, sheep, horses, pigs, camels, donkeys, mice, dogs, rabbits, and ostriches (Mangombi et al. 2020, Spengler et al. 2016, Pak 1972). The presence of CCHFV antibodies in a diverse host spectrum indicate that CCHFV is endemic in these areas and will continue to circulate in nature for many years (Ceianu et al. 2012). Thus, testing for specific antibodies against CCHFV by serological tests is crucial for establishing the fact of current or prior viral infection. This is the first research ever conducted on equids in Türkiye, and the results provide crucial baseline information on the prevalence of CCHF in the region. This study is expected to provide current data on the detection of antibodies against CCHF in equidae animals such as horses *(Equus caballus*) and donkeys (*Equus asinus*) bred for various purposes.

MATERIAL and METHOD

Animals

Samples were obtained for this investigation from 11 horses and 86 donkeys used as mounts in rural parts of Türkiye's Western Mediterranean region (Figure 1), which were used for transportation in livestock and daily works. Anamnesis information for asymptomatic animals was collected from their owners during sampling. All procedures were approved by the Animal Ethics Committee (AEC) Burdur Mehmet Akif University, Türkiye (102/912, 25.05.22).

Sample Preparation

Vena jugularis was used to collect blood samples into a sterile, vacuumed kaolin tube. It was transported to the virology laboratory under cold chain conditions for analysis on the same day. Serum samples were transferred to sterile tubes after being centrifuged at 3000 rpm for 10 minutes at room temperature. All samples were stored at -20°C until analyzed.

ELISA Assay

The detection of antibodies specific to CCHFV in a total of 97 serum samples was investigated according to the commercial ELISA kit (ID Screen® CCHF Double Antigen Multi-species, Grabels, France) procedure. The diagnostic sensitivity and specificity of the ELISA kit are %96.8-99.8 and %99.8-100, respectively (Sas et al. 2018). In summary, after adding 50 µl of dilution buffer to all wells, 30 µl of negative and positive controls were placed in 2 wells. 30 µl serum samples were distributed to the remaining wells.

Then, the incubation conditions and procedures stated in the test procedure were followed. Following the validation controls, the data were measured in an ELISA microplate reader at 450 nm and interpreted based on the optical density (OD) values. OD values less than or equal to 30% were defined as negative, whereas those greater than 30% were regarded as positive. The detection of CCHFV-specific antibodies in collected serum samples was recorded.

Figure 1: Distribution of the samples according to the provinces from which they were obtained and some tick species reported to be present in these provinces

RESULTS

The distribution of the OD values obtained after the analysis is shown in Figure 2. Forty-six out of a total of 86 donkey serum samples were positive for the presence of the antibody.

A total of fifty animals found to be seropositive, including 33 females and just 17 males. Four horses were found to be seropositive out of a total of 11 serum samples. Three of them found to be seropositive were female, whereas just one was male. It is found that the average age of male donkeys was 8.8 years and that of females was 5.2 years, whereas the average age of male horses was 6.9 years and that of females was 5.2 years. Briefly, the median age of seropositive female animals was lower than that of seronegative male animals, and the seroprevalence of female animals was greater than that of male animals. Overall, 51.54% (50/97) of the animals tested positive for the presence of the antibody (Table 1).

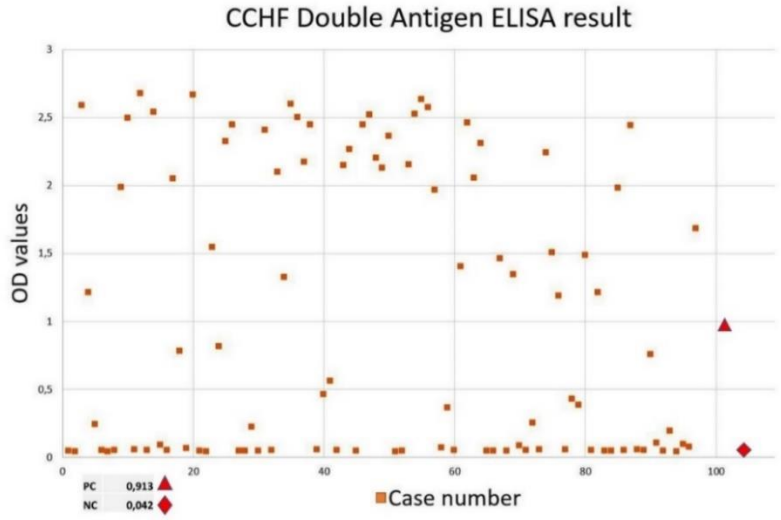

Figure 2: OD values distrubitons. PC: Positive control, NC: Negative control.

Table 1. Distribution of test results by animal species, sex, and age.

	Donkey		Horse	
	Male	Female	Male	Female
Age (Mean)	8.8	5.2	6.9	5.2
Total number of samples	31	55	5	6
CCHF positive numbers	16	30	1	3
CCHF negative numbers	15	25	4	3
Rate of seroprevalance (%)	51.61	54.54	20.0	50.0

DISCUSSION

Human actions, including increasing deforestation, invasion of natural animal habitats, and climate change, dramatically enhance the possibility for exposure and spillover of novel zoonotic diseases like CCHF. Newer research has also put an emphasis on the dangers of epidemics and pandemics because of their potential effects on human health (Aydin et al. 2020, Karamese et al. 2020). When healthcare authorities like the World Health Organization (WHO) include zoonotic diseases as CCHFV that have caused outbreaks on their lists, it demonstrates the disease's significance for human and animal health (Gilbride et al. 2021).

This study is the first to report on the prevalence of CCHF in equids, located in Türkiye's Western Mediterranean region. According to the findings of this investigation, a total of 51.54% (50/97) were found to be seropositive, including 53.48% (46/86) donkeys and just 36.36% (4/11) horses (Table 1). Recently, in northern Türkiye, Albayrak et al. (2012) reported an antibody prevalence of 66% in sheep and 85% in goats. In the Marmara region, Tuncer et al. (2014) also reported the seroprevalence of CCHFV was 13% in cattle, 31.8% in sheep, and 66% in goats. Despite the lack of comparable data from Türkiye, only a small number of studies were undertaken on the other species. Among them, there is no study on equids about CCHF seroprevalence in Türkiye. On the other hand, an investigation conducted in Senegal, indicated that the CCHF seroprevalence rate was high in horses but low in donkeys, supporting the results of the current study (Mangombi et al. 2020). It was also noted that positive animals had a higher mean age than negative animals. Even though this research shows that horses have a high seroprevalence, which seems to match the results of other studies, we think that the small number of samples may be misleading. However, high levels of antibodies were detected in donkeys in this study. We concluded that the significant seroprevalence we found was most likely to be attributed to the length of time these animals lived and how long the antibodies lasted in their bodies. When average age is considered, the animals with positive are younger than the animals with negative, according to this data. Since young animals are preferred over old animals in terms of energy and endurance in daily work, the chance of viral exposure increases. We believe that high antibody positivity in young animals is related to this scenario. Donkeys exposed to tick bites in rural settings are regarded to be possible sources of CCHF in people (Lwande et al. 2012). Therefore, it has been suggested that animals such as horses and donkeys play an important role in the epidemiology of the disease (Ibrahim et al. 2015). There is also a high potential of tick species transfer from animals to people in the areas where the samples were taken, *Hyalomma marginatum*, *Rhipicephalus bursa*, and so on (Figure 1). The fact that animals used as transportation in rural areas of these regions are likely to be exposed to ticks shows that these animals will play a big role in how CCHFV spreads.

While studies of the virus are commonly conducted in high-risk populations or places, the real prevalence of the disease remains unclear. Despite the fact that Türkiye was infection-free before the 2000s, it has become the global "epicenter" of the disease, with over a hundred new cases in recent years (Ozturk et al. 2017). In Türkiye, particularly in the Black Sea and Central Anatolia regions, CCHF is prevalent (Leblebicioglu et al. 2016). Despite this, new cases have been reported in the western regions in recent years due to changing climatic conditions, movement and trade of infected livestock, and migratory bird routes (Elaldı 2004, Öztürk et al. 2017, Simsek et al. 2018). Due to the rising human population in the Eastern Mediterranean region (Al-Abri et al. 2017), the favorable circumstances for vectors, and the migration of animals and wild birds transporting infected ticks from Syria, Iraq, and Iran, CCHFV has become endemic in the southwest of Türkiye. In the study areas, equids are commonly used for everyday labor due to the relative ease of access to land, which in turn increases the likelihood of vector-host contact. This investigation determined that there were a high specific antibodies in equidae in the Western Mediterranean region. The foregoing findings support the hypothesis that CCHF may be spreading in these places.

In many parts of the world, the domestication of donkeys, mules, and horses was crucial to the progress of civilisation. These animals have served numerous purposes for humans throughout history, including transportation, race, warfare, entertainment, friendship, labor, and sport. They are still in use for work today, especially in countries with low and middling incomes and in places with steep hills. In Türkiye, these animals are still used as riding animals, draught animals in agricultural operations, sources of power for wheeled vehicles, and pack animals. They carry firewood, water, grain, hay, and a multitude of other goods over short distances. They escort sheep flocks and carry the equipment of shepherds, and a weak newborn lamb that cannot follow the flock in rural areas (Yılmaz and Wilson, 2013). These mentioned events are very common in rural areas, which increases the chance of being exposed to vectors like ticks. Thus, it is crucial to study infectious agents that can be detected in or transmitted by these animals, for the benefit of both animal and human health (Alkan et al. 2013, Aydin et al. 2020, Gilbride et al. 2021; Timurkan et al. 2019). The reason that the seroprevalence of CCHFV infection is so high in this study is due to a number of factors, including hot weather conditions, global warming, the existence of asymptomatic animals, a continuous movement of hosts in and out of these regions, their living conditions, and the fact that horses or donkeys are kept together with other livestock. The foregoing data provide support to the hypothesis that equids contribute significantly to the maintenance of high CCHFV prevalence despite the fact that they likely do not act as direct sources of viral transmission in the same way that viraemic livestock do.

It was found that the incidence of CCHFV infection during spring-summer was the highest among samples animals. On the other hand, CCHFV is considered to be seasonal but can cause infections because of reservoir animals (Spengler et al. 2016). The sera samples in this study were collected at spring and summer seasons, and this could be a factor in the high seroprevalence because of the high activity of ticks. In eastern Türkiye, CCHF cases are often reported between June and August. However, western Türkiye has a longer tick season because of the warmer and milder weather compared to the east. This results in a seasonal pattern of vector incidence, watt the peak occurring between April and November. The western Türkiye region has recently become endemic for CCHF. Therefore, it indicates that the natural foci of CCHFV may spread further to other provinces in western Türkiye. Furthermore, it is crucial to take a variety of anti-epidemic actions targeted at reducing epizootic activity and boosting population immunity to CCHFV. Even though most people in the Western Mediterranean provinces live in cities, they mostly have their own farms where they grow their requirements of vegetables and fruits. Although the residents of the region do not farm, there is an increase in agricultural activity throughout the spring and summer months for this reason. This condition also has a high potential for enhancing the interaction of tick and host (Sorvillo et al 2020). For future seroprevalence studies that will be done in both rural and urban areas, like the provinces of Afyonkarahisar and Burdur, it may be helpful to think about the routines, ways of life, and interactions of the rural area. Additionally, it is clear that animal owners should be informed about such arboviral diseases and that they need to learn how to deal with vectors in such rural areas. Ticks, in particular, act as both mechanical and biological vectors. They allow pathogens to spread, which leads to re-transmission, and so they keep infections with them through all of their life stages. Increased CCHFV instances and recent environmental changes, such as global warming, that favored larger tick numbers, suggest that public health concerns regarding tick-borne illnesses will become more urgent in the western areas of Türkiye.

CONCLUSION

In endemic regions, CCHF is a threat to anyone who works closely with animals, including animal herders, livestock workers, and those who work in slaughterhouses. Individuals working in healthcare in endemic regions are at risk of contracting an infectious disease due to their unprotected contact with patients' blood and other bodily fluids. Travelers and locals alike who come into contact with hosts in endemic areas are at risk. According to the results of the study, CCHFV has the potential to function in equidae as a reservoir. We believe that the findings will aid in future research to manage the disease from a global public health perspective.

Ethical Permission: All procedures were approved by the Animal Ethics Committee (AEC) at Burdur Mehmet Akif University, Turkey (No: 102 / 912).

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RESEARCH ARTICLE

Genetic Animal Heritage of Anatolia: Short-beaked Pigeon Genotypes

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ABSTRACT

This study aimed to determine the morphological and morphometric characteristics of Bango, Mısıri and Baska pigeons, which have been preferred to breed as ornamental and diver pigeons in Anatolia. The ages of the pigeons were classified into four groups: 12-24 months of age (age group I), 25-36 months of age (age group II), 37-48 months of age (age group III), and 48 months of age and over (age group IV). These three pigeon genotypes were included in the bird group with short beak and small body structure. There were statistically significant differences among the genotypes in terms of body weight, head length-width, beak length-depth, chest depth-width, thoracic perimeter, tail and body length, wing span-length, tarsus diameter ($p \leq 0.01$). Considering the body plumage color of pigeons, Mısıri and Bango pigeons show more similar appearance, however Baska pigeons has a different appearance from both genotypes. However, it is thought that it would be appropriate to evaluate morphological data together with genetic analysis. We think that these three pigeon genotypes should be taken under immediate protection in order to protect the domestic gene resources of Turkey.

Keywords: Diver pigeons, Morphological characteristics, Ornamental pigeons, Short-beaked pigeons

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Anadolu'nun Genetik Hayvan Mirası: Kısa Gagalı Güvercin Genotipleri

ÖZ

Bu çalışma ile Anadolu'da süs ve dalgıç güvercini olarak yetiştirilmek üzere tercih edilen Bango, Mısıri ve Baska güvercinlerinin morfolojik ve morfometrik özelliklerinin belirlenmesini amaçlanmıştır. Güvercinlerin yaşları 12-24 aylık (yaş grubu I), 25-36 aylık (yaş grubu II), 37-48 aylık (yaş grubu III) ve 48 aylık ve üzeri olarak (yaş grubu IV) dört gruba ayrılmıştır. Bu üç güvercin genotipi kısa gagalı ve küçük vücut yapısına sahip kuş grubuna dahil edilmiştir. Vücut ağırlığı, baş uzunluğu-genişliği, gaga uzunluğuderinliği, göğüs derinliği-genişliği, göğüs çevresi, kuyruk ve vücut uzunluğu, kanat açıklığı-uzunluğu, tarsus çapı açısından genotipler arasında istatistiksel olarak anlamlı farklılıklar tespit edilmiştir ($p \leq 0.01$). Güvercinlerin vücut tüy rengi göz önüne alındığında Mısıri ve Bango güvercinleri daha benzer görünüm gösterirken Baska güvercinleri her iki genotipten farklı bir görünüme sahiptir. Türkiye yerli gen kaynaklarının korunması amacıyla bu üç güvercin genotipinin ivedilikle koruma altına alınması gerektiğini düşünmekteyiz.

Anahtar Kelimeler: Dalıcı güvercinler, Kısa gagalı güvercinler, Morfolojik özellikler, Süs güvercinleri

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INTRODUCTION

The location on the earth and regional topographic differences, Anatolia have a historical past with a wide biological diversity and local animal genetic resources (Şekercioğlu et al. 2011). The current situation has caused genetic differentiation within the species (Çiplak et al. 1993, Şekercioğlu et al. 2011). Considering these differences, it is possible to come across a large number of pigeon genotypes bred in different regions in Turkey. Mısıri, Bango, and Baska pigeons are short-beaked pigeons that have been widely bred for many years, especially around Istanbul in Turkey. The three genotypes used in the present study are in the subgroup of diving pigeons belonging to the performance bird group. After reaching a certain height in the sky, these pigeons dive immediately when a rotating tool made of a metal called a "glitter" or a female pigeon in the nest is shown (Yılmaz and Boz 2012). In the interviews made with the pigeon breeders, it was stated that due to the environmental changes such as urbanization and tall buildings in the regions where they were raised, the pigeons are bred as "ornamental birds" nowadays. They show similarities in terms of both their external appearance and flight characteristics, and there is a great demand for these genotypes from within the country and abroad. Obtaining some data based on observations and measurements in genotypes and classifying races with similar characteristics under a group is a method that has been used by variousresearchers for many years (Balcı et.al. 2018, Helms and Schneider 2003, Soysal et al. 2011). To date, no research has been conducted to morphologically define the genotypes of short-beaked pigeons, which are the genetic animal heritage of Anatolia. Therefore, the study aimed to compare the morphological and morphometric characteristics of the Bango, Mısıri, and Baska pigeon genotypes, which have been bred for many years in Anatolia. This research is very important for the identification of shortbeaked pigeons, which are among animal genetic resources in Turkey.

MATERIAL AND METHODS

Study area, Birds and Their Management

This research was conducted in İstanbul province (located at 41°00'19.0"N 29°00'43"E), Turkey in 2020. The animal materials of this study consisted of Bango (46 male and 54 female), Mısıri (52 male and 48 female), Baska (49 male and 51 female) pigeon genotypes. During the research, the birds were kept in the hands of the breeder and no change was made in the management, care, and feeding conditions of the pigeons. The sex and age of the pigeons were determined according to the records kept at the breeders. The ages of the pigeons were classified into four groups: 12-24 months of age (age group I), 25-36 months of age (age group II), 37-48months of age (age group III), and 48 months of age and over (age group IV).

Morphological Characteristics

Each pigeon was morphologically examined in detail concerning their head type, head marks, eye color, plumage color, body marks, wing and tail marks, presence or absence of muffs (foot-feathered). Tail and wing feather numbers were recorded. The wing feathers were counted in the order of the primary-axial and secondary (p-a-s) feathers (Erdem et al. 2021).

Morphometric Characteristics

Morphological measurements were carried out by visiting 16 private enterprises (5 enterprises for Bango pigeons, 7 enterprises for Mısıri pigeons, and 4 enterprises for Baska pigeons) in total. The body weights of the pigeons were individually weighed using a scale with sensitive to 0.01 g. While determining the morphometric characteristics, a metal ruler was used to detect body length, measuring tape was used to determine trunk length, wingspan, wing length, thoracic perimeter, and tail length. A digital caliper was used to determine the head length and head width, beak length and depth, chest width and depth, and tarsus diameter (Atasoy et al. 2013, Özbaşer et al. 2021).

Statistical Analysis

The general linear model (GLM) was used to identify the differences between age and sex groups. When a significant difference was found among groups for post hoc multiple comparisons, Tukey's test was used 4 (Özdamar 2015). A value of $p<0.05$ was considered statistically significant. Statistical analyses were performed using SPSS (IBM Corp., Armonk, NY, USA) for Windows.

RESULTS

Morphological Characteristics

As a mutual trait in all three pigeon genotypes examined, it was determined that the head structures were large and slightly oval-shaped. The forehead is wide and angular in all three genotypes. All three pigeon genotypes have a soft tissue on the upper side of their beaks, which is the thickened part of the skin (integument), called the 'cere' or 'nasal cere' which surrounds the nostrils. Cere shape and color are similar in all three genotypes, and it was described as small in structure, lime-white color, and powdery appearance. In addition, in pigeons, there was a featherless area around the eyes surrounded by a white or pale pink color, soft skin fold called as the 'orbital ring' or 'eye cere' (Figure 1 d, e, f). The

eyelids were fleshy. Iris colors were determined as color tones from light to dark brown in all of the pigeons examined (100%) (Table 1). The condition of having brown and black color tones in the irisis called "bull". The beak is short, thick and curved downward in all three genotypes. However, the beak structure of Baska pigeons was slightly longer than the other two short-beaked pigeon genotypes (Mısıri and Bango pigeons). It was determined that Mısıri and Bango pigeons were morphologically very similar to each other, but Baska was different from these two genotypes. The difference between the Mısıri and Bango genotypes is the curled feather structure called the "rosette". Mısıri pigeons have curly, frill-shaped feathers on the chest (Figure 1c). It was determined that all pigeons in the three genotypes examined in the present study were free from crest and muffs. Head, body and wing primary feathers are white, wings and/or tail are colored in Mısıri and Bango pigeon genotypes (Fig. 2a-j). While the colors seen on the wings and/or tail was considered by some researchers as 'marks' (Akçapınar and Özbeyaz 2021), fanciers refer to it as the plumage color used to identify the pigeon individually. The plumage color of the pigeons is given in Table 1 according to the wing and/or tail color declared by the pigeon breeders. It was determined that the coloration seen in either body parts (wing and tail) of these pigeons is uniform. It was determined that when the bird had colored wings in 5 both pigeon genotypes, varying numbers of white feathers were observed in the primary feathers on the wings with completely white plumage color were found in 5% and 3% of the Mısıri and Bango genotypes, respectively. In the Mısıri and Bango pigeon genotypes, the ratio of birds with colored wings only was determined as 40 % and 41 %, respectively. The ratio of colored tail birds in the same genotypes is 20 % and 26 %, respectively. In all three genotypes, white primaries, which we describe as 'mark', can be found on the wing, the number of which can vary between 4 and 8 (Table 1). In Baska pigeon, the head is white, the body, wings, and tail are colored (Figure 3 a-f). White feathers on the head cover the area to the beak line from the top of the head. This situation is defined as a mark. However, it is a breed characteristic in Baska pigeons. In some Baska pigeons, while the line view the of white feathers is straight (straight-mark line) (35%) (Figure 4a), this line in other is dashed and curved with a slight inward curve at the eye level (camber-mark line) (65%) (Figure 4b). Some Baska pigeons (43%) may have white primary feathers known as wing markings (Figure 1a,b). If this structure is not seen, the pigeons are called "Akbaş (white-head)" in Turkey. Red, yellow, black, chocolate (feather color is dark brown), blue (feather colour is blue, and the tip of wing primaries are covered with dark black bars), chickpea (nohudi in Turkish) (plumage color is blue and the tip of wing primaries are covered with light gray bars), lemon-yellow (limoni in Turkish) (feather colour is light yellow and tip of wing primaries are covered with dark yellow bars) colors were determined in all three pigeon genotypes (Figure 2, 3). In Baska genotype, as in the other two genotypes, no white feather color was observed on the trunk.

Morphometric Characteristics

The difference among genotypes was statistically significant in all morphological characteristics except the trunk length. Baska genotype had higher values than other genotypes in terms of body weight, head length, beak depth, tail length, wing span, tarsus diameter and body length ($p<0.01$). In all three genotypes, males had higher values than females in terms of body weight, head length, wing span-length, chest depthwidth, thoracic perimeter, and tarsus diameter ($p\leq0.01$). While body weight and head length were significantly affected by age group in all three genotypes ($p\leq0.05$), head width, body weight-length, head length-width, tail length and wing span in Bango and Mısıri genotypes were significantly affected by age 6 group ($p\leq0.05$). In the Baska pigeon, body weight, head length, chest depth-width, thoracic perimeter and tarsus diameter were significantly affected by the age group ($p\leq0.01$). In the present study, the morphological characteristics of all three pigeon genotypes are shown in Table 1 and the morphometric characteristics are shown in Table 2, 3.

Table 1. Morphological characteristics of Bango, Mısıri and Baska pigeons.

Morphological characteristics	Bango	Mısıri	Baska
	Ratio (%)		
Eye color			
Bull	100	100	100
Plumage color			
Black	26	23	17
Red	11	12	15
Yellow	23	18	10
Chocolate	9	14	16
Blue	13	15	25
Chickpea (Nohudi)*	10	5	10
Lemon-yellow (Limoni)**	5	8	7
White	3	5	-
Number of white feathers on wings			
4 to 6	36	37	27
6 to 8	31	31	16
8 and above	4	7	-
No white feathers on the wings	-	-	57
Number of wing feather			
9-1-8	73	69	-
10-1-9	27	31	-
9-1-11	-	-	55
10-1-9	-	-	25
10-1-10	-	-	20
Number of tail feather			
11	55	51	59
12	45	49	41

Chickpea*: Plumage colour is blue and tip of wing primaries are covered with light gray bars
Lemon-yellow**: Feather colour is light yellow and tip of wing primaries are covered with dark

Figure 1: a, b: The appearance of white feathers on the wings of Mısıri and Baska pigeons, c: Frill structure on chest in Mısıri pigeon, d, e and f: Eye cere and nasal cere in Bango, Mısıri and Baska pigeons, respectively.

Table 2. Body weight, wing, head and beak morphometric characteristics of Bango, Mısıri, Baska pigeons.

Variable		n	Body weight (g)	Wing span (cm)	Wing length (cm)	Head length (mm)	Head width (mm)	Beak depth (mm)	Beak length (mm)
	Sex		**	***	***	**	-	-	*
	Male	46	272.53 ± 2.94^{a}	56.56 ± 0.58^{a}	25.97 ± 0.25^{a}	33.17 ± 0.26^{a}	26.49 ± 0.20	5.36 ± 0.05	7.97 ± 0.09^{a}
	Female	54	260.38 ± 3.10^{b}	54.00 ± 0.47^{b}	24.86 ± 0.19^{b}	32.07 ± 0.24^{b}	26.53±0.41	5.22 ± 0.05	8.20 ± 0.07^{b}
	Age group		**	**	-	***	***	-	*
Bango	I	29	254.93 ± 1.39^{a}	53.27 ± 0.32^{a}	24.58 ± 0.15^{a}	31.15 ± 0.15^{a}	24.74 ± 0.28^{a}	5.23 ± 0.04	7.96 ± 0.12^{a}
	II	11	275.18 ± 5.98^{b}	54.00 ± 1.12^{ab}	24.72 ± 0.41^{ab}	33.77 ± 0.59^{c}	26.68 ± 0.12^{b}	5.36 ± 0.18	8.14 ± 0.09^{ab}
	III	28	269.08 ± 4.61^{b}	55.70 ± 0.79^{bc}	25.58 ± 0.33^{bc}	32.60 ± 0.29^{b}	26.03 ± 0.23^{b}	5.34 ± 0.07	7.94 ± 0.07^{a}
	IV	32	270.09 ± 4.74^{b}	56.85 ± 0.77^{c}	26.12 ± 0.32^{c}	33.44 ± 0.29^{bc}	28.49 ± 0.50^{c}	5.27 ± 0.05	8.32 ± 0.10^{b}
	Grand mean	100	265.97 ± 2.22	55.18 ± 0.39	25.37 ± 0.16	32.58 ± 0.18	25.74 ± 0.28	5.29 ± 0.04	8.09 ± 0.06
	Sex		*	***	***	*	-	-	-
	Male	52	274.16 ± 2.86^{a}	60.18 ± 0.30^{a}	27.00 ± 0.11^{a}	32.64 ± 0.29^{a}	27.22 ± 0.22	5.34 ± 0.37	8.10 ± 0.04
	Female	48	266.25 ± 2.71^{b}	56.23 ± 0.59^{b}	25.07 ± 0.26^{b}	31.88 ± 0.23^{b}	26.83 ± 0.38	5.22 ± 0.05	8.05 ± 0.06
	Age group		**	***	***	*	***	-	-
Mısıri	I	24	257.00 ± 1.38^{a}	54.36 ± 0.75^{a}	24.22 ± 0.34^{a}	31.43 ± 0.28^{a}	25.35 ± 0.31^{a}	5.24 ± 0.05	7.94 ± 0.11
	II	18	276.33 ± 4.02^{b}	58.77 ± 0.71^{b}	26.11 ± 0.30^{b}	31.96 ± 0.55^{ab}	26.95 ± 0.16^{b}	5.31 ± 0.12	8.11 ± 0.06
	III	27	274.05 ± 4.61^{b}	59.59 ± 0.57^{bc}	26.72 ± 0.24^{bc}	32.46 ± 0.36^{ab}	27.02 ± 0.28^{b}	5.32 ± 0.07	8.06 ± 0.06
	IV	31	270.36 ±2.00^{b}	59.91 ± 0.48^{c}	26.94 ± 0.11^{c}	32.94 ± 0.33^{b}	28.40 ± 0.48^{c}	5.26 ± 0.06	8.17 ± 0.04
	Grand mean	100	270.36 ± 2.00	58.29 ± 0.37	26.08 ± 0.17	32.27 ± 0.19	27.03 ± 0.21	5.28 ± 0.04	8.08 ± 0.04
	Sex		-	-	*	*	-	-	-
	Male	49	290.30 ± 1.84	59.36 ± 0.29	26.68 ± 0.09^{a}	35.73 ± 0.21^{a}	22.37 ± 0.11	5.12 ± 0.03	9.73 ± 0.08
	Female	51	286.37 ± 1.56	59.71 ± 0.20	27.00 ± 0.09^{b}	35.14 ± 0.19^{b}	22.02 ± 0.15	5.09 ± 0.02	9.62 ± 0.07
	Age group		*	-	-	***	-	-	-
Baska	I	24	291.57 ± 1.83^{b}	59.52 ± 0.18	27.10 ± 0.01	35.91 ± 0.35^{b}	21.79 ± 0.26	5.12 ± 0.04	9.65 ± 0.10^{ab}
	II	22	289.95 ± 1.96^{b}	59.68 ± 0.21	26.77 ± 0.07	35.49 ± 0.26^{ab}	21.77 ± 0.26	5.13 ± 0.04	9.43 ± 0.09^{a}
	III	35	288.92 ± 2.13^{b}	59.74 ± 0.32	26.84 ± 0.13	34.94 ± 0.19^{a}	22.45 ± 0.15	5.13 ± 0.03	9.72 ± 0.07^{ab}
	IV	19	281.10 ± 3.54^{a}	59.05 ± 0.65	26.60 ± 0.16	35.67 ± 0.38^{ab}	22.71 ± 0.57	5.01 ± 0.01	9.91 ± 0.15^{b}
	Grand mean	100	288.30 ± 1.21	59.54 ± 0.17	26.84 ± 0.06	35.43 ± 0.14	22.19 ± 0.09	5.11 ± 0.02	9.68 ± 0.05
			***	***	***	***	***	***	***
Genotype	**Bango**	100	267.18±1.88^{b}	53.59±0.29^{a}	25.38±0.13^{c}	32.63±0.17^{b}	26.42±0.17^{a}	5.30±0.03^{a}	8.05±0.05^{b}
	Mısıri	100	270.52±1.84^{b}	54.04±0.29^{a}	26.02±0.12^{b}	32.24±0.17^{b}	26.92±0.17^{a}	5.28±0.03^{a}	8.08±0.05^{b}
	Baska	100	289.32±1.84^{a}	51.37±0.29^{b}	26.84±0.13^{a}	35.55±0.17^{a}	22.32±0.17^{b}	5.10±0.03^{b}	9.68±0.05^{a}

ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. a–c: means within a column with different letters are significantly different ($p < 0.05$)

Table 3. Body length, chest, trunk, tail and Tarsus morphometric characteristics of Bango, Mısıri, Baska pigeons.

Variable			Body length (cm)	Chest depth (mm)	Thoracic perimeter (cm)	Chest width (mm)	Trunk length (cm)	Tail length (cm)	Tarsus diameter (mm)
	Sex		*	***	***	***	-	-	***
	Male	46	25.58 ± 0.37^{a}	56.23 ± 0.45^{a}	22.02 ± 0.16^{a}	54.69 ± 0.50^{a}	7.84 ± 0.09	12.04 ± 0.01	4.91 ± 0.06^{a}
	Female	54	26.48 ± 0.24^{b}	52.33 ± 0.41^{b}	21.12 ± 0.12^{b}	52.61 ± 0.45^{b}	7.77 ± 0.06	11.77 ± 0.14	4.66 ± 0.05^{b}
	Age group		***	***	-	-	-	***	-
Bango	I	29	24.71 ± 0.28^{a}	52.67 ± 0.72^{a}	21.30 ± 0.14	52.68 ± 0.54^{a}	7.75 ± 0.10	11.15 ± 0.20^{a}	4.67 ± 0.05
	II	11	27.34 ± 0.63^{b}	52.27 ± 0.54^{a}	21.54 ± 0.28	53.66 ± 0.85^{a}	7.90 ± 0.13	11.59 ± 0.20^{ab}	4.74 ± 0.12
	III	28	26.27 ± 0.51^{b}	54.06 ± 0.67^{a}	21.47 ± 0.18	51.90 ± 0.58^{a}	7.69 ± 0.11	11.78 ± 0.16^{b}	4.79 ± 0.07
	IV	32	26.68 ± 0.29^{b}	56.15 ± 0.52^{b}	21.79 ± 0.24	55.79 ± 0.63^{b}	7.92 ± 0.07	12.59 ± 0.13^{c}	4.87 ± 0.09
	Grand mean	100	26.09 ± 0.22b	54.13 ± 0.36	21.53 ± 0.11	53.56 ± 0.35	7.80 ± 0.05	12.10 ± 0.10	4.78 ± 0.04
	Sex		**	***	-	-	-	***	*
	Male	52	27.18 ± 0.12^{a}	57.03 ± 0.43^{a}	21.53 ± 0.15	54.47 ± 0.58	7.77 ± 0.07	12.40 ± 0.12^{a}	4.76 ± 0.04^{a}
	Female	48	26.63 ± 0.15^{b}	54.70 ± 0.46^{b}	21.45 ± 0.12	53.72 ± 0.40	7.91 ± 0.07	11.77 ± 0.14^{b}	4.62 ± 0.04^{b}
	Age group		***	-	-	**	**	***	-
Mısıri	I	24	26.51 ± 0.22^{a}	54.57 ± 0.63	21.45 ± 0.20	53.17 ± 0.42^{a}	7.88 ± 0.10^{ab}	11.62 ± 0.23^{a}	4.59 ± 0.04
	II	18	26.55 ± 0.19^{a}	55.99 ± 0.81	21.36 ± 0.15	53.41 ± 0.75^{a}	8.08 ± 0.12^{b}	11.47 ± 0.17^{a}	4.67 ± 0.09
	III	27	26.75 ± 0.22^{a}	56.20 ± 0.73	21.58 ± 0.16	53.22 ± 0.62^{a}	7.72 ± 0.11^{a}	12.12 ± 0.16^{b}	4.73 ± 0.05
	IV	31	27.58 ± 0.11^{b}	56.65 ± 0.53	21.53 ± 0.22	56.01 ± 0.36^{b}	7.77 ± 0.07^{ab}	12.80 ± 0.11^{c}	4.74 ± 0.07
	Grand mean	100	26.91 ± 0.10a	55.91 ± 0.34	21.49 ± 0.10	54.11 ± 0.36	7.84 ± 0.05	12.10 ± 0.09	4.69 ± 0.03
	Sex		*	*	-	-	-	-	-
	Male	49	29.18 ± 0.24^{a}	56.62 ± 0.48^{a}	19.97 ± 0.11	51.12 ± 0.23	8.01 ± 0.05	12.30 ± 0.11	4.33 ± 0.06
	Female	51	28.64 ± 0.12^{b}	53.02 ± 0.40^{b}	19.81 ± 0.10	51.26 ± 0.25	7.87 ± 0.06	12.36 ± 0.12	4.31 ± 0.06
	Age group		-	***	**	***	-	-	**
Baska	I	24	28.87 ± 0.17	54.73 ± 0.50^{a}	19.47 ± 0.10^{a}	50.31 ± 0.30^{a}	7.95 ± 0.08	12.62 ± 0.16	4.54 ± 0.01^{b}
	II	22	28.61 ± 0.26	56.86 ± 0.45^{c}	20.00 ± 0.13^{b}	52.35 ± 0.41^{b}	7.90 ± 0.07	12.31 ± 0.21	4.13 ± 0.05^{a}
	III	35	29.12 ± 0.31	52.46 ± 0.67^{b}	19.90 ± 0.12^{ab}	51.18 ± 0.21^{a}	7.97 ± 0.07	12.24 ± 0.12	4.28 ± 0.06^{a}
	IV	19	28.90 ± 0.17	56.75 ± 0.65^{c}	20.28 ± 0.23^{b}	51.04 ± 0.44^{a}	7.92 ± 0.01	12.15 ± 0.17	4.33 ± 0.11^{ab}
	Grand mean	100	28.91 ± 0.13c	54.79 ± 0.36	19.89 ± 0.77	51.19 ± 0.17	7.94 ± 0.04	12.33 ± 0.08	4.32 ± 0.04
			***	**	***	***	-	***	***
Genotype	**Bango**	100	25.98±0.19^{c}	54.37±0.30^{b}	21.55±0.09^{a}	53.59±0.3^{a}	7.82±0.05	11.78±0.09^{b}	4.80±0.04^{c}
	Mısıri	100	26.89±0.18^{b}	55.87±0.30^{a}	21.48±0.09^{a}	54.04±0.3^{a}	7.84±0.05	12.04±0.09^{b}	4.66±0.04^{b}
	Baska	100	28.82±0.16^{a}	55.01±0.30^{ab}	19.90±0.09^{b}	51.37±0.3^{b}	7.97±0.05	12.36±0.09^{a}	4.34±0.03^{a}

ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. a–c: means within a column with different letters are significantly different ($p < 0.05$)

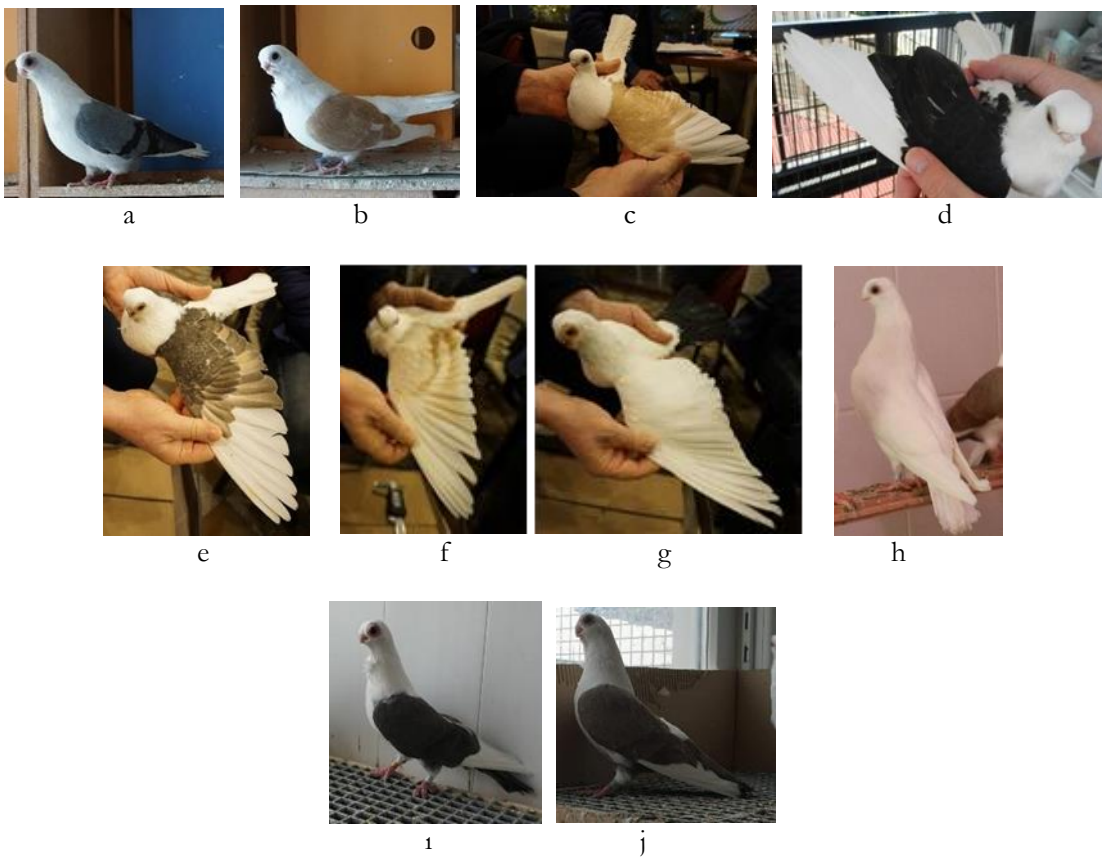

Figure 2: Plumage colors in Mısıri and Bango pigeons. a: Blue wing (Blue), b: Red wing (red), c: Yellow wing (yellow), d: Black wing (Black), e: Chocolate wing (chocolate), f: Lemon-yellow (Limoni) wing, g: Black tail (Black), h: White, ı: Black wing and tail (Black), j: Red wing and tail (red).

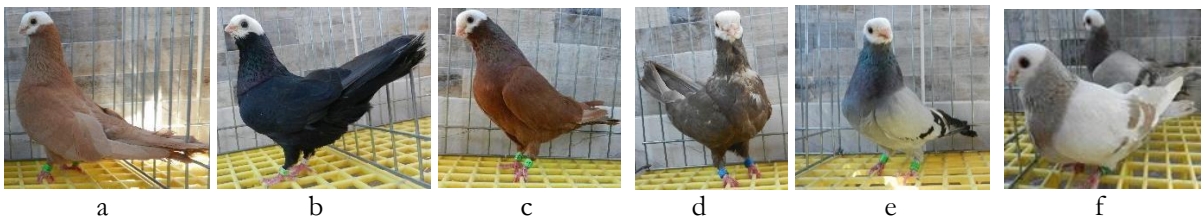

Figure 3: Plumage colors in Baska pigeons. a: Yellow, b: Black, c: Red, d: Chocolate, e: Blue, f: pigeon in front; Lemon-yellow pigeon in the back: Nohudi or chickpea.

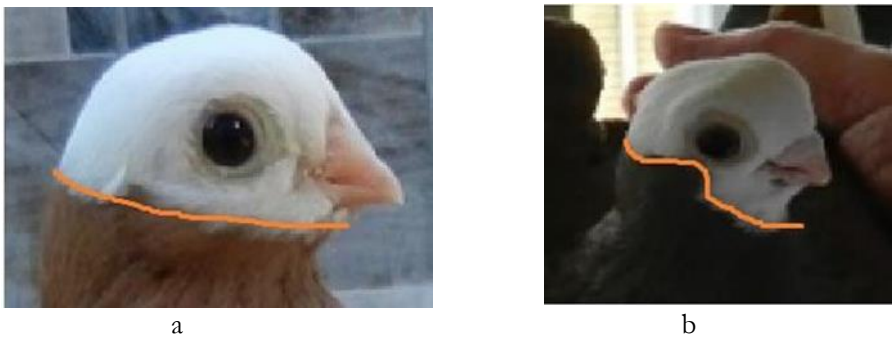

Figure 4: Baska pigeon a: Straight mark line, b: Camber mark line.

DISCUSSION

With the artificial selection applied by pigeon breeders according to behavior and body structure in the World, many pigeon breeds/genotypes have emerged that they are related but have different phenotypic diversity (Pacheco et al. 2020; Young et al. 2017). The genetic mechanism of variation in cranio-facial morphology in pigeons has been explained, and it has been argued that variation in headstructure, and variation in beak structure may be related. The head structure was slightly oval in all three pigeons (Mısırı, Bango, and Baska pigeons), the fore head was wide and angular. This structure of the head resembles the Turbit pigeons. Many authors have argued that there may be kinship between bird genotypes with similar head structures (Bailleul and Horner 2016, Felice and Goswami 2018, Levi 1992, Young et al 2017). The short beak structure seen in some pigeon breeds is one of the cranio-facial differences that enable the distinction between races. It is a feature that occurs as a result of variations in some gene locus and it is seen in many breeds (Adamčík et al 2021, Boer et al. 2021, Pacheco et al. 2020, Stringham et al. 2012).

In the present study, eye cere color (white or pale pink) was determined in three genotypes, and it was different from the yellow-orange, light blue, and red colors reported by Baptista et al. (2009) in different pigeon genotypes (Jambu Fruit-dove, Bare-faced Ground-dove, Diamond dove). The nasal cere, which can be seen among various bird species and breeds, can show differences in terms of texture, appearance, and color (Baptista et al. 2009, Purton 1989). It was determined that the nasal cere color determined in all three genotypes was similar to that domestic racing pigeon reported by Purton (1989), and the appearance of the nasal cere (flat and small in structure) was different from the exaggerated, hypertrophic and cauliflower-shaped appearance reported in Dragon and Carrier pigeons (Baptista 2009). It was determined that all three pigeon genotypes were short-beaked, non-muffled, non-crested and had a small body weight (under 300 g). Darwin (1875) classified pigeons into four groups based primarily on morphological traits, especially beak size. When Anatolian short pigeon genotypes were compared with some short-beaked pigeon breeds used in taxonomic classifications, it was determined that the body weight and non-crested appearance in the head were similar in Old German Owl and Berlin short-faced Tumbler pigeons. Moreover, it was determined that the pigeon genotypes examined in our study were similar to the body weight of Oriental Frill pigeons and similar to body weight, non-crested, non-muffled of African Owl, and Chinese Owl pigeons (Pacheco et al. 2020, Stringham et al. 2012). The frill structure determined in the breast region of Mısırı pigeons is also found in pigeons such as Turbit, Tubiten, Oriental Frill, African Owl, Cheese Owl, and Old German Owl (Pacheco et al. 2020, Levi 1992). However, the frill structure in Mısırı pigeons had less and sparse feathers.

Eye color variation in birds can be shaped by the pattern of melanin pigment or non-melanin pigments (pteridines, purines, carotenoids) in the iris (Edwards et al. 2012, Oliphant 1987). Bull, pearly white and orange iris colors are common in domestic pigeons (Maclary et al. 2021; Si et al. 2021). Blue, white and powder pink eye colors have also been determined in different genotypes of pigeons bred for performance purposes in Turkey (Balcı et al. 2018, Erdem et al. 2021, Özbaşer et al. 2021, Soysal et al. 2011). Hollander and Owen (1939) reported that the bull color is common in pigeons with white feather color. In our study, the fact that the head region of all three genotypes is white and the bull eye color is observed in all pigeons suggests that there may be a relationship between the pigmentation formed in the head and eye color. However, more studies are required on this subject.

Researchers report that there are many variations in feather pigmentation in pigeons due to artificial selection shaped over many years, mutations in gene loci, and the dominance or recession effects of colors (Domyan et al. 2014, Guernsey et al. 2013). In nature, some bird species can show some sexual dimorphism in terms of their color and color patterns. Generally, females have a monochromatic appearance to stay hidden in the nest, while males have a multicolored appearance for courtship behavior (Madeira 2018). In the present study, no dimorphism was found in terms of the plumage colors seen in males and females in all three genotypes. This situation may be related to the fact that these pigeons were reared under human control for many years and artificial selection. While body color was defined in the genotypes examined in our current study, it was observed that a pigmentation-like structure, which some researchers call 'Bar', was formed on the wings of pigeons with blue, chickpea plumage colors (Haag- WackernageL et al. 2006, Vickrey et al. 2018). T pattern and checker among the primary patterns reported by Haag-Wackernagel et al. (2006) were not found in all three genotypes. Similar body plumage colors were also found in Bango and Mısırı pigeons different from Baska. This suggests that it may be related to the fact that the pigeons were raised in the same region and that the breeders were influenced by each other. In addition, this suggests that these two genotypes may belong to similar subgroups of the same lineage in the phylogenetic tree. However, we think that this situation should be investigated with genetic studies.

It was determined that the average head length (32.58, 32.27 and 35.43 mm, respectively) and beak length (8.09, 8.08 and 9.68, respectively) values in Bango, Mısırı, and Baska pigeons were lower than the Owl (6.00 and 1.50 cm), Satinette (6.00 and 1.50 cm),

Suachandan (5.70 and 1.85 cm), Lakkha (6.00 and 1.95 cm) and Jacobin pigeons (5.00 and 1.85 cm) defined in the short-beak pigeons (Parvez et al. 2016). In addition to feeding, the beak in birds is involved in various functions such as warbling, fighting, thermoregulation, and preening (Van Wassenbergh and Baeckens 2019). Apart from genetic variations, it is thought that these factors may be effective in the differences in beak shape and morphology. Head width values of Bango, Mısıri, and Baska pigeons (25.74; 27.03 and 22.19 mm) were higher than Muradiye Dönek (18.20 mm) and Alabadem (18.51 mm) pigeons, which were defined as diving and ornamental birds (Erdem et al. 2021, Özbaşer et al. 2021). Flight style, optimum flight speed and power, flight distance in birds can vary depending on many factors such as the bird's body weight, chest depth, wingspan, and tail length (Bruderer et al. 2010, Dial 2009, Mercieca et al. 2017, Pennycuick et al. 1996, Shatkovska and Ghazali 2017, Thomas 1996). Average body weight (265.97 g; 270.36 g, and 288.30 g, respectively), wing length (25.37 cm, 26.08 cm and 26.84 cm, respectively), chest depth (54.13 cm, 55.91 cm and 54.79 cm, respectively) and tail length (12.10 cm, 12.10 cm and 12.33 cm, respectively) in Bango, Mısıri, and Baska pigeons in the present study were generally lower than some diver-spinner pigeons [Muradiye Dönek (319.74 g, 35.10 cm, 57.81 cm, and 13.14 cm)], tumbler [Ankara tumbler (321.62 g, 31.55 cm, 62.98 cm, and 13.45 cm)], roller [Mülakat pigeon (328.96 g, 65.43 cm, 59.02 cm, and 13.61 cm)] and ornamental bird [Alabadem (332.0 g, 32.0 cm, 58.12 cm and 12.71 cm)] in the group with a body weight of 250-350 g bred in Turkey (Atasoy et al. 2013, Erdem et al. 2021, Özbaşer et al. 2020, Özbaşer et al. 2021). In addition, the body weight and wingspan values of all three genotypes were found to be lower than the results obtained in Owl (301.80 g and 63.20 cm), Satinette (332.85 g and 60.80cm), Suachandan (336.50 g and 64.70 cm) pigeons raised abroad (Parvez et al. 2016). In birds, the tail is a structure that plays a role in body stability and maintaining balance during the flight (Thomas 1996). It was stated that long-tailed birds fly slower than short-tailed ones to save energy and to compensate for air resistance during flight (Baptista et al. 2009, Norberg 1995). The fact that especially the tail length and wingspan values were lower than the birds bred for performance purposes in the study suggests that these birds are currently bred for form purposes and are subject to selection in this direction. In addition, as a result of the data obtained and a general comparison with different pigeons, it is thought that these birds can be evaluated among low body weight pigeon breeds.

CONCLUSION

As a result of the present study, it was determined that there were statistically significant morphometric differences among the genotypes. Considering the body plumage color of pigeons, Mısıri and Bango pigeons show more similar appearance, however, Baska pigeons have different appearance from both genotypes. Despite these results, we think that it would be appropriate to evaluate morphological data together with genetic analysis. Therefore, we have performed the phylogenetic analysis studies using DNA samples taken from three genotypes. In addition, it can also be interpreted that these genotypes are well preserved by the breeders. However, for safer and guaranteed results, protected and controlled breeding should be carried out as soon as possible in order to protect these genotypes.

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Ethical Approval: This study was approved by the Tekirdağ Namık Kemal University, Animal Experiments Local Ethics Committee (Approval no: 2017-09)

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RESEARCH ARTICLE

Evaluation of Some Quality Characteristics of Fermented Sucuks Produced by Butchers with Traditional Methods in terms of Compliance with Standards

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ABSTRACT

This research was carried out to determine some microbiological and physicochemical characteristics of fermented sucuks produced by butchers in Siirt with traditional methods and reveal whether fraudulent practices were applied during production. It was determined that the mean total aerobic mesophilic microorganism count of sucuks was 7.06±0.47, coliform group microorganism count 4.66±1.50, *Escherichia coli* count 3.79±1.59, *Staphylococcus aureus* count 4.08±2.13, yeast and mold count 5.88±1.02 $\log_{10}$ cfu/g; moisture content 30.92%±8.20, pH value 5.41±0.45, water activity value 0.888±0.05. In addition, starch was encountered in twenty of the samples, putrefaction in twenty-eight, and blood presence in all of them. According to TS 1070, 6.67% of the examined sucuks were moisture content, 20% *E. coli*, 60% *S. aureus*, 93.33% coliforms, pH value and putrefaction, and according to Turkish Food Codex, 26.67% of them were not suitable in terms of pH value and 66.67% of starch presence. In conclusion, it was concluded that some samples with insufficient hygienic quality might pose a potential risk for public health. In order to obtain hygienic and standards-compliant products; producers should be made aware, hygienic measures should be taken at all stages from production to consumption, and inspections by competent authorities should be increased.

Keywords: Fermented Sucuk, Quality Characteristics, Traditional Production

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Kasaplar Tarafından Geleneksel Yöntemlerle Üretilen Fermente Sucukların Bazı Kalite Özelliklerinin Standartlara Uygunluk Yönünden Değerlendirilmesi

ÖZ

Bu araştırma, Siirt'teki kasaplar tarafından geleneksel yöntemlerle üretilen fermente sucukların bazı mikrobiyolojik ve fizikokimyasal özelliklerinin belirlenmesi, ayrıca üretim sırasında hile amaçlı uygulamaların yapılıp yapılmadığının ortaya konması amacıyla yapılmıştır. Sucukların ortalama toplam aerob mezofilik mikroorganizma sayısı 7.06±0.47, koliform grubu mikroorganizma sayısı 4.66±1.50, *Escherichia coli* sayısı 3.79±1.59, *Staphylococcus aureus* sayısı 4.08±2.13, maya-küf sayısı 5.88±1.02 $\log_{10}$ kob/g; rutubet miktarı %30.92±8.20, pH değeri 5.41±0.45, su aktivitesi değeri 0.888±0.05 olarak belirlenmiştir. Ayrıca, örneklerin yirmisinde nişastaya, yirmisekizinde kokuşmaya ve tamamında kan varlığına rastlanmıştır. İncelenen sucukların TS 1070'e göre %6.67'si rutubet miktarı, %20'si *E. coli*, %60'ı *S. aureus*, %93.33'ü de koliformlar, pH değeri ve kokuşma yönünden; Türk Gıda Kodeksi'ne göre ise %26.67'si pH değeri ve %66.67'si nişasta varlığı yönünden uygun bulunmamıştır. Sonuç olarak; yetersiz hijyenik kaliteye sahip bazı örneklerin halk sağlığı açısından potansiyel bir risk oluşturabileceği kanaatine varılmıştır. Hijyenik ve standartlara uygun ürünlerin elde edilebilmesi için; üreticiler bilinçlendirilmeli, üretimden tüketime kadar geçen tüm safhalarda hijyenik tedbirler alınmalı ve yetkili otoriteler tarafından yapılacak denetimler artırılmalıdır.

Anahtar kelimeler: Fermente Sucuk, Geleneksel Üretim, Kalite Özellikleri

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INTRODUCTION

Processing meats in different ways, especially conservation by fermenting it, has been practiced since ancient times (Anar 2015, Medić 2017, Demirok Soncu and Kolsarıcı 2019). Curing these meats also ensures the obtainment of reliable products in terms of chemical and microbiological properties (Ranken 2000, Öztan 2011, Gökalp et al. 2015b, Turantaş 2015, Leroy and De Vuyst 2016). According to the Turkish Food Codex Communique on Meat, Prepared Meat Mixtures, and Meat Products (Anonymous 2019); sucuk is defined as a fermented meat product that has a mosaic appearance and has not undergone heat treatment, after which bovine and/or ovine carcass meats and fats are minced, mixed with flavorings, and filled into natural or artificial casings, fermentation and drying processes under certain conditions. In other words, sucuk is a meat product produced with the minced meat of various animals or their mixtures (bovine, ovine, poultry) and fat after mincing through a meat grinder, mixed with salt, spices, and additives, filled into natural or artificial casings, kept in a certain temperature, moisture, and air circulation for a while, then drying by ripening (Bulduk 2013, Anar 2015, Medić 2017). The shelf life of fermented sucuks are more longer, furthermore, these products' taste, aroma, and texture properties become more pronounced due to the probiotic microorganisms and the biological substances formed (Anar 2015, Malo and Urquhart 2016, Lücke 2017, Ockerman and Basu 2017, Demirok Soncu and Kolsarıcı 2019).

The spices used in the production of sucuks have an antimicrobial effect, albeit limited, on the microflora that causes deterioration. At the same time, the development of lactic acid bacteria is supported by the effect of manganese ions in the spices (Turantaş 2015, Danilović and Savić 2017). Although fermented sucuks, which are unique to Turks, have similarities with sucuks produced in multiple European countries, the smoking process applied in production and the benefit from some molds in ripening (Nout 2007, Gökalp et al. 2015b) cause differences. Fermented sucuks can be processed industrially as well as can be produced by traditional methods. Therefore, depending on climatic conditions, socio-economic lifestyle, cultural differences, and consumer habits, different characteristic of sucuks are found in many parts of the world. While traditionally produced sucuks are only drying in Mediterranean countries, these in Middle and Northern European countries are applied drying and smoking process (Arslan 2013, Gökalp et al. 2015b, Lücke 2017, Medić 2017).

Sucuk, which is sold in natural or artificial casing in the forms of coil, baton, and finger is among they meat products have a high consumption rate in Türkiye (Arslan 2013, Tayar and Yıldırım 2020).

This meat product is classified as dry (<35%) and semi-dry (~50%) sucuks according to their moisture content (Gökalp et al. 2015b, Turantaş 2015) and heat-treated and untreated (fermented) according to temperature applications (Warriss 2010, Candoğan and Çarkçıoğlu 2015, Anonymous 2019, Tayar and Yıldırım 2020).

It has been reported in the TS 1070 fermented sucuk standard (Anonymous 2016) that sucuks should have maximum moisture of 40% by mass and pH values between 5.4-5.8, furthermore that there should be no pathogenic microorganisms and no putrefaction in the products. According to the Turkish Food Codex (Anonymous 2019), it is stated that the highest pH value in sucuks can be 5.4, also substances containing starch and non-meat-derived proteins cannot be added to the products (the sum of amount of spice-originated starch and herbal protein cannot exceed 1% by mass).

It has been reported in some studies on sucuks in Türkiye (Sancak et al. 1996, Atasever et al. 1998, Erdoğrul and Ergün 2005, Öksüztepe et al. 2011, Gürbüz and Çelikel Güngör 2018) that products with low microbiological quality and which may adversely affect public health were encountered in the productions made in technologically and hygienically unsuitable workshops. In addition, there are studies in sucuks that do not comply with the standards in terms of physicochemical properties were detected (Atala 1992, Yücel and Karaca 1993, Sancak et al. 1996, Pehlivanoğlu et al. 2015, Gürbüz and Çelikel Güngör 2018, İnce et al. 2018). This research was carried out to with the aim of some quality characteristics of fermented sucuks produced by traditional methods offered for consumption in butchers in Siirt, also to reveal whether there are fraudulent practices in production and determine whether these sucuks pose a risk in terms of public health. Thus, it is thought that raising the producers' awareness will contribute to the obtain of higher quality products, therefore consumers will be able to obtain healthier products.

MATERIAL AND METHODS

In this research, approximately 300-400 g thirty pieces fermented sucuks produced by traditional methods taken from the butchers in the city center of Siirt were used as material. Firstly, the microbiological analyses of fermented sucuks brought to the laboratory under cold conditions were made and the samples were kept at +4 ^{o}C until other analyses were complete

Microbiological Analyses

10 g of sample and 90 ml of buffered peptone water (Oxoid CM0509B) taken into sterile stomacher bags under aseptic conditions were homogenized in the stomacher (SJIA-04C, China) for two minutes, and decimal dilutions up to 10^{-8} were prepared (Harrigan 1998). Used in microbiological analyses the media, cultivation methods and incubation conditions are presented in Table 1. All colonies grown on Plate Count Agar (PCA) were counted as total aerobic mesophilic microorganism (TAMM). Purple/black color and colorless colonies grown on Eosin Methylen-Blue Lactose Sucrose Agar (EMBA) were defined as coliform group microorganisms, gas forming at 44.5 °C in EC Broth and metallic bright green colonies grown on EMBA were defined as *E. coli*. Brilliant black-colored colonies without halo (atypical) and halo (typical) growing on Baird-Parker Agar (BPA) were considered as *S. aureus* and colonies growing on Potato Dextrose Agar (PDA) were counted as yeast and mold (Harrigan 1998, Temiz 2010, Halkman 2019).

Physicochemical Analyses

The moisture amounts of the examined fermented sucuks was determined according to the method reported by Gökalp et al. (2015a). The pH values of the samples (Honikel 2014) were determined in the pH-meter (Mettler Toledo, SevenCompact™ S220, China) and the water activity (a_w) values (Welti-Chanes et al. 2007) were determined in the water activity device (Novasina, LabTouch®-a_w, Switzerland).

Biochemical Analyses

The presence of starch and indicate of putrefaction in sucuks were determined according to the method reported by Gökalp et al. (2015a), and the presence of blood was determined according to the method reported by Kayaardı et al. (2017).

Statistical Analyses

Statistical analyses of the findings obtained in the analyses were made in the SPSS 23.0 program (Anonymous 2015).

RESULTS

Microbiological analysis findings of fermented sucuks produced by traditional methods in butchers in Siirt were shown in Table 2, frequency distributions of microorganisms are in Table 3, physicochemical analysis findings are in Table 4, frequency distributions of the physicochemical findings are in Table 5, and biochemical analysis findings are in Table 6.

Table 1. Used in microbiological analyses the media, cultivation methods and incubation conditions

Microorganism	Medium	Cultivation	Incubation
TAMM	PCA (Oxoid, CM463)	Pouring	30 °C (24-48 h)
Coliforms	EMBA (Merck, 1.01347)	Pouring	37 °C (24 h)
E. coli	ECB (Merck, 1.10765), EMBA	Spreading	37 °C (24 h)
S. aureus	BPA (Merck, 1.05406)	Spreading	35-37 °C (18-24 h)
Yeast and mold	PDA (Oxoid, CM139)	Pouring	25 °C (4-5 day)

Table 2. Microbiological analysis findings of the examined fermented sucuks (log_{10} cfu/g)

Microorganism	Number of samples (n)	Minimum	Maximum	Mean±SD
TAMM	30	5.70	7.70	7.06±0.47
Coliforms	30	<1.00	6.30	4.66±1.50
E. coli	30	<2.00	4.60	3.79±1.59
S. aureus	30	<2.00	5.48	4.08±2.13
Yeast and mold	30	3.70	6.78	5.88±1.02

Table 3. Frequency distributions of microorganisms determined in the examined fermented sucuks

Number of microorganism (log_{10} cfu/g)	TAMM		Coliforms		*E. coli*		*S. aureus*		Yeast-mold	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
<1.00	-	-	2	6.67	-	-	-	-	-	-
1.00-1.99	-	-	-	-	-	-	-	-	-	-
<2.00	-	-	-	-	24	80.00	12	40.00	-	-
2.00-2.99	-	-	-	-	-	-	-	-	-	-
3.00-3.99	-	-	8	26.66	4	13.33	12	40.00	4	13.33
4.00-4.99	-	-	12	40.00	2	6.67	4	13.33	4	13.33
5.00-5.99	2	6.67	6	20.00	-	-	2	6.67	6	20.00
6.00-6.99	12	40.00	2	6.67	-	-	-	-	16	53.33
7.00-7.99	16	53.33	-	-	-	-	-	-	-	-

Table 4. Physicochemical analysis findings of the examined fermented sucuks

Physicochemical	Number of samples (n)	Minimum	Maximum	Mean±SD
Moisture (%)	30	12.93	42.82	30.92±8.20
pH	30	5.07	6.68	5.41±0.45
a_w	30	0.722	0.938	0.888±0.05

Table 5. Frequency distributions of physicochemical findings determined in the examined fermented sucuks

Moisture	**10.00-20.00**		**20.01-30.00**		**30.01-40.00**		**40.01-50.00**		**50.01-60.00**	
	n	%	n	%	n	%	n	%	n	%
	4	13.33	8	26.67	16	53.33	2	6.67	-	-
pH	**5.00-5.40**		**5.41-5.80**		**5.81-6.20**		**6.21-6.60**		**6.61-7.00**	
	n	%	n	%	n	%	n	%	n	%
	22	73.33	2	6.67	4	13.33	-	-	2	6.67
a_w	**0.700-0.750**		**0.751-0.800**		**0.801-0.850**		**0.851-0.900**		**0.901-0.950**	
	n	%	n	%	n	%	n	%	n	%
	-	-	2	6.67	2	6.67	10	33.33	16	53.33

Table 6. Biochemical analysis findings of the examined fermented sucuks

Biochemical	Negative		Less evident		Evident		Much evident	
	n	(%)	n	(%)	n	(%)	n	(%)
Starch	2	6.67	8	26.66	18	60.00	2	6.67
Putrefaction	2	6.67	22	73.33	6	20.00	-	-
Blood	-	-	16	53.33	8	26.67	6	20.00

DISCUSSION

Generally, total count of microorganism in foods is around 10^6-10^7 cfu/g is accepted as an indicator of the deterioration of these products. However, when the probiotics in fermented products are considered, this count can be up to 10^8 (Temiz 2015a, Danilović and Savić 2017, Nova et al. 2017). The mean count of total aerobic mesophilic microorganisms (TAMM) was determined as 7.06±0.47 $\log_{10}$ cfu/g in the fermented sucuks examined in this research (Table 2). The mean count of TAMM determined in traditionally produced these sucuks is similar to the findings of some researchers (Çon et al. 2002, Erkmen and Bozkurt 2004, Erdoğrul and Ergün 2005, Ed-dra et al. 2017). However, this value determined in fermented sucuks is higher than the values reported by Atasever et al. (1998) and Pehlivanoğlu et al. (2015), and lower than the values reported by Sancak et al. (1996), Öksüztepe et al. (2011), Gürbüz and Çelikel Güngör (2018), and Kaval et al. (2020). The fact that the count of TAMM was generally low in the sucuks examined in this research suggests that meat with antibiotic residues may have been used in production and that the sucuks were not sufficiently fermented according to the pH values determined in the samples. The differences between the studies may be due to the microbial load of the meat used as raw material and the act of different ripening processes applied to the sucuks.

The presence of coliforms group microorganisms in sucuks gives an idea about the hygiene of the enterprise and the reliability of these products (Erol 2007, Temiz 2015a). The mean count of coliform group microorganism in the samples examined in this research was determined as 4.66±1.50 $\log_{10}$ cfu/g (Table 2). This determined value is lower than the values reported by Pehlivanoğlu et al. (2015), Ed-dra et al. (2017), Gürbüz and Çelikel Güngör (2018) and Kaval et al. (2020), and higher than the values reported by Sancak et al. (1996), Çon et al. (2002), Erdoğrul and Ergün (2005), and Öksüztepe et al. (2011). It is thought to be important factors that the examined sample count in the studies, the hygiene approaches of the personnel during production, and the hygienic conditions of the enterprises are significant in the emergence of these differences. Along with these, it is also evaluated that preservatives may have been used during production in some enterprises. A significant correlation was determined between the count of coliform group microorganism in the sucuk samples with the count of *E. coli* and the amount of moisture at the level of $p<0.05$, and between the coliforms and the count of TAMM at the level of $p<0.01$. The amount of moisture available in foodstuffs and the mesophilic microorganisms in these foods affect coliform group microorganisms which are generally hygiene index, and therefore the count of *E. coli*. Since it is stated in the TS 1070 standard (Anonymous 2016) that there should be no pathogenic microorganisms in fermented sucuks, it is seen that 93.33% of the samples examined
in this research do not comply with this criterion (Table 3). This situation suggests that is not much attention to hygienic conditions during production. As a matter of fact, the detection of *E. coli* at the level of 3.00-3.99 $\log_{10}$ cfu/g in four samples and 4.00-4.99 $\log_{10}$ cfu/g in two samples (Table 3) supports this opinion.

The presence of indicator microorganisms in foods is evaluated as a sign of fecal contamination, and in this respect the most important microorganism is stated to be *E. coli* (Erol 2007, Laury et al. 2009, Temiz 2015a). The mean count of *E. coli* was determined as 3.79±1.59 $\log_{10}$ cfu/g in the samples examined in this research. This value; the mean values reported by Sancak et al. (1996) in Van (Türkiye) and Ed-dra et al. (2017) in Meknes (Morocco) as $4.6x10^3$ cfu/g and 3.69 $\log_{10}$ cfu/g, respectively were found similar. In terms of *E. coli*, it was observed that 20% of the samples examined did not comply (Table 3) with the criterion specified in the TS 1070 standard (Anonymous 2016). The detection rate of *E. coli* in fermented sucuks traditionally produced in Siirt was higher than the rate reported as 15% by some researchers (Erdoğrul and Ergün 2005, Öksüztepe et al. 2011), and lower than the rate reported as 30% by Kaynarca and Gümüş (2020). These differences in the detection rates of *E. coli* in studies, may have been caused by together with the hygiene of the enterprise and personnel, the facilities of the enterprises and the techniques used in the conservation of the products. Statistically, a significant correlation was determined between the count of *E. coli* and the count of yeast and mold at the level of $p<0.05$, between the count of *E. coli* with the presence of starch and putrefaction at the level of $p<0.01$. The starch in sucuks is broken down to simple sugars by microorganisms during fermentation. Pathogenic microorganisms such as *E. coli*, and yeast and mold also increase their activities by using these sugars. Also, compounds that cause putrefaction, such as sulfur, indole, and ammonia may arise especially depending on the proteolytic activity of microorganisms (Ranken 2000, Laury et al. 2009, Ünlütürk and Turantaş 2015). Because of these it is thought that the presence of starch determined in the samples examined supports the development of microorganisms and creates undesirable changes in the chemical composition of these sucuks.

In this research, the mean count of *S. aureus* in fermented sucuks was determined as 4.08±2.13 $\log_{10}$ cfu/g (Table 2), and eighteen of these sucuks were found non-compliant (Table 3) with the criterion specified in the TS 1070 standard (Anonymous 2016). This mean value determined in fermented sucuks is show similarities to the values specified in different sucuks by Sancak et al. (1996), Çon et al. (2002),

Öksüztepe et al. (2011), and Gürbüz and Çelikel Güngör (2018). Nevertheless, this count is higher than the value (3.33 $\log_{10}$ cfu/g) reported by Kaynarca and Gümüş (2020), and lower than the values reported as $3.2x10^5$ cfu/g and 4.85 $\log_{10}$ cfu/g, respectively, in some studies (Atasever et al. 1998, Pehlivanoğlu et al. 2015) where *Staphylococci/Micrococci* were detected together. As a result of the analyses, the detection rate (60%) of *S. aureus* determined in fermented sucuks; was higher than the rates reported by Erdoğrul and Ergün (2005) as 6.67% and by Öksüztepe et al. (2011) as 10%. The fact that the count of positive samples is very high in the sucuks examined in this research indicates that the necessary care is not given to the personnel and enterprise hygiene in the workshops where sucuk is produced in Siirt. The fact that a significant correlation ($p<0.01$) was determined between the count of *S. aureus* in fermented sucuks with the putrefaction, coliform group microorganisms, and the count of *E. coli* in the samples also supports this opinion. In addition, in parallel with the low TAMM count detected in the samples examined and the idea that the sucuks are not sufficiently fermented, it is evaluated that even if there are antibiotic residues in the meat used in production, *Staphylococci* resistant to these residues can reproduce. Therefore, it should not be forgotten that *S. aureus* and other pathogenic microorganisms will continue their activities under unsuitable hygienic conditions and pose potential hazards for public health, without being forgotten all enterprises should take finically necessary precautions. As a matter of fact, *S. aureus* found in meat products is one of the most important pathogens that cause food infections and poisonings in humans (Erol 2007, Nørrung et al. 2009, Ünlütürk and Turantaş 2015).

Except for some products (Italian type salami, roquefort cheese, camembert cheese, herbal-originated tempe, bogkrek, miso, soy sauce) produced using special molds (Perrone et al. 2015, Turantaş 2015, Magistà et al. 2017), mold growth in foods is an undesirable situation (Ünlütürk and Turantaş 2015, Kameník 2017, Lücke 2017, Halkman 2019). The yeast and mold count, which was determined as 5.88 ± 1.02 $\log_{10}$ cfu/g on mean in the sucuk samples examined in this research (Table 2), it was found similar to Sancak et al. (1996), Erdoğrul and Ergün (2005) and Pehlivanoğlu et al. (2015), but lower than the findings of some researchers (Atasever et al. 1998, Çon et al. 2002, Erkmen and Bozkurt 2004, Gürbüz and Çelikel Güngör 2018, Kaval et al. 2020). It is thought that the microbiological load of raw materials and additives used in production and also the ripening and storage conditions of sucuks may be effective in the differences between the studies. A significant correlation at the level of $p<0.05$ was determined between the yeast and mold count and presence of blood in fermented sucuks. However, this correlation between yeast and mold count with coliform group microorganism count, moisture content, a_w value and putrefaction were found at the level of $p<0.01$. The high moisture amount and a_w value in sucuks, together with the chemical composition of the blood support the development of microorganisms with proteolytic activity (Arslan 2013, Temiz 2015b, Danilović and Savić 2017, Kameník 2017). Therefore, also in the putrefaction determined in the samples examined in this research, it is thought that the low microbiological quality of the raw material used in the production and the presence of blood in the sucuks are effective.

The moisture content in meat and its products reveals the physical, chemical, microbiological and sensory quality and nutritional value of the product, also gives information about whether the ripening is done completely (Erol 2007, Warris 2010, Gökalp et al. 2015a). The mean moisture amount, which was determined as $30.92\%\pm8.20$ in this research (Table 4), is similar to the findings reported by Kuyumcu and Yuttagül (2000) and Erdoğrul and Ergün (2005). However, this amount was found higher than Atasever et al. (1998) findings, and lower than the findings of some researchers (Atala 1992, Sancak et al. 1996, Öksüztepe et al. 2011, Pehlivanoğlu et al. 2015, Gürbüz and Çelikel Güngör 2018). It is thought that, in the emergence of these differences, the use of high moisture content and undervalued in production of meat, the removal status of blood from these meats used in pieces, the amount of fat in the sucuk dough, and the drying, ripening and storage conditions during production may be effective. It was stated in the TS 1070 fermented sucuk standard (Anonymous 2016) that the maximum moisture amount in sucuks could be 40%, and it was observed that, in terms of moisture amount, two samples examined in this research (Table 5) did not comply with the criterion specified in the relevant standard.

The pH and a_w values of foods are the most important internal factors that affect the growth of microorganisms (Erol 2007, Warriss 2010, Temiz 2015b, Karastogianni et al. 2016). These values provide information regarding the freshness and quality of the food, also the suitability of the storage and conservation conditions applied to the food (Ranken 2000, Warriss 2010, Gökalp et al. 2015a). In this research, the mean pH value of fermented sucuks was determined as 5.41 ± 0.45 (Table 4), and it was seen that eight (26.67%) of the samples did not comply with the Turkish Food Codex (Anonymous 2019), and twenty-eight (93.33%) of them did not comply with TS 1070 (Anonymous 2016) (Table 5). The mean pH value determined in the traditionally produced fermented sucuks produced in Siirt was similar to the values reported by some researchers (Sancak et al. 1996, Erdoğrul and Ergün 2005, Gürbüz and Çelikel Güngör 2018, Kaynarca and Gümüş 2020). However, this value is higher than the values reported by Pehlivanoğlu et al. (2015), Poçan et al. (2015) and Kara et al. (2021), and lower than the values reported by Erkmen and Bozkurt (2004) and

Kaval et al. (2020). The determination of different pH values in the studies on sucuks may have resulted from the microbiological quality of the meat used in production and therefore the sucuks, their moisture amount, and the fermentation conditions in the ripening of the products. As a matter of fact, in this research the detection of high blood levels in samples with low pH values, which adversely affects the microbiological quality, supports this idea.

The mean a_w value of 0.888±0.05 in fermented sucuks (Table 4) was lower than the findings reported by Sancak et al. (1996) and Kaval et al. (2020). Generally, a_w values are between 0.850-0.920 in dried and cured fermented sucuks, and 0.930-0.970 in moist sucuks. When a_w value in foods is >0.800, yeast and molds increase their activity, causing foods to deteriorate and putrefaction more quickly (Ranken 2000, Bulduk 2013, Temiz 2015b). In this research, a statistically significant correlation at the level of $p<0.05$ was determined between the a_w value and putrefaction in the fermented sucuks. In addition, in some of the samples with high a_w values, the count of yeast and mold was observerd to be high, and a significant correlation ($p<0.01$) was also found between the a_w value in the samples with the number of yeast and mold, the amount of moisture and the pH value. In this research on the traditionally produced fermented sucuks in Siirt, the a_w value was determined ≤0.800 in two (6.67%) examined samples (Table 5). When this situation and statistical analysis results were evaluated together, it was concluded that most of the sucuks examined were not sufficiently dried, and ripening was done under unsuitable conditions. Because of these, also products with low economic value and poor quality can be encountered in the market.

Presence of starch was not found in only two of the samples examined in this research, and in eight of the samples, in less evident level presence of starch, which is thought to be caused by the spices used in production, was detected (Table 6). In the studies on sucuks in Izmir and Bursa, it was reported that starch was encountered in 2% (Atala 1992) and 28.5% (Yücel and Karaca 1993) of the samples examined, respectively. The incidence rate of starch (66.67%) in fermented sucuks in this research was higher than the findings of the relevant researchers. In the Turkish Food Codex (2019), it has been stated that non-meat proteins, starch and starch-containing substances, soy and soy products cannot be added in the production of sucuks, however the total amount of starch and herbal protein originating from spices cannot exceed 1% by mass. Accordingly, it was observed that 66.67% of fermented sucuks produced by traditional methods by butchers in Siirt did not meet the relevant criterion in terms of presence of starch (Table 6). Especially in emulsified products such as sausage and salami, to bind absorb excess water and give the product a good texture, starch, different grain flours, some protein products, and skimmed milk powder can be added in certain proportions (Anar 2015, Gökalp et al. 2015a, Leroy and De Vuyst 2016, Medić 2017, Anonymous 2019). According to the findings obtained and these properties that starch can add to the product, it is thought that this additive detected in the examined samples may have been used for fraudulent purposes.

Only two of fermented sucuks (6.67%) produced by the butchers in Siirt with traditional methods did not encounter any putrefaction. However, it was determined that twenty-two of the samples (73.33%) had less evident and six of them (20%) had evident putrefaction (Table 6). It was observed that 93.33% of the samples examined did not comply with the relevant standard (Table 6) since it is stated in the TS 1070 fermented sucuk standard (Anonymous 2016) that the putrefaction tests on sucuks should be negative. In addition, the detection of indicates of putrefaction in most of the fermented sucuks examined in this research does not coincide with the researchers' (Yücel and Karaca 1993, Erdoğrul and Ergün 2005) findings, who stated that no putrefaction was found in any of the products they examined. This situation suggests that meat with indicates of putrefaction and poor hygienic quality may have been used to produce sucuks or that the produced sucuks were stored under non-appropriate conditions. As a matter of fact, in meat products, which are easily decomposition as a result of physical and chemical reactions, begin to rot rapidly, especially with the effect of proteolytic microorganisms (Paramithiotis et al. 2009, Gökalp et al. 2015a, Ünlütürk and Turantaş 2015).

In order to ensure standard and quality sucuk production, pale, soft, and exudative (PSE), and dark, firm and dry (DFD) meats should be used at a maximum of 20%, the meats should be rested at 4 °C for 24-48 hours (rigor mortis), and the meats to be processed in pieces should be kept in steel strainers, and the blood is required to be drained thoroughly (Warriss 2010, Arslan 2013, Anar 2015, Gökalp et al. 2015b). Although, in the literature review, no research was found examining the presence of blood in sucuks, blood presence was observed less evident in 53.33%, evident in 26.67%, and much evident in 20% of fermented sucuks in this research. In addition, a significant correlation ($p<0.01$) was determined between the presence of blood in the samples examined with the putrefaction and the presence of starch. This situation suggest that starch and similar products, offal with high blood content and low value (head meat, lung, spleen, heart meat, diaphragm muscle) or unrested meat may have been added to sucuks.

CONCLUSION

As a result, it was determined that some fermented sucuks traditionally produced in Siirt do not comply with the criteria specified in the relevant standards in terms of coliform group microorganism, *E. coli* and *S. aureus* count, moisture amount, pH value, presence of starch, and putrefaction. Moreover, in all of the examined sucuk samples, the presence of blood was encountered at an extent that is enough to cause negativities in terms of food hygiene and technology. According to the findings, it has been evaluated that unhygienic and non-standard manufactured these products can pose potential public health problems. However, it has been concluded that some manufacturers are trying to gain an unfair advantage by means of poor-quality products. Because of these, technological conditions in the region should be improved and this product with high economic value production should be provided in a standard way. In addition, awareness-raising activities should be increased for the butchers manufacturing in sucuk production and the personnel working in the enterprises, and together with the inspections made by the authorized institutions should be contribute to expanded to preventive medicine.

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RESEARCH ARTICLE

Determination of Lipid Profile in Anatolian Native Horses

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ABSTRACT

The aim of this study was to evaluate the lipid profile of Anatolian native horses. The study material consisted of 100 Anatolian native horses of different sexes, between the ages of 3-10, living under similar nutritional conditions in Kars, Ardahan and Iğdır regions. All results are given as Mean±SE. To determine the lipid profile of Anatolian native breed horses, total cholesterol (TC), triglyceride (TG), high-density lipoproteincholesterol (HDL-C), very low-density lipoprotein-cholesterol (VLDL-C) and low-density lipoprotein-cholesterol (LDL-C) levels were determined (respectively; 117.19±2.73, 75.36±1.11, 57.45±1.21, 19.13±0.30, 30.75±6.45 mg/dL,). As a result; In this study, lipid profile was determined for the first time in Anatolian native horses.
Keywords: Anatolia, Cholesterol, Horse, Lipid profile, Triglyceride

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Anadolu Yerli Atlarında Lipit Profilin Belirlenmesi

ÖZ

Bu çalışmanın amacı, Anadolu yerli ırkı atlarının lipid profilini değerlendirmekti. Çalışma materyalini Kars, Ardahan ve Iğdır bölgesinde benzer beslenme şartlarında yaşayan, 3-10 yaş aralığında, 100 adet farklı cinsiyetteki Anadolu yerli ırkı attan oluşmuştur. Tüm sonuçlar Mean±SE olarak verildi. Anadolu yerli ırkı atlarının lipid profilini belirlemek için ölçülen total kolesterol (TC), trigliserid (TG), yüksek yoğunluklu lipoproteinkolesterol (HDL-C), çok düşük yoğunluklu lipoprotein-kolesterol (VLDL-C) ve düşük yoğunluklu lipoprotein-kolesterol (LDL-C) seviyeleri sırasıyla 117,19±2,73, 75,36±1,11, 57,45±1,21, 19,13±0,30, 30,75±6,45 mg/dL olarak belirlendi. Sonuç olarak; yapılan bu çalışma ile Anadolu yerli atlarında ilk kez lipid profil belirlenmiştir.
Anahtar Kelimeler: Anadolu, At, Kolesterol, Lipid profil, Trigliserit

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INTRODUCTION

Horses have been bred for agricultural, transportation, military and sportive purposes throughout history and are one of the important elements of animal husbandry. However, due to technological developments in recent years, there has been a serious decrease in their number. While the number of horses in Turkey was 90,007 in 2020, it decreased by 7.2% in 2021 to 83,568 (TÜİK 2022).

The Anatolian native horse, which is one of the basic Turkish horse breeds, is considered to be the breed with the highest number in the Turkish horse population (Hendricks 1995, Kırmızıbayrak et al. 2004, Bayram et al. 2005, Yılmaz and Ertuğrul 2011, Yılmaz 2012, Çelik et al. 2015). These horses, which are small in size and commonly seen in central Anatolia, have all the color and decorations (Taşkın and Koçak 2010). Anatolian native horses, which show slow development, are also very resistant to diseases and adverse environmental conditions (Yılmaz and Ertuğrul 2011). In addition, these horses have strong legs and nails (Taşkın and Koçak 2010, Yılmaz, 2012). In studies on the effects of environmental conditions on horses, changes in lipid metabolisms are mentioned (Hasso et al. 2012). Therefore, geographic lipid profile is important in animal breeds and physiological effect assessments.

Cholesterol, cholesterol esters, triglycerides and phospholipids are the main plasma lipids (Kocaman and Fidancı 2016). Lipids and lipoprotein profile are used to evaluate the nutritional status of animals and to diagnose metabolic diseases such as ketosis, abomasum displacement and hypocalcemia (Raphael et al. 1973, Nazifi et al. 2002, Civelek et al. 2007, Kocaman and Fidancı 2016, Tunc et al. 2017). It has also been reported that lipid profile may differ according to breeds (Kedzierski and Bergero, 2006).

With this study, it was aimed to determine the lipid profile [total cholesterol (TC), triglyceride (TG), high-density lipoproteincholesterol (HDL-C), very low-density lipoprotein-cholesterol (VLDL-C), and low-density lipoprotein-cholesterol (LDL-C)] of Anatolian native horses and to evaluate the effects of age and gender differences on the lipid profile.

MATERIALS AND METHOD

Animal Material

In this study, it was carried out between January 01, 2022 and February 1, 2022, in Kars, Ardahan and Iğdır regions, which are important in terms of animal husbandry in Turkey, to determine the lipid profile of Anatolian native horses. In the study, 100 Anatolian native horses of different ages (between 3-10 years of age) and gender were used. Blood samples taken from the vena jugularis of the horses used in the study were centrifuged at 3000 g for 10 minutes and stored at -20 °C until analysis. The horses used in the study were selected by random sampling method.

Measuring Lipid Profile

Values of serum TC, TG, HDL-C, VLDL-C and LDL-C were measured with Cobas C501 autoanalyzer (Roche-Cobes, Switzerland).

Statistical Analysis

SPSS 20 package program was used for statistical analysis of the obtained data. In order to compare the changes in the parameters used to determine the lipid profile in horses; T-test was used independent of gender and ANOVA was used according to age changes. All results are given as Mean±SE. Results were presented as a table in all data.

RESULTS

In the study, the mean levels of TC, TG, HDL-C, VLDL-C and LDL-C in Anatolian native horses were determined as 117,19±2,73, 75,36±1,11, 57,45±1,21, 19,13±0,30, 30,75±6,45 mg/dL, respectively.

In the study, 100 horses of Anatolian native breed were used and 92% (92/100) of these animals were female and 8% (8/100) were male.

When Table 1 was examined, although there was a numerical increase in TC and LDL-C levels in female horses, no statistically significant difference was found ($P>0.05$).

The horses used in the study; 40% (40/100) are 5 years old and under, 36% (36/100) are 6-7 years old and 24% (24/100) are 8 years old and over.

When Table 2 was examined, although TC, HDL-C and LDL-C levels were higher in horses aged 6-7 years, no statistically significant difference was found ($P>0.05$).

Table 1. Changes of lipid profile by gender

Parametres	Gender	N	Mean±SE	T/P
TC (mg/dL)	Male	8	112,15±3,72	T= 0,852 P>0,05
	Female	92	117,49±1,82	
TG (mg/dL)	Male	8	76,66±3,67	T= 0,344 P>0,05
	Female	92	75,24±1,17	
HDL (mg/dL)	Male	8	58,06±4,82	T= 0,146 P>0,05
	Female	92	57,40±1,26	
VLDL (mg/dL)	Male	8	19,73±1,20	T= 0,593 P>0,05
	Female	92	19,07±0,31	
LDL (mg/dL)	Male	7	22,30±2,54	T= 0,360 P>0,05
	Female	92	31,39±6,93	

TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoproteincholesterol, VLDL-C: Very low density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol.

Table 2. Changes of lipid profile parameters according to age

Parametres	Age	N	Mean±SE (Min-Max)	P
TC (mg/dL)	≤5	40	117,19±2,73 (89,60-149,80)	P>0,05
	6-7	36	118,83±2,91 (85,70-168,40)	
	≥8	24	114,21±3,30 (83,70-136,80)	
	Total	100	117,06±1,70 (83,70-168,40)	
TG (mg/dL)	≤5	40	73,76±1,65 (55,20-91,40)	P>0,05
	6-7	36	76,05±2,23 (58,60-110,60)	
	≥8	24	76,97±1,65 (62,90-92,40)	
	Total	100	75,36±1,11 (55,20-110,60)	
HDL (mg/dL)	≤5	40	57,49±1,74 (38,20-81,60)	P>0,05
	6-7	36	58,73±2,24 (39,20-81,90)	
	≥8	24	55,48±2,48 (39,20-80,60)	
	Total	100	57,45±1,21 (38,20-81,90)	
VLDL (mg/dL)	≤5	40	19,26±0,45 (14,60-26,50)	P>0,05
	6-7	36	19,27±0,54 (13,80-24,00)	
	≥8	24	18,69±0,60 (13,80-23,80)	
	Total	100	19,13±0,30 (13,80-26,50)	
LDL (mg/dL)	≤5	39	23,22±1,38 (10,90-44,20)	P>0,05
	6-7	36	40,38±1,63 (9,80-55,20)	
	≥8	24	28,52±2,35 (9,60-55,20)	
	Total	99	30,75±6,45 (9,60-65,20)	

TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoproteincholesterol, VLDL-C: Very low density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol.

DISCUSSION AND CONCLUSION

Anatolian native horse; it's one of the basic Turkish horse breeds with a small build, slow development, all color and insignia, resistant to diseases (Taşkın and Koçak 2010, Yılmaz and Ertuğrul 2011, Çelik et al. 2015).

Lipid profile is used to evaluate the nutritional status of animals and to diagnose metabolic diseases (abomasum displacement, hypocalcemia, ketosis, etc.) (Raphael et al. 1973, Nazifi et al. 2002, Kocaman and Fidancı 2016). Therefore; reference hematological and biochemical values are needed to compare them with abnormal values indicative of a disease state.

It has been reported that the level of TG in horses is between 4-44 mg/dL (Altıntaş and Fidancı 1993). In different studies, TG levels were reported as 36.9±2.25, 25.50±42.03 and 23.63±9.09 mg/dL in British, Arabian and Turkmen horses, respectively (Özcan et al. 2002, Mohri et al. 2005, Oktay and Eren 2014). In another study, TG levels were reported as 11.88, 22.86, 38.70 and 41.04 mg/dL in Yili, Kazakli, Yanqi and Crossbred horses, respectively (Xinxin et al. 2021). In Iranian racehorses, the TG level was determined as 93.81±1.77 (Hasso et al. 2012). In our study, TG levels in Anatolian native horses were lower than Iranian racehorses, and higher than British, Arabian, Turkmen Yili Kazakh, Yangi and Crossbreed horses.

It has been reported that the level of TC in horses is 75-150 mg/dL (Kaneko et al. 1997). In different studies, TC levels in Turkmen, American Morgan and Arabian horses have been reported as 93.84, 98.6, 98.88 mg/dL, respectively (Mohri et al. 2005, Nadeau et al. 2006, Kocaman and Fidancı 2016). In another study, the TC level was found to be 116.22±1.93 in Iranian racehorses (Hasso et al., 2012). Although the TC level determined in our study was within the reference value range and parallel to the level of Iranian racehorses, it was found to be higher than the Turkmen, American Morgan and Arabian horses.

High-density lipoproteincholesterol, VLDL-C and LDL-C levels has been reported 47.87, 3.72, 114.28 mg/dL in Turkmen horses, 50.11±2.11, 2.42±1.37, 38.68±3.98 mg/dL in Arabian horses and 57.92±0.03, 21.24±0.02, 35±1.54 mg/dL in Iranian racehorses (Nazifi et al. 2003, Kocaman and Findancı 2016). In a different study, Yili, Kazakh, Yanqi and Crossbred horses HDL-C levels were reported as 16.02, 24.12, 20.7, 28.44 mg/dL, and LDL-C levels as 39.24, 36.72, 60,12, 57.96 mg/dL, respectively (Xinxin et al. 2021). In our study, HDL-C, VLDL-C and LDL-C levels in Anatolian native horses were found to be similar to Iranian racehorses. However, when compared to other breeds, HDL-C and VLDL-C levels were higher in Anatolian native horses, and LDL-C levels were lower than other breeds. HDL-C and VLDL-C reveal the rate of lipid processing that can be used when needed. Low levels of LDL-C may be due to high lipid flows (Weber 2009). This situation can be explained by the resistance of horses to geographical conditions.

In a study conducted on Turkmen horses, it was reported that TC, TG, HDL-C, VLDL-C and LDL-C levels increase with increasing age (Nazifi et al. 2003). In another study, it was reported that TC, TG and VLDL-C levels increased until the age of 5 years and then decreased (Hasso et al. 2012). In our study, although TC, HDL-C and LDL-C levels were found to be high in horses aged 6-7 years, no statistically significant difference was found.

Factors such as gender can affect blood parameters (Lumeij and Bruijne 1985). However, in a study conducted on Turkmen horses, it was reported that gender had no effect on the lipid profile of horses (Nazifi et al. 2003). In our study, although there was a numerical increase in TC and LDL-C levels in female horses, no statistically significant difference was found. It is thought that this may be due to the insufficient number of males in the animals used in the study.

As a result; In this study, lipid profile was determined for the first time in Anatolian native horses. It has been reported that lipid profile may differ according to breeds (Kedzierski and Bergero 2006). The results of the study revealed that the lipid profiles of Iranian racehorses and Anatolian native horses are similar. It can be said that this is due to the fact that the vegetation and plant-rich soils of the Iran-Turan region are similar to the Kars, Ardahan and Iğdır regions. It has been reported that lipid profile may differ according to breeds (Kedzierski and Bergero 2006). In our study, it can be said that breed changed the lipid profile, while age and gender changes did not. It has been reported that animals can reveal unique mechanisms in energy storage and transport depending on effort and environmental conditions (Weber 2009). Although high effort and work do not change the gross muscle mitochondrial composition ratios, it has been reported that the mitochondrial content can increase by as much as twofold when looking at physiological integration (Davies et al. 1981). It has also been reported that the season increases the TC level (Gündüz et al. 2000). For these reasons; it can be said that care, feeding methods and environmental conditions (altitude, climate, etc.) cause changes in the lipid profile of animals.

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RESEARCH ARTICLE

The Comparison of Two Different Intrauterine Treatment Efficiency in Repeat Breeder Cows

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ABSTRACT

It is known that repeat breeder cases in cows cause severe economic losses. This study was purposed to evaluate the efficacy of different treatment protocols in intrauterine applications for subclinical genital tract infections in repeat breeder cows. In the present study, 75 Holstein-Fleckvieh cows were used. Cows that constitute the material of the study were divided into three groups randomly; 10 ml of cefquinom sulfate + 5 ml of dexamethasone sodium phosphate + 10 ml of 30% dextrose three times with an interval of one day by intrauterine route in Group I; spray containing 100 mg of rifaximin (13.4 grams) was applied to Group II. Animals in Group III did not undergo any treatment procedures and were evaluated as a control group. The pregnancy rate results of the first artificial insemination performed after intrauterine applications were determined as 32% (8/25) in Group I, 20% (5/25) in Group II and 20% (5/25) in Group III (P>0.05). It was concluded that it would be appropriate to investigate the intrauterine drug combination used in the presented study more thoroughly by increasing the number of animal materials and evaluating different factors affecting the repeat breeder case.

Keywords: Cow, Intrauterine Treatment, Repeat Breeder

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Repeat Breeder Gözlenen İneklerde İki Farklı İntrauterin Tedavi Yönteminin Etkinliklerinin Karşılaştırılması

ÖZ

İneklerde repeat breeder olgularının ciddi ekonomik kayıplara neden olduğu bilinmektedir. Çalışmada repeat breeder tanısı konulan ineklerde subklinik genital kanal enfeksiyonlarına yönelik intrauterin uygulamalarda farklı iki tedavi seçeneğinin etkinliklerinin karşılaştırılması amaçlandı. Sunulan çalışmada 2-8 yaş aralığında 75 adet Holstein-Fleckvieh ırkı inek kullanıldı. Çalışmanın materyalini oluşturan inekler rastlantısal olarak üç gruba ayrıldı. İntrauterin yolla bir gün ara ile üç kez 10 ml sefkuinom sülfat + 5 ml deksametazon sodyum fosfat + 10 ml %30 dekstroz Grup I'e; 100 mg rifaksimin ihtiva eden (13,4 gram) sprey Grup II'ye uygulandı. Grup III'teki hayvanlara herhangi bir tedavi prosedürü uygulanmadı, kontrol grubu olarak değerlendirildi. İntrauterin uygulamalardan sonra ilk suni tohumlamaların gebelik oran sonuçları Grup I'de %32 (8/25), Grup II'de %20 (5/25) ve Grup III'de %20 (5/25) olarak belirlendi (P>0,05). Yapılan bu çalışmada elde edilen bulgular sonucunda, repeat breeder tanısı konulan hayvanlarda intrauterin yolla uygulanan sefkuinom sülfat + deksametazon sodyum fosfat + dekstroz kombinasyonunun gebelik oranları ve fertilite üzerine olumlu etkisi olacağı düşünülmektedir. Çalışma sonucunda uygulanan intrauterin ilaç kombinasyonunun repeat breeder nedeni olan farklı faktörler de göz önünde bulundurularak daha kapsamlı şekilde araştırılmasının faydalı olacağı kanısına varıldı.

Anahtar Kelimeler: İnek, İntrauterin Tedavi, Repeat Breeder

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GİRİŞ

Repeat breeder (RB) tüm dünyada ciddi ekonomik kayıplara sebep olan önemli bir yetiştiricilik sorunudur (Pothmann ve ark 2015). En az bir kez doğum yapmış, on yaşından genç, düzenli olarak östrus siklusu gösteren, yapılan muayenelerde herhangi bir genital organ patolojisi belirlenmemiş, fertil bir boğa ile çiftleştiği ya da motilite sorunu bulunmayan kaliteli sperma ile üç sefer tohumlama işlemi uygulanmasına rağmen gebelik elde edilemeyen ineklere RB tanısı konulmaktadır (Abdisa 2018). Yetersiz östrus takibi, uzayan östrus ve lüteinleştirici hormon (LH) pikinin gecikmesi, enfeksiyonlar, fertilizasyonun şekillenmemesi, erken dönem embriyonik ölüm, genetik faktörler, bakım ve beslenme yetersizlikleri RB'nin başlıca sebepleri arasında sayılmaktadır (Bogado Pascottini ve ark 2017). Repeat breeder oluşumunda etkili risk faktörleri arasında genital kanal enfeksiyonları önemli bir araştırma konusudur ve subklinik endometritislerin bu patolojide rol oynadığı bilinmektedir. İneklerde özellikle doğum sonrası dönemde uterusta kolonize olan patojen veya nonpatojen bakteriler uterusun sağlıklı ortamını bozmakta, uterusta yangıya, uterus endometriyumunda hasara, endometriyal bezlerin fonksiyonunda bozulmaya, oosit ve spermatozoonun yaşamsal fonksiyonlarını olumsuz etkileyerek embriyonik ölümlere yol açmakta ve fertilizasyon oranını düşürmektedir (Dinç 1990, El-Khadmwy ve ark 2011, Gümen ve ark 2012).

Postpartum uterus enfeksiyonları ve özellikle subklinik endometritis kaynaklı RB olgularında gebelik oranlarını arttırmak amacıyla çok farklı tedavi seçenekleri araştırılmaktadır. Bu amaçla sıklıkla reprodüktif hormonlar, antiseptik solüsyonlar ve antibiyotikler tercih edilmektedir. Repeat breeder ineklerde farklı etken maddeye sahip antibiyotikler tohumlamadan önce veya sonra değişik sürelerde parenteral ve intrauterin olarak uygulanabilir (Aköz ve Dinç 2001, Purohit 2008, Gümen ve ark 2012, Perez-Marin ve ark 2012). Subklinik endometritislerde pek çok antibiyotik ve antiseptik intrauterin yolla sıklıkla kullanılmaktadır. Bu amaçla gentamisin, rifaksimin, oksitetrasiklin ve sefapirin grubu antibiyotikler ve lügol benzeri irritan antiseptik solüsyonlar kullanılarak farklı tedavi başarı oranları elde edilmektedir (Öztürkler ve Uçar 2003). Günümüzde klasik tedavi yöntemlerindeki düşük başarı oranları nedeniyle antibiyotik ve antiseptik uygulamalarına alternatif olabilecek tekli ya da kombine tedavi seçenekleri geliştirilmektedir. Bu araştırmalarda kullanılan in vitro mannoz (bir şeker monomeri) uygulaması ile uterus içi dekstroz (%50'lik) uygulamasının endometriyumun epitel hücrelerinde ve intrauterin ortamda bakteriyel üremeyi sınırlandırdığı belirlenmiştir (King ve ark 2000, Brick ve ark 2012). Yine benzer amaçlarla, deksametazonun antienflamatuar etkisi ile prostaglandin F2 alfa (PGF2α) üzerine baskılayıcı etkisi araştırılarak ineklerde embriyo transferini takiben uterus içine deksametazon uygulamasının gebelik oranlarını olumlu etkilediği saptanmıştır (Roh ve ark 2016).

Sunulan çalışmada, tüm dünyada ineklerde ciddi bir reprodüktif problem olan ve önemli ekonomik kayıplarla seyreden RB olgusunun tedavisinde, iki farklı intrauterin tedavi seçeneğinin etkinliklerinin karşılaştırılması ve tedavi sonrası gebelik oranlarının ortaya konulması amaçlanmıştır.

MATERYAL ve METOT

Sunulan çalışma Konya ili Karapınar ilçesinde yer alan özel bir işletmede, Ocak-Aralık 2021 tarihleri arasında yapıldı. İnekler total miks rasyon (TMR) ile günde iki kez beslendi, suya erişimleri ad libitum olarak sağlandı.

Hayvan Materyali

Çalışmamız Afyon Kocatepe Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun onayı ile (49533702/37) gerçekleştirildi. Çalışmada en az bir kez doğum yapmış, yaşları iki ve sekiz arasında olan, yaklaşık 28-30 lt/gün süt verimine sahip, vücut kondisyon skoru 2,5-4 arasında değişen (5'lik skorlama sistemi) 75 adet Holstein-Fleckvieh ırkı inek kullanıldı. Çalışmadaki hayvanlar en az üç kez tohumlama yapılmış olmasına rağmen gebe kalmamış, klinik açıdan sağlıklı, RB teşhisi konulmuş inekler arasından rastgele seçildi. İneklere yapılan rektal muayene ve transrektal ultrasonografi muayenesi sırasında uterusun involüsyonu, büyüklüğü, pelvis boşluğundaki konumu, ovaryumlardaki korpus luteum, folikül, kistler ve inaktif ovaryum ile akıntı varlığı yönünden detaylı olarak incelendi. Muayene sonucunda gebe kalmasına engel olabilecek herhangi bir bulguya rastlanmayan hayvanlara RB teşhisi konularak çalışmaya alındı.

Çalışma Gruplarının Dizaynı

Çalışmaya dahil edilen RB tanısı konmuş toplam 75 adet inek rastlantısal olarak üç gruba ayrıldı. Buna göre;

Grup I (n=25): Tedavi başlangıcından itibaren gün aşırı olarak, günde bir kez steril şartlarda hazırlanmış 10 ml sefkuinom sülfat (Cobactan® %2,5, MSD, Almanya) + 5 ml deksametazon sodyum fosfat (Vetakort® 2 mg, HIPRA, İspanya) + 10 ml %30 dekstroz (Polifleks®, Polifarma, Türkiye) içeren solüsyon intrauterin yolla üç uygulama halinde yapıldı.

Grup II (n=25): Günde bir kez, gün aşırı olarak 100 mg rifaksimin içeren sprey (Fatroximin®, Fatro, İtalya) intrauterin yolla toplamda üç kez verildi.

Grup III (n=25): Kontrol grubu olarak belirlenen bu ineklere bir tedavi uygulanmadan takip edildi.

Tedavi sonunda ineklere Ovsynch senkronizasyon protokolü (GnRH+PGF2α+GnRH) kullanılarak suni tohumlama gerçekleştirildi. Suni tohumlamadan sonraki 35. günde transrektal ultrason muayenesi yapılarak kornu uterilerde asimetri, embriyonun görüntülenmesi ve korpus luteumun varlığı kontrol edildi. Sayılan bulguların gözlendiği inekler gebe olarak kayıt altına alındı. Gebe olmadığı belirlenen ineklere işletmede uygulanan senkronizasyon protokollerinden biri tercih edilerek tekrar senkronizasyon yapıldı ve tohumlamalar uygulandı. Çalışma sonunda belirlenen gebelik oranlarına göre çalışma bulguları değerlendirildi.

İstatistiksel Değerlendirme

Çalışmada elde edilen bulgular Microsoft Excel ve Windows SPSS 20.0 Paket Programı (SPSS Inc., Chicago, IL, USA) ile analiz edildi. Gruplar arasındaki gebelik oranları Ki-kare testi kullanılarak karşılaştırıldı. Derlenen veriler çoklu uyum analizi ile incelendi. Tedavi gruplarının (Grup I ve Grup II) kontrole göre "göreli etkinlikleri" gebelik oranları dikkate alınarak aşağıda verilen yöntemle incelendi. P değeri <0,05 istatistiksel olarak anlamlı kabul edildi.

$$Göreli\ Etkinlik = \frac{Tedavi\ Yöntemi}{Kontrol}$$

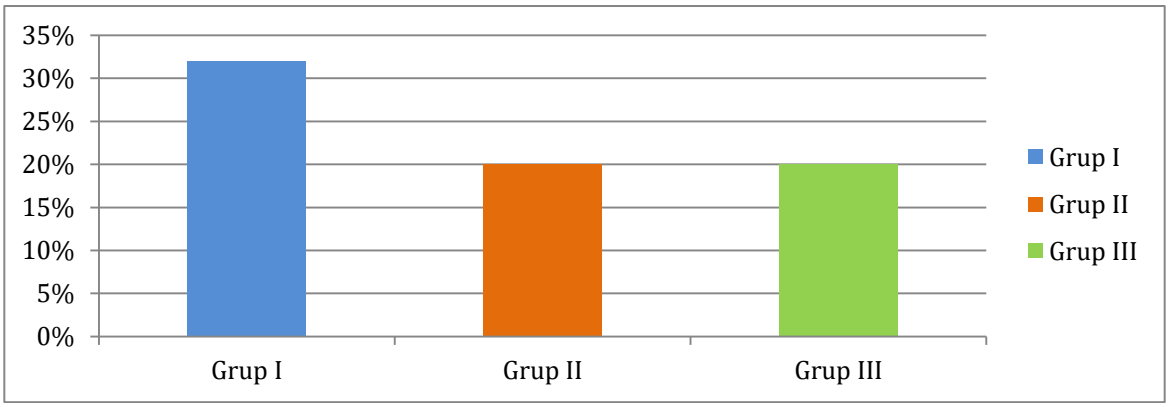
BULGULAR

Çalışmada uygulanan tedavilerin ardından gerçekleştirilen ilk suni tohumlama sonuçlarına göre gebelik oranları sırasıyla %32 (Grup I: 8/25), %20 (Grup II: 5/25) ve %20 (Grup III: 5/25) olarak tespit edildi. Çalışmada yer alan hayvanlardan gebe olmayanlar, çalışmanın gerçekleştirildiği işletmenin rutin uygulamaları nedeniyle farklı tedavi gruplarına dahil edilerek yeniden tedaviye alındığından, bulgularda yalnız ilk suni tohumlama sonrası gebelik oranları değerlendirildi (Tablo 1; Şekil 1).

Yapılan istatistiksel incelemeler sonucunda, gebelik oranları bakımından gruplar arasında farka rastlanmadı (P=0.518). Bununla birlikte, intrauterin tedavi uygulanan her iki grupta tedavilerin, kontrol grubuna göre göreli etkinlikleri değerlendirildiğinde, birinci grupta sefkuinom sülfat+deksametazon sodyum fosfat+%30 dekstroz tedavisinin gebelik oranlarını artırdığı gözlendi (Tablo 2).

Tablo 1. Çalışma gruplarında tedavi sonrası gebe kalma bulguları

		Grup			Toplam
		Grup I (%)	Grup II (%)	Grup III (%)	
Gebelik	+	8 (32)	5 (20)	5 (20)	18
	–	17 (68)	20 (80)	20 (80)	57
Toplam		25 (100)	25 (100)	25 (100)	75



Şekil 1: Çalışma sonrası belirlenen gebelik bulguları (%).

Tablo 2. Intrauterin tedavi uygulanan grupların kontrol grubuna göre göreli etkinlik düzeyi bulguları

Yöntem	Gebe inek sayısı	Göreli Etkinlik
Grup I	8	160
Grup II	5	100
Grup III	5	100

Çalışmada hayvanların laktasyon sayıları karşılaştırıldığında, birinci grupta yer alan ineklerin birinci ve beşinci laktasyonlar arasında olduğu, ikinci ve üçüncü grupta ise hayvanların bir ile dördüncü laktasyonlar arasında oldukları tespit edildi. Gebelik oranlarının laktasyon sayısından etkilenmediği ve istatistiksel bir fark olmadığı belirlendi (P>0,05).

TARTIŞMA

İneklerde önemli bir üreme ve sürdürülebilirlik sorunu olan RB birden fazla patolojik durumun etkisiyle oluşmakta, yapıcı nedenin kesin tanısında ayrıntılı laboratuvar analizleri ve geniş çaplı muayeneler gerekmektedir. Repeat breeder tanısı konulmuş olan hayvanlar klinik bulgu göstermediğinden, saha şartlarında ayrıntılı tanı için zaman kaybetmemek amacıyla hormon ve antibiyotik uygulamaları ve/veya östrus senkronizasyonu sonrası tekrar suni tohumlama yapılmaktadır (Erdoğan ve Alaçam 2003, Sarıbay ve ark 2018, Lopez-Gaitus ve Garcia-Ispierto 2020). Repeat breeder sendromunun nedenleri arasında sıklıkla subklinik endometritis görülmekte ve klinik belirtiye neden olmaksızın infertiliteye yol açmaktadır. Subklinik endometritisin kesin tanısı endometriyal biyopsi yapılarak elde edilen dokunun histopatolojik analizi, endometriyal sitoloji, uterus lavajı sırasında elde edilen hücrelerin analizi ile konulmaktadır. Belirtilen yöntemler özel laboratuvar şartlarına ihtiyaç duyan, zaman alan, maliyetli ve özel klinik şartlarında yapılması güç işlemlerdir (Salasel ve ark 2010, Pothman ve ark 2015). Çalışmaya alınan ineklere saha şartlarına uygun olarak planlanan intrauterin uygulamalar rastlantısal olarak yapılmış, iki farklı intrauterin tedavinin RB üzerine etkinliği araştırılmış ve elde edilen gebelik oranları değerlendirilmiştir.

Repeat breeder tanısı konulan ineklerde subklinik genital kanal enfeksiyonu tespit edilse de, uterustan alınan örneklerde her zaman patojen bakteriyel üreme tespit edilmeyebilmektedir. İnfertilite olgularında klinik endometritis görülme sıklığı %54 ve subklinik endometritis %1,4 oranında bildirilmekte, en sık izole edilen patojenler Escherichia coli, Corynebacterium spp., Staphylococcus aureus, Enterobacter spp., Proteus spp., Bacillus spp. ve Pseudomonas aeruginosa olarak belirlenmektedir (Lafi ve Kaneene 1988, Sing ve ark 2017). Sunulan çalışmada RB tanısı konulan ineklerde, hastalığın oluşumunda etkili faktörlerden subklinik uterus enfeksiyonu tedavisi ve tedavi sonrası fertilitenin yükseltilmesi hedeflenmiştir. Çalışmanın gerçekleştirildiği işletmenin şartları ve mevcut imkanlar doğrultusunda uterus enfeksiyonunun kesin tanısı için ayrıntılı analizler yapılmadan, saha şartlarına göre tedavi grubuna alınmıştır. Bununla birlikte, RB olgusuna genel yaklaşım ve daha önce yapılmış araştırmalar ayrıntılı olarak incelendiğinde, kesin tanı konulmadan intrauterin tedavi uygulamalarının RB tedavisinde etkili olabileceği ve fertilite oranını yükselteceği gözlenmiş, çalışmamızdan elde edilen veriler ile gebelik oranlarında artış sağlanabileceği desteklenmiştir.

İneklerde RB olgularında mikrobiyolojik analiz ve antibiyogram uygulaması sonuçlarına göre tetrasiklin, penisilin, ampisilin, gentamisin, streptomisin, kloramfenikol, kanamisin, neomisin, oksitetrasiklin ve eritromisin gibi etken maddelere duyarlılığın yüksek olduğu belirtilmektedir (Singh 1998). Ancak bu bilgilerin aksine ampisiline dirençli patojenlerin izole edildiğini bildiren araştırmalar da mevcuttur (Sharma ve ark 2009). Bu nedenle RB'ye yol açan patojenlerin farklı antibiyotik tedavilerine verdikleri yanıtlar değişiklik sergilemektedir. Repeat breeder belirlenen hayvanlarda Ovsynch protokolü ile birlikte sefapirin benzatinin intrauterin olarak uygulandığı bir çalışmada, sefapirin benzatinin gebelik oranlarını artırmada yeterince etkili olmadığı bildirilmektedir (Gümen ve ark 2012). Öztürkler ve ark (2001) tarafından gerçekleştirilen bir araştırmada rifaksimin ve oksitetrasiklinin intrauterin tedavi etkinlikleri karşılaştırılmış, rifaksimin uygulamasının gebelik oranları üzerinde daha olumlu etkisi olduğu tespit edilmiştir. Sunulan çalışmadan elde edilen gebelik oranları karşılaştırıldığında sefkuinom kombinasyonu ile intrauterin tedavinin, rifaksimin uygulamasından

rakamsal olarak daha yüksek gebelik bulgusu sağladığı, ancak istatistiksel olarak önemli bir farka yol açmadığı görüldü.

İneklerde klinik ve subklinik uterus enfeksiyonları ile infertilitenin tedavisi amacıyla antibiyotik uygulamaları haricinde çeşitli hormonlar, irritan antiseptikler ve bazı bitkisel ekstraktlar intrauterin yolla kullanılmaktadır (Ahmed ve Elsheikh 2013, Mellado ve ark 2012, Lehimcioğlu ve ark 2019, Lopez-Gaitus ve Garcia-Ispierto 2020). Antibiyotik ve hormon kullanımının dışında farklı alternatif tedavi seçeneklerinin araştırıldığı çalışmalarda dekstroz ile mannoz gibi şeker türevlerinin hem hücre kültürü araştırmalarında in vitro olarak hem de in vivo uygulanmasının uterus enfeksiyonlarında sık izole edilen E. coli ve C. pyogenes gibi bazı patojenlerin kolonizasyonunu önlediği tespit edilmiştir (King ve ark 2000, Brick ve ark 2012). İntrauterin yolla uygulanan ve PGF2α sentezini durduran deksametazonun embriyo transferi çalışmalarında gebelik oranlarını, embriyonun yaşam şansını arttırarak olumlu yönde etkilediği bildirilmektedir (Roh 2016). Kısrakta deksametazonun intrauterin yolla uygulanmasının endometriyumun savunma sistemini desteklediği, aktin ve albümin yapısına katılarak enfeksiyonlarda proteomiklerin yapısını değiştirdiği görülmüştür (Arlas ve ark 2015). Sunulan çalışmada, dekstroz ve deksametazon yukarıda belirtilen faaliyetlerinin RB tanısı konulan ineklerde fertiliteyi artırmadaki etkisinin araştırılması için sefkuinom ile kombine olarak intrauterin yolla kullanıldı. Detaylı literatür taramasında RB tanısı konulan ineklerin tedavisinde intrauterin yolla uygulanan sefkuinom sülfat + deksametazon sodyum fosfat + %30 dekstroz kombinasyonunun daha önce kullanılmadığı görüldü. İlk kez yapılan bu tedavinin rifaksimin ve kontrol grubuna göre daha yüksek gebelik oranına sahip olması ve herhangi bir yan etki gözlenmemesi, veteriner jinekoloji pratiğinde yeni ve etkili bir uygulamanın varlığını ortaya koymuştur. Repeat breeder ineklerde tedavi prosedürü olarak intrauterin yolla sefkuinom + deksametazon + dekstroz kombinasyonunu kullanımının olumsuz bir etkisinin bulunmadığı, güvenle ve başarıyla kullanılabileceği kanısına varıldı.

Sonuç olarak, RB tanısı konulan ineklerde intrauterin sefkuinom sülfat, deksametazon sodyum fosfat ve dekstroz (%30) kombinasyonu uygulamasının sayısal olarak gebelik oranlarını ve fertiliteyi artırıcı etkisi olabileceği tespit edildi. Uterus enfeksiyonu tedavisinde rutin olarak kullanılan rifaksimin ile karşılaştırıldığında, bu çalışmada uygulanan intrauterin ilaç birleşiminin daha olumlu etki yarattığı gözlendi. Uygulanan tedavi kombinasyonun endometriyumda neden olabileceği histolojik değişiklikler ve uterus patojenlerine göre terapötik etkilerinin ayrıntılı olarak saptanabilmesi için daha büyük hayvan grupları ile ve RB sendromuna neden olan diğer etmenler de değerlendirilerek daha kapsamlı şekilde araştırılmasının faydalı olacağı kanısına varıldı.

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RESEARCH ARTICLE

Determination of Leptin, Some Vitamins and Biochemical Parameters in Different Breeds of Sheep

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ABSTRACT

Leptin is a hormone that coordinates body weight control and is produced by adipocytes. In this study, Yüzüncü Yıl University research and application farm of leptin in the Nord and Tahirov grown on sheep breeds, and some vitamins and the correlation between them for the purpose of determining the levels of some biochemical parameters was made. In the study, 2 different healthy sheep breeds (Norduz, Tahirova) were used. A total of 26 sheep, 13 of each sheep breed, were included in the study. The levels of Leptin, Vitamins A, D and E, glucose, urea, total protein, FT3 and FT4 were examined in blood samples taken from animals. The leptin and FT4 levels were 15.92 ng/ml and 1.07 µg/dl, respectively, in the Tahirova sheep breed, while these values were 5.22 ng/ml and 0.91 µg/dl in the Norduz sheep, and the difference in value was found to be statistically significant ($p<0.001$). A negative correlation was found between alpha Decopherol and total protein and FT3 in Tahirova sheep, and a positive correlation was found between vitamin D3 and retinol. Decibels were found to be negative in Tahirova sheep. In the Norduz sheep, a negative correlation Dec detected between retinol, vitamin D3 and leptin, and a positive correlation Dec detected between FT4 and FT3. By looking at the results of this study, it shows that the detection of high serum leptin levels in the Tahirova breed, which is one of the thin-tailed sheep, theoretically does not depend on the tail fat ratio of leptin production. In order to explain the biochemical mechanisms of such phenomena in sheep with thin and fat tails, additional feeding-hormone studies are needed.

Key Words: Glucose, Leptin, Sheep, Tiroid Hormones,Vitamins

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Farklı Koyun Irklarında Leptin, Bazı Vitaminler ve Biyokimyasal Parametre Düzeylerinin Belirlenmesi

ÖZ

Leptin, vücut ağırlığı kontrolünü koordine eden ve adipositler tarafından üretilen bir hormondur. Bu çalışma, Yüzüncü Yıl Üniversitesi Araştırma ve Uygulama Çiftliğinde yetiştirilen Norduz ve Tahirova koyun ırklarında leptin, bazı vitaminler ve bazı biyokimyasal parametrelerin seviyelerini ve aralarında korelasyonların saptanması amacıyla yapıldı. Çalışmada sağlıklı 2 farklı koyun ırkı (Norduz, Tahirova) kullanıldı. Her bir koyun ırkından 13'er adet olmak üzere toplam 26 koyun çalışmaya dahil edildi. Hayvanlardan alınan kan örneklerinde Leptin, Vitamin A, D ve E , glukoz , üre, total protein, FT3 ve FT4 düzeylerine bakıldı. Leptin ve FT4 düzeyleri Tahirova koyun ırkında sırasıyla 15.92 ng/ml, 1.07 µg/dl iken bu değerler Norduz koyunlarında 5.22 ng/ml, 0.91 µg/dl olarak tesbit edildi ve değer farkı istatistiksel önemde bulundu ($p<0.001$). Tahirova ırkı koyunlarda alfa tokoferol ile total protein ve FT3 arasında negatif, vitamin D3 ile retinol arasında pozitif korelasyon tespit edildi. Norduz ırkı koyunlarda ise retinol, vitamin D3 ile leptin arasında negatif korelasyon, FT4 ile FT3 arasında pozitif korelasyon saptandı. Bu araştırmanın sonuçlarına bakarak ince kuyruklu koyunlardan olan Tahirova ırkında serum leptin düzeyinin yüksek olarak saptanmasının teorik olarak leptin üretiminin kuyruk yağ oranına bağlı olmadığını göstermektedir. İnce ve yağlı kuyruklu koyunlardaki bu gibi olayların biyokimyasal mekanizmalarını açıklanması için ilave besleme-hormon çalışmalarına gereksinim vardır.

Anahtar Sözcükler: Glukoz, Koyun, Leptin, Tiroid Hormonları,Vitaminler

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GİRİŞ

Norduz koyunu kombine verimli bir ırk olup adını Van'ın Gürpınar İlçesinde bulunan Norduz Bölgesi'nden almaktadır. Van'ın dağlık bölgelerinde yetiştiriciliği yapılmaktadır. Norduz koyunlarının renkleri, beyaz, çoğunlukla kül renginde, ancak az miktardada gri-beyaz ve kahverengi-beyaz renk kombinasyonlarından oluşmaktadır. Koyunlarda kuyruk kısmı üç kısımdan oluşmuş olup ve ortadaki kısım daha uzundur (Ocak et al., 2009). Yüksek süt verimi, erken gelişim özellikleri, soğuk iklim koşullarına ve zoonoz hastalıklara yüksek adaptasyonları nedeniyle genellikle 450-500 başlık homojen sürüler halinde yetiştirilmektedirler. Laktasyon süresi yaklaşık olarak 180 gündür. Bu dönemde ortalama süt verimi 135-140 kg dır (Bingöl, 1998).

Tahirova koyun ırkı; Doğu Friz ve Kıvırcık kombinasyon melezlemesiyle oluşturulmuş olup, %75 Doğu Friz, %25 Kıvırcık genotipi içermektedir. Süt ve döl verimi oldukça yüksektir. Tahirova koyunu, Kıvırcık ve Kıvırcık melezi koyunların yetiştirildiği her yerde yaşayabilir. Mera durumu iyi olan işletmelerde rahatlıkla yetiştirilmektedir. Kuyrukları ince ve yapağısızdır. Memeleri geniş meme yapısındadır. Koyunların doğum ağırlıkları 4-4.5 kg'dır. Yapağısı bir örnek ve kemp kıl oranı çok düşüktür. Bu koyunların ortalama canlı ağırlıkları 55-60 kg, süt verimi 250-300 kg, laktasyon süresi 200-240 gün, kirli yapağı verimi ise 3-4 kg' dir (Tuncel, 1995).

Leptin, beslenme ve enerji homeostazisinde önemli görevlere sahip bir hormon olup sitokinlere yapısal benzerlik göstermektedir. Eksikliğinde veya direnç durumlarında obezite, diyabet ve infertilite görülmektedir. Endokrin ve immün fonksiyonların yanısıra leptin enerji metabolizmasında önemli rol oynar. (Hekimoğlu, 2006). Leptinin; üreme (Chehab ve ark. 1996), hematopoez (Bennet ve ark. 1996), sempatik sinir sistemi aktivasyonu (Pelleymounter ve ark. 1995), gastrointestinal fonksiyonların düzenlenmesi (Bado ve ark. 1998), anjiogenez ve osteogenezisde (Iwaniec ve ark. 1998) de önemli metabolik etkileri saptanmıştır. Kemiricilerde kanda leptinin yüksek değerleri, vücut yağının büyük miktarlarının belirteci olarak ifade edilir. Gıda alınımının azalması ve enerji tüketimi artışı yoluyla vücut ağırlığı azalır, düşük leptin düzeyleri, küçük enerji depolarının göstergesidir (Karlsson, 2000).

Dolaşımdaki leptin miktarının doğrudan yağ dokudaki leptin mRNA düzeyi ile alakalı olduğu saptanmıştır (Baile et al., 2000).

Leptin organizmada yağ miktarını dengede tutmada önemli bir role sahiptir. Leptin vücutaki yağ düzeyini sabit tutmakta görevli olduğundan leptin miktarının düşmesi, beyinde enerji açığının sinyalini verir. Leptin intrasellüler lipit düzeyini iskelet kasları, karaciğer ve pankreasın beta hücrelerindeki insülinle etkileşerek azalmaktadır (Klaus, 2004). Leptinin gıda alımı, yağ depolanması ve metabolizması, kardiovasküler ve immun fonksiyonların düzenlenmesi üzerine önemli etkileri bulunmaktadır (Dulloo et al., 2002).

Leptinin fazla lipit deposunun bir regülatörü olması haricinde dengesiz beslenmeye hayvanların uyumunda da görevli olduğu belirtilmiştir. Yetersiz ve dengesiz beslenen hayvanlarda plazma leptin miktarındaki hızlı düşüşü takiben, üreme problemleri, hipo tiroidi, enerji tüketilmesi ve protein sentezinde azalma görülmüştür (Chelikani et al., 2004). Geviş getiren hayvanlarda leptini düzeyi azaltıp kortizolü miktarı artırılarak yetersiz beslenmeye karşı şekillenecek metabolik uyuma yardımcı olur. Normal ve dengeli beslenmeye başlanınca insülin salgılanması uyarılır ve hali hazırdaki yüksek kan kortizol miktarı leptin salgılanmasını situmule eder. Leptin salgılanmasını düzenleyen en önemli faktör olarak canlı vücut ağırlığına işaret edilmiştir (Fruhbeck et al., 1998). Lipit depolarının azalmasıyla leptin miktarı azalır. Lipit depolarının artması leptin düzeyini yükseltip iştahı keser. Böylece besin alınması da azalır. Leptinin miktarındaki artış negatif enerji balansı ile sonlanırken, enerji tüketimi besin alınmasını geçer.

Açlık uygulamasıyla memeli hayvanların çoğunda kan glukoz ve insülin konsantrasyonları azalırken, plazma serbest yağ asitleri (FFA) miktarlarının arttığı bildirilmiştir. Plazmada bulunan proteinlerin hepsine birden total protein denir. Serum protein miktarları, protein metabolizması bozukluklarında, karaciğer, böbrek ve kemik iliği ile ilgili ciddi patolojilerin tanısında önemlidir (Mert, 1996). Hayvanlarda metabolizma hızını artırarak önemli etkilere neden olan hormonlar arasından tiroid hormonları (TH) en ön sırada yer alır. Hücrelerin gelişmesi, büyümesi, metabolizmalarının ayarlanması, enerjetik işlemlerin koordine edilmesi, kardiyovasküler sisteminin normal fizyolojik çalışmasının temini ve tiroid uyarıcı hormon sekresyonunun baskılanması gibi başka önemli fonksiyonları da vardır. TH' nın seviyesindeki değişimler, hayvansal metabolizmanın farklı çevre koşullarına, besin gereksinimi ve sağlanmasındaki farklılıklara ve hayvanın içinde bulunduğu fizyolojik dönemlere homeostazı yani değişimlere karşı uyum göstermesini sağlamaktadır (Todini, 2007). Tiroidden troid hormonlarının salınımı tiroid stimülan hormonunun (TSH) kontrolü altındadır. TSH, troid hormon salınımını uyarırken, kandaki T3 ve T4 artışı hipofizden TSH salınımını baskılar (negatif feed-back) ve salınımı ise tirotropin salgılatıcı hormonunun (TRH) kontrolü altındadır. Karaciğerde sitoplazmik ve mitokondriyal basamakları içeren üre siklüsü ile üreye dönüştürüldükten sonra idrar yoluyla atılmakta, amonyağın zararlı etkileri önlenmiş olmaktadır (Mert, 1999). Üre gerçekten renal atılım azaldığı zaman, plazmada biriken ve protein olmayan azotlu bileşiklerden biridir. Organizmada karbonhidrat eksikliği, yüksek protein diyeti, intestinal hemoraji, ateş, nekrozis, hipertiroidizm, uzun süren ekzersiz, katabolik ilaçlar anabolizmadaki azalma, şiddetli hemoraji, şok, hipoadrenokortisizim, primer akut

renal yetmezlik, akut interstitial nefritis, kronik renal yetmezlik ve neoplazi gibi birçok durumlarda düzeyi yükselir (Mert, 1996).

Vücudun sağlıklı gelişimi, sindirim fonksiyonları, enfeksiyonlara karşı bağışıklık kazanması açısından oldukça gereklidir. Ayrıca karbonhidrat, yağ ve proteinin kullanmasını da sağlarlar. Vitaminler, özel hücresel fonksiyonların yerine getirilmesinde vücudun eser miktarlarda gerek duyduğu organik bileşiklerdir. Çözünürlüklerine ve metabolizmadaki fonksiyonlarına göre sınıflandırılabilirler. Vitaminler çözünürlüklerine göre; yağda ve suda çözünenler diye 2 grupta toplanırlar (Champe ve Harvey 1997).

Vitamin A doğada alkol (retinol), aldehit (retinal) ve asit (retinoik asit) yapılarında bulunur. Memeliler provitamin halinde aldıkları maddelerden vitamin A sentezi yaparlar. ß-karotenin parçalanması ile oluşan retinol, kanda retinol-bağlayan proteine bağlanarak taşınır. Bu protein plazmada az miktarlarda bulunan, prealbumin'e bağlanır. Oluşan protein-protein kompleksi retinol için doğal bir koruyucu rol oynar. 1993 yılında vitamin A' nın besinlerle alınan ve yağda eriyen bir vitamin olduğu tespit edilmiştir. E vitamininin kimyasal yapısı bir tokol olup, antisterilite yani kısırlığı engelleyen vitamin olarak da bilinir. E vitamini yağda çözünür ve çok önemli antioksidan görevlere sahiptir. Özellikle hücre zarları ve lipoproteinlerin korunmasında önemli antioksidan işlevler görmektedir. Epidemiyolojik ve diğer çalışmalarda, E vitamininin kardiyovasküler hastalıkların, bazı kanserlerin ve bazı kronik hastalıkların riskini azalttığını belirlenmiştir. Tokoferoller işlevini sonlandırdıktan sonra yeniden sisteme katılamazlar, bu nedenle hücredeki biyolojik görevlerini yapabilmek için devamlı olarak yenilenmektedir. Tokoferolün antioksidan tesiri yüksek oksijen miktarlarında etkilidir. Özellikle yüksek oksijen basıncına uğrayan lipit oluşumları, örneğin eritrosit ve respiratorik zarlarında yoğunlaşmışlardır (Kesseb and Hamliri, 1986). Vitamin E' nin öncelikle en önemli görevi antioksidant etkiye sahip olmasıdır. Membranlar içinde bulunan doymamış yağ asitlerinin oksitlenmesini önleyen vitamin E, membranlarda meydana gelebilecek yıkımlanmayı önlemektedir. E vitamini hücre zarları ve lipoproteinlerin korunmasında önemli antioksidan işlevler görmektedir. (Kalaycıoğlu et al., 2000).

D vitamini yağda eriyen ve kemik sağlığı için gerekli olan bir vitamin türüdür, kalsiyum ve fosforun sindirim yollarında kullanımı ve emilimi ile özellikle çocuklarda büyüme için gerekli bir vitamindir. Kan basıncının düzenlenmesi, kalp hastalıklarının önlenmesi, kalp krizi ve felç riskini düşürmek içinde son derece önemlidir (Norman, 2006).

Beslenme, fizyolojik ve endokrin faktörler ruminantlarda leptin düzeyini etkilemekte ve leptin düzeyi vücuttaki yağ dokusu ile orantılıdır gibi klasik bilgi ve genellemeler, ince kuyruklu ve yağlı kuyruklu koyun ırkları arasında vücudun farklı yerlerinde yağ depolanmasının, moleküler mekanizmalarını açıklamada yetersiz kalmaktadır. Bu çalışma aynı bölgede yaşayan, aynı bakım, besleme şartlarına maruz kalan ve yaşları birbirine yakın olan, sağlıklı ve farklı kuyruk yapısına sahip iki farklı koyun ırklarından toplanacak kan serumlarında, leptin, bazı vitaminler (retinol, alfatokoferol, vitamin D3) ve bazı biyokimyasal parametrelerin (glikoz, üre, total protein, T3, T4) seviyelerini saptamak ve aralarında korelasyon olup olmadığını araştırmak amacıyla yapılmıştır.

MATERYAL ve METOT

Deney Grubunun Sağlanması

Tüm hayvan kullanım protokolleri, Avrupa Parlamentosu ve Konseyi'nin 22 Eylül 2010 tarihli, bilimsel amaçlarla kullanılan hayvanların korunmasına ilişkin 2010/63/EU sayılı Direktifi uyarınca gerçekleştirilmiştir (ABD. 2010). Bu çalışmanın materyalini; Van Yüzüncü Yıl Üniversitesi Araştırma ve Uygulama Çiftliği'nde yetiştirilen, aynı bakım ve besleme şartlarına sahip, 2-3 yaşlarında, gebe olmayan, sağlıklı, 2 farklı koyun ırkı (Norduz, Tahirova) oluşturdu. Her ırktan 13'er adet olmak üzere toplam 26 koyun çalışmaya dahil edildi. Bunların içinden canlı ağırlığı en yüksek olan koyunlar seçildi ve Temmuz ayı boyunca tüm hayvanlara ilave hiçbir besin maddesi verilmedi. Sadece sabah ve öğleden sonra meraya çıkartıldı.

Kan Örnekleri

Sabah yayılımdan gelen koyunlar önce tartıldı, sonra usulüne uygun olarak vena jugularisten 5 ml'lik antikoagulansız biyokimya tüplerine kanlar alındı. Soğuk zincir şartlarında muhafaza edilen kanlar laboratuvara götürüldü.

Pıhtılaşma gerçekleştikten sonra, tüpler +4 0C, 3000 devirde 10 dk. santrifüj edilip, kanların serumu çıkarıldı. Çıkarılan serumlar eppendorf tüplerine aktarılıp -20 0C' de saklandı. En kısa sürede serum leptin, total protein, üre, glukoz, T3, T4, retinol, α-tokoferol ve vitamin D3 seviyelerine bakıldı.

Glukoz ölçümü Abbott marka glukoz kiti (Katalog Numarası:1600809) ,Üre ölçümü Abbott marka üre kiti (Katalog Numarası:1600809), Total protein seviyesinin Abbott marka total protein kiti (Katalog Numarası:1600809) FT3 ve FT4 ölçümü Abbott marka FT3-FT4 kiti (Katalog Numarası:1600809) ile Architect plus 160000 cihazında kullanılarak ölçüldü. Leptin düzeyleri ise; Cusabio marka Sheep Leptin Elisa kiti (Katolog Numarası: CSB-EL 012870 SH) kullanılarak, Stat Fax 2100 ELISA Reader' da saptandı.

Vitamin Analizleri

Vitamin A, D ve E analizleri için, 200 µl serum plastik tüplere alındı. Üzerlerine 200 µl etanol eklenip bir dakika vorteksle karıştırıldı. Bunların üzerine 800 µl n-hekzan ilave edilip tekrar bir dakika vortekslendi ve 2000 rpm'de 10 dakika santrüfüj edildi. Oluşan

hekzan fazı alınarak azot gazı altında kurutuldu. Kalıntı 100 µl metanolde çözündürüldü ve HPLC kolonuna enjekte edildi (Zaspel ve ark. 1983; Miller ve ark. 1985). Vitamin A, D ve E'lerin tanıları DAD (diode-array detector) dedektörü kullanılarak 325, 265 ve 290 nm dalga boylarında yapıldı. Mobil faz olarak metanol-su (98:2) 1.5 ml/dak akış hızında kullanıldı. Vitaminlerin ayrılmasında C18 kolonundan (4.6 mm x 25 cm, Supelco) faydalanıldı (Zaspel ve ark. 1983; Miller ve ark. 1985) Analizler Yüksek performanslı sıvı kromatografisi (HPLC) (Terma Sentific Pinligan Surveyor) cihazı ile gerçekleştirildi. Hesaplamalar vitamin A, D ve E standartlarının pik alan ve konsantrasyonlarına göre yapıldı.

İstatistik Analiz

Üzerinde durulan özellikler için tanımlayıcı istatistikler; ortalama ve standart sapma olarak ifade edildi. Bu özellikler bakımından grupları karşılaştırmada Mann- Whitney U testi kullanıldı. Değişkenler arasındaki ilişkiyi belirlemede gruplarda ayrı ayrı olmak üzere Spearman Korelasyon Katsayıları hesaplandı. Hesaplamalarda istatistik anlamlılık düzeyi %5 olarak alındı ve hesaplamalar için SPSS istatistik paket programı kullanıldı.

BULGULAR

Norduz ve Tahirova ırklarındaki koyunların serum leptin, total protein, üre, glukoz, FT3, FT4, retinol, α-tokoferol ve vitamin D3 seviyelerinin ortalamaları Tablo 1' de verildi. Serum leptin düzeyi, Tahirova ırkı koyunlarda 15.92 ng/ml ve Norduz koyun ırkında ise 5.22 ng/ml olarak bulundu. Yağlı kuyruklu koyun ırkı olan Norduz ile ince kuyruk olan Tahirova koyun ırkları arasında ortalama değerler arasındaki farklılık istatistiksel ($p<0.001$) önemli saptandı. Yine serum FT4 düzeyi Tahirova ırkı koyunlarda 1.07 µg/dl, Norduz ırkı koyunlarda ise 0.91 ± 0.07 µg/dl ve ortalama değerler arasındaki farklılık istatistiksel olarak önemli bulundu $p<0.001$. Tablo 1 incelendiği zaman; Tahirova ve Norduz koyun ırklarında sırası ile serum glukoz düzeyi 37.54 ve 38.00 mg/dl olarak saptanırken, serum üre ortalamaları (32.69-34.15 mg/dl), serum total protein düzeyi 7.55 g/dl -7.38 g/dl, serum retinol düzeyi 0.90 µg/dl- 1.09 µg/dl serum α-tokoferol düzeyi 1.38-1.69 µg/ml serum vitamin D3 düzeyi 0.02 µg/ml olarak aynı değerde bulundu. Serum glukoz, üre, total protein, retinol α-tokoferol ve vitamin D3 ortalamaları farklı kuyruk tiplerine sahip bu iki ırk koyunda istatistiki önemli bulunmadı ($p\geq0,05$). Tahirova ırkı koyunlarda incelenen parametreler arasındaki korelasyon katsayıları Tablo 2'de gösterildi. Tablo 2 incelendiğinde Tahirova ırkı koyunlarda alfa tokoferol ile total protein ve FT3 arasında negatif (sırasıyla $p<0.05$, $p<0.01$), vitamin D3 ile retinol arasında pozitif korelasyon ($p<0.05$) tespit edildi. Norduz ırkı koyunlarda incelenen parametreler arasındaki korelasyonkatsayıları Tablo 3'de sunuldu. Tablo 3 incelendiğinde Norduz ırkı koyunlarda ise FT3 ile üre ($p<0.01$), FT4 ile üre ($p<0.01$), retinol ile leptin ($p<0.05$), vitamin D3 ile leptin ($p<0.05$) arasında negatif korelasyon, FT4 ile FT3 ($p<0.05$) arasında pozitif korelasyon saptandı.

Tablo 1. Norduz ve Tahirova ırklarındaki koyunlara ait bazı biyokimyasal parametrelerin ortalamaları.
Table 1. Averages of some biochemical parameters of sheep in Norduz and Tahirova breeds.

	Tahirova		Norduz		
Parametreler	**n**	**$X \pm S_x$**	**N**	**$X \pm S_x$**	**p değeri**
Glukoz (mg/dl)	13	37.54 ± 8.69	13	38.00 ± 8.82	.857
Üre (mg/dl)	13	32.69 ± 3.50	13	34.15 ± 5.84	.796
Leptin (ng/ml)	13	15.92 ±6.39	13	5.22 ± 2.45	.001
Total protein (g/dl)	13	7.55 ± 0.40	13	7.38 ± 0.64	.643
FT_3 (pg/dl)	13	3.38 ± 0.52	13	3.10 ± 0.62	.144
FT_4 (ng/dl)	13	1.07± 0.07	13	0.91 ± 0.07	.001
Retinol (µg/ml)	13	0.90 ± 0.37	13	1.09 ± 0.26	.200
α -Tokoferol (µg/ml)	13	1.38 ± 0.80	13	1.69 ± 1.42	.778
Vitamin D_3 (µg/ml)	13	0.02 ± 0.02	13	0.02 ± 0.01	.440

Tablo 2. Tahirova ırkı koyunlarda incelenen parametreler arası korelasyon katsayıları
Table 2. Correlation coefficients between parameters examined in Tahirova sheep

	Glukoz	Üre	Leptin	Total Protein	FT_3	FT_4	Retinol	α-Tokoferol	Vitamin D_3
Glukoz	1								
Üre	-0.181	1							
Leptin	-0.531	0.016	1						
Total Protein	-0.460	0.489	0.122	1					
FT_3	-0.224	0.393	-0.135	0.506	1				
FT_4	-0.074	-0.098	-0.212	0.367	0.326	1			
Retinol	-0.444	0.525	0.028	0.353	0.445	-0.217	1		
α-Tokoferol	-0.093	-0.489	0.314	-0.600*	-0.725**	-0.428	-0.084	1	
Vitamin D_3	-0.153	0.433	0.273	0.346	0.487	-0.170	0.663*	-0.344	1

* $p<0.05$, **$p<0.01$

Tablo 3.Norduz ırkı koyunlarda incelenen parametreler arası korelasyon katsayıları.
Table 3. Correlation coefficients between parameters examined in Norduz sheep.

	Glukoz	Üre	Leptin	Total Protein	FT_3	FT_4	Retinol	α-Tokoferol	Vitamin D_3
Glukoz	1								
Üre	0.196	1							
Leptin	-0.240	-0.285	1						
Total Protein	-0.308	0.469	-0.035	1					
FT_3	0.192	-0.743**	0.095	-0.478	1				
FT_4	0.040	-0.700**	0.310	-0.142	0.638*	1			
Retinol	0.358	0.247	-0.670*	-0.251	0.104	-0.341	1		
α-Tokoferol	-0.108	0.124	0.169	-0.380	-0.087	-0.482	0.231	1	
Vitamin D_3	0.270	0.478	-0.599*	0.091	-0.078	-0.377	0.530	0.365	1

* $p<0.05$, ** $p<0.01$

TARTIŞMA

Koyun ırklarına ait kan parametreleri ile ilgili olarak bir çok çalışma yapılmış, çeşitli araştırıcılar bunun bir gereklilik olduğunu da belitrmişlerdir (Mert et al., 1998). Çünkü bu değerler hem ırkların farklılıklarını ve hem de bakım beslenme yaş cinsiyet gibi faktörlerin fizyolojik olaylara etkisini açıklanmasına yardım eder. Yağlı ve ince kuyruklu hayvanların biyokimyasal kan değerleri hep ilgi konusu olmuş, bazı parametrelerin ırk özelliği hariç tutulduğunda farklı oldukları araştırılmıştır (Görgülü, 1994).

Çimen ve ark. (2001) yaptıkları bir çalışmada ince kuyruklu Karayaka koyunu ile yağlı kuyruklu Gıcık ve Akkaraman koyunlarının serum glukoz, trigliserid, total protein, kolestrol VLDL değerleri arasındaki ortalamalarının birbirine yakın olduğunu, değişimlerin istatistiki öneme sahip olduğunu bildirmişlerdir (Görgülü, 1994). Sunulan bu çalışmada da glukoz, üre ve total protein düzeyleri Tahirova ve Norduz koyun ırklarında birbirine çok yakın olarak bulunmuş ve istatistiki önem saptanamamıştır ($p\geq0,05$). Glukoz koyunlarda embriyonik hayatta ve sonrasında enerji metabolizması ve doku gelişimine C kaynağı olması açısından oldukça önemlidir. Koyunlarda normal glukoz değerleri %50-80 mg arasında değişebilir (Mert 1996). Mert ve ark. (1990) sığırlarda canlı ağırlık artışını etkileyen bir parametrenin de glukoz olduğunu bildirmişlerdir. Özyurtlu ve ark. (2007) İvesi koyunlarda glukoz değerini %42-44 mg olarak, total

protein düzeylerini ise % 5,85-6,13 g/dl olarak ölçmüşlerdir. Altınsaat (2000) koçlarda ve koyunlarda kan glukoz değerlerini sırasıyla 54.75 ve 52.25 mg/dl total protein düzeylerinin ise 7.54 ve 7.17 g/dl olarak bulmuş, yine aynı çalışmasında koç ve koyunlarda üre miktarlarını 36 ve 39.45 µmol/l olduğunu bildirmiştir. Bir diğer araştırmada ise Akkaraman koyunlarında 7 farklı sürüde kan üre değerleri 19.87 - 33.22 mg/dl ve total protein miktarını 7.24-8.03 g/dl olarak saptanmıştır (Kurt, 2008). Sunulan bu çalışmada Tahirova ve Noduz ırkı koyunların kan glikoz ve üre düzeylerinde fark tepit edilmemiştir. Ancak Eryavuz ve ark. (2007) bildirdiği anlamda çok az da olsa kan glikoz düzeyi yüksek olan Norduz'da, üre miktarı yüksek görülmekte olup istatistiksel bir önem yoktur. Daha önce farklı araştırıcılar arasından bildirilen ve genotip olarak yağsız koyunların yağlı olanlara göre daha yüksek glikoz düzeyine sahip olabildiği görüşü sunulan bu çalışmadaki sonuçları desteklememektedir. Araştırmada kullanılan koyunların glikoz düzeyleri sadece ortalama 0.48 mg/dl fark göstermektedir.

Ruminantlarda kaynak ne olursa olsun alınan azot proteine çevrilir. Rumende gerçekleşen bu mikrobiyel protein sentezinde amonyak temel olarak kullanılır. Vücutta üre sentezi sonucunda böbrekte atılan üre farklı klinik patolojik olaylarda artar. Avcı ve ark. (2000) dört farklı rasyonla besledikleri koyunlarda kan glukoz düzeyini deneme başında 16-17,50 mg/dl, deneme sonunda 16,50-24,40 mg/dl arasında tespit etmişlerdir. Aynı araştırıcılar serum üre değerini sırasıyla 22,27-25,50 mg/dl ve 11,00-17,36 mg/dl olarak bulmuşlar. çeşitli araştırıcılar serum protein düzeylerini Akkaraman koyununda 6.82 g/dl ve Karayaka koyunlarında 6.56 g/ dl (Çimen et al., 2001), Kırmızıgül ve ark. (2005) de sağlıklı koyunlarda 8.66±0.58 g/dl olarak bulmuşlardır. Sunulan bu çalışmada Tahirova ve Norduz ırk koyunlarda serum üre, total protein ve glukoz düzeyleri normal değerler arasında olup, grupların ortalamaları arasındaki farklılık istatistiksel önem göstermemiştir ($p \geq 0,05$)

Keçeci (1995) kuzu ve koyunlarda troid hormonları ve kan pH sı arasında yaptıkları çalışmalarında yeni dogan kuzularda, annelerine göre zamana bağlı olarak T3 düzeyi artarken T4 düzeyinin azalmış oldukları bulmuşlardır. Ateşşahin ve ark. (2002), Akkaraman ırkı koyunlarda Se ilavesi ile tiroid hormonları arasındaki ilişkiyi araştıran çalışmalarında kontrol grubu koyunlarda T3 düzeyini 57,1± 8,63 ng/dl T4 düzeyini ise 5,70 mg/dl, T4/T3 oranını ise 9,98 ng/ml olarak bildirmişlerdir.

1994 yılında ob geninin kodladığı, 16 kd bir protein olan, adipoz dokudan salınan ve belki de homeostatik sistemin en büyük parçası olarak tanımlanan leptin, son yıllarda enerji ve metabolik dengede en çok çalışılan bir hormon olmuştur. Leptin insülin ve tiroid hormonları gibi bazal metabolizma üzerine etkili olan hormonların aktivitelerini düzenler ve iştahın regulasyonunda görev alır. Bir obese (ob) gen ürünü olup yağ dokularından sentezlenen leptin üreme, bağışıklık gibi bir takım genel etkilerini yanı, sıra enerji metabolizmasında, depo ve harcanma işlemlerinde aktif rol alır. Her zaman vücut yağı ile ilişkilendirilmeye çalışılmış olup, vücut yağı değişimleri ile bağlantılı belirteç olarak kullanılmıştır (Delavaud, 2000).

Yapılan çalışmalarda farklı dokularda leptin miktarlarının değişkenliği insülin direnci veya yağ doku miktarlarıyla ilgilendirilip, Eryavuz ve ark. (2007) Avcı ve ark. (2013) gibi araştırıcılar kuyrukta depolanan yağın vucut leptin düzeyini etkilemediklerini bildirmişlerdir. Rasyona çinko ilave edilince iştah ve vucut kondisyonu iyileşmiş, leptin düzeyi artmış, yağ dokusundan geç salgılanması azalmış olması gibi sonuçlar bulunmuştur (Ott and Shay 2001).

D vitamini, son yılların üzerinde en fazla çalışılan bir hormon görevini gören vitamin olarak karşımıza çıkmaktadır. D vitamini eksikliğinde bilinen klinik hastalıkların yanında vücudun birçok önemli metabolik işlevleride etkilenmektedir. D vitamini yokluğunda leptin hormonu etkisi bozulmakta ve böyle bireylerde kilo verilmesi daha az olmaktadır. Aynı kaloriyi alanlarda D vitamini düzeyindeki en küçük bir artış kilo kaybını 200 grama kadar çıkarabilmektedir. Genel olarak bilim adamları tarafından D vitamini yağ hücresi dahil herşeyi etkilediğine inanılmaktadır (Norman, 2006).

Obezite ile ilgili Vitamin D eksikliğinin leptin tarafından ayarlanabileceği, IL-6 'nın işe daha az karıştığı, adipoz dokudan salgılanan IL-6 ve leptin reseptörleri yoluyla 25-(OH) D sentezi üzerine inhibe edici etki bulunduğu bildirilmiştir (Ding et al., 2010). Leptinin, D vitamini üzerine azaltıcı etkisinin böbrekte I-25 (OH) D3 - I α hidroksilaz enziminin gen ekspresyonunu azaltarak yaptığı, farelerde leptin reseptörünün aktif formunun tespit edilmesiyle açıklanmıştır. (Matsunuma and Horiuchi 2007). Sunulan bu tezde vitamin D düzeyleri her iki ırk koyunda da aynı değerde bulunmuştur ($p>0.05$).

Tokoferoller hücresel oksidatif hasarı önleyici olarak önemli görevlere sahiptir. Bu yolla kanser ve yaşlanma ile ilgili hastalıklara karşı koruyucu rol yaptıkları düşünülmektedir. α-Tokoferol en yüksek biyoaktiviteye sahip olmasına rağmen, son yıllarda yapılan çalışmalarda α-Tokoferollerin hayvan modellerinde kanser önleyici etkileri bulunmuştur (Chai et al., 2010). Bazı görüşlere göre α-tokoferol, oksijen bazlı oksidantlara karşı temel madde olarak kabul edilirken antienflamatuar etkisinin yetersiz olduğu ifade edilmiştir (Jiang, 2003). α-Tokoferol ile 25(OH) D arasındaki ters ilişki obezitede rastlantısal olabileceği ve direkt olarak vitamin D durumu ile ilgili olamayacağı bildirilmiştir. Leptin ile vitamin D ilişkisi ve regulasyonu konusunda da kesin bir kanıt yoktur (Menendez, 2001)

Dolaşımdaki retinol düzeyleri kışın yükselip yazın azalırken, vitamin D de de tersi izlenir. Bu vitamin D ve A arasındaki muhtemel ilişki ve birbirini kompanse etmesi halini düşündürür (Cooney et al., 1995)

Sunulan bu çalışmada α-Tokoferol düzeyleri Tahirova ve Norduz koyunlarında 1.06-1.40 µg/ml olarak saptanmış ırklar arasındaki bu ortalamalar istatistiksel olarak önemsiz bulunmuştur. Ayrıca retinol düzeyleri yine ırklar arasında çok farklılık (0.98-1.06 µg/ml) arz etmemiştir.

Avcı ve ark. (2013), Akkaraman ve Merinos koyunlarında yaptıkları çalışmada kontrol grubunda vitamin A ve β-karoten düzeyleri 0.37-0.56 µg/l , 0.03-0.17 µg/l ve Merinoslarda β-karotenin önemle yüksek olduğunu bildirmişlerdir. Yağlı ve ince kuyruklu ırklar arasında vitamin A düzeylerinde farklılık bulunmazken β-karoten miktarları farklılık gözlenmiştir

Avcı ve ark. (2013) ırklar arasında tiroid hormon düzeyleri önemli farklılık bulunurken, Keçeci (1999) Akkaraman ve Merinos koyunlarında ırklar arasında farklılık bildirilmiştir. Serum T3 ve T4 düzeylerini Akkaraman ırkı koyunlarda 222 ng/dl ve 5.33 mcg/dl, FT3 4.00 ng/dl FT4 1.10 mcg/dl, Merinos koyunlarında ise T3 169.00, FT3 4.83ng/dl, T4 5.00 mcg/dl, FT4 0.94 mcg/dl olarak bildirilmiş olup ırklar arasında bir farklılık, istatistiksel önem bulunmamıştır (Avcı et al., 2013). Sunulan bu çalışmada ise Tahirova ve Norduz koyunları T3 düzeyleri 3.42-2.94 pg/dl olarak bildirilirken T4 miktarı 1.08-0.92 ng/dl arasında saptanmıştır. İki ırk arasındaki T4 düzey farklılığı $p \leq 0.01$ düzeyinde önemli bulunmuştur.

Bu çalışmadaki Tahirova ve Norduz koyunlarında incelenen parametreler arasında ilişki veya etkileşim olup olmadığını anlamak için yapılan korelasyon analizlerinde; Tahirova ırkı koyunlarda alfa tokoferol ile total protein ve FT3 arasında negatif (sırasıyla $p<0.05$, $p<0.01$), vitamin D3 ile retinol arasında pozitif korelasyon ($p<0.05$) tespit edildi. Norduz ırkı koyunlarda ise FT3 ile üre ($p<0.01$), FT4 ile üre ($p<0.01$), retinol ile leptin ($p<0.05$), vitamin D3 ile leptin ($p<0.05$) arasında negatif korelasyon, FT4 ile FT3 ($p<0.05$) arasında pozitif korelasyon saptandı. Daha önce de belirtildiği gibi literatürde T3 ile leptin düzeyleri arasında negative, alfa tokoferol ile Vitamin D ve Leptin ile Vitamin D arasında negative ilişki saptanırken, mevsimsel olarak Vitamin A ve D arasında negative ilişki saptanmıştır. Yüksek vitamin A verilmesinin Vitamin D etkisini azalttığı ileri sürülmüştür (Cooney et al., 1995).

SONUÇ

Sonuç olarak Norduz ve Tahirova koyunlarında incelenen parametrelerden glukoz, üre, total protein, T3 retinol, α-tokoferol, Vitamin D3 ortalama değerleri arasında önemli bir farklılık bulunamadı. Tahirova ırkı koyunlarında leptin ve T4 düzeyleri Norduz ırkı koyunlarından daha yüksek ve ırklar arasındaki farklılık istatistiki öneme sahipti. Bilindiği gibi; beslenme, fizyolojik ve endokrin faktörler ruminantlarda leptin düzeyini etkilemekte ve leptin düzeyi vücuttaki yağ dokusu ile orantılıdır gibi klasik bilgi ve genellemeler, ince kuyruklu ve yağlı kuyruklu koyun ırkları arasında vücudun farklı yerlerinde yağ depolanmasının, moleküler mekanizmalarını açıklamada yetersiz kalmaktadır. Leptin düzeyi ile vücut yağ dokusu arasında belli bir orantı olmasına karşın, birbirlerine kesin bir bağımlılık ta yoktur. Bu, açlık sırasında yağ dokusunda ciddi olarak azalma şekillenmeden leptin miktarında gözlenen düşmenin, normal beslemeye geçildiğinde yağ depoları dolmadan leptin düzeyinin yükselmesi ile açıklanabilir. Sunulan araştırmanın sonuçlarına bakarak, ince kuyruklu koyunlardan olan Tahirova ırkında serum leptin düzeyinin yüksek olarak saptanmasının teorik olarak leptin üretiminin kuyruk yağ oranına bağlı olmadığını göstermektedir. İnce ve yağlı kuyruklu koyunlardaki bu gibi olayların biyokimyasal mekanizmalarını açıklanması için ilave besleme-hormon çalışmalarına gereksinim vardır.

Teşekkür: Bu araştırma yüksek lisans tezinden özetlenmiştir.

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

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

KAYNAKLAR

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RESEARCH ARTICLE

Effect of Vinegar and Lemon Juice on Survival of *Salmonella* in Outer Surface of Chicken Eggshell

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ABSTRACT

In this study, experimentally contaminated eggs with *Salmonella* were immersed for 3 min in distilled water (DW), lemon juice (L), vinegar (S) and lemon juice+vinegar (LS), and then stored at three different conditions such as: i) 4°C for 28 days, ii) 25°C for 28 days, iii) 4°C for 10 days following 25°C for 18 days. On day 0, *Salmonella* in control (K, without any treatment), DW, L, S and LS groups were detected 3,2±1,0, 3,6±0,4, 1,7±0,5, 1,9±0,8 and 1,8±0,8 log cfu/eggshell, respectively. The decrease in the number of *Salmonella* at day 0 in L and LS groups was statistically significant compared to K and DW groups (P<0,05). The *Salmonella* number decreased below the level of detection on the 18th day of storage in L and LS groups and on the 21st day in S group at 25 °C and 25 °C (18 days)+4 °C (10 days). The difference between *Salmonella* numbers at 4°C was found significant between DW and S and LS groups on the 6th day of storage (P<0,05). According to SEM observation of eggshell, it was determined that washing with L, S and LS caused significant damage to the cuticle layer of the eggshell. This study demonstrated that the use of lemon juice and vinegar in eggs resulted significant reductions in the number of *Salmonella.*

Keywords: Egg, lemon juice, immersion, *Salmonella*, vinegar

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Sirke ve Limon Suyunun Tavuk Yumurta Kabuğu Dış Yüzeyinde S*almonella*'nın Yaşamı Üzerine Etkisi

ÖZ

Bu araştırmada deneysel olarak *Salmonella* ile kontamine edilen yumurtalar kontrol (K) grubu hariç, distile su (DS), limon suyu (L), sirke (S) ve limon suyu+sirke (LS) yıkama sıvılarına daldırılıp 3 dakika bekletildikten sonra i) 4°C'de 28 gün, ii) 25 °C'de 28 gün, iii) 25 °C'de 18 gün ve sonrasında 4°C'de 10 gün olmak üzere üç farklı koşulda muhafaza edildi. K ve DS gruplarında 0. günde *Salmonella* sayısı sırasıyla 3,2±1,0 ve 3,6±0,4 log kob/yumurta kabuğu, L, S ve LS gruplarında ise sırasıyla 1,7±0,5, 1,9±0,8 ve 1,8±0,8 log kob/yumurta kabuğu olarak saptandı. L ve LS gruplarında 0. günde *Salmonella* sayısındaki azalma K ve DS gruplarına göre istatistiksel açıdan önemli bulundu (P<0,05). *Salmonella* sayısının 25 °C ve 25 °C (18 gün)+4 °C (10 gün) muhafazası sırasında L ve LS gruplarında muhafazanın 18. gününde, S grubunda ise 21. gününde tespit seviyesinin altına düştüğü belirlendi. Muhafaza sırasında (4 °C) *Salmonella* sayıları arasındaki farklılık DS ile S ve LS grupları arasında muhafazanın 6. gününde istatistiksel açıdan önemli bulundu (P<0,05). Tarayıcı elektron mikroskop görüntülerine göre L, S ve LS uygulamasının yumurtalarda kütikül tabakasında hasarlara neden olduğu saptandı. Bu çalışma; limon suyu ve sirkenin yumurtalarda *Salmonella* sayısında önemli derecede azalmalara neden olduğunu göstermektedir.

Anahtar Kelimeler: Daldırma, limon suyu, *Salmonella*, sirke, yumurta

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GİRİŞ

Yumurtanın ucuz ve yüksek besin kaynağı (biyoyararlı bileşiklerden karotenoidler, lutein, zeaksantini bulundurması, iyi bir kolin kaynağı vb.) içeriğine bağlı fonksiyonel bir gıda olarak kabul edilmesi dünyada yumurtanın en çok tüketilen gıdalar arasında yer almasına neden olmaktadır (Réhault-Godbert ve ark. 2019). Dünyada her yıl yaklaşık 86 milyon ton (1 ton = 18.995 adet) yumurta üretimi yapılırken 700 milyar adet yumurta tüketilmektedir (Réhault-Godbert ve ark. 2019, Mattioli ve ark 2020). Türkiye'de 2020 yılında tavuk yumurtası üretimi yaklaşık 1,2 milyon ton (19,8 milyar adet) olmuştur (Anonim 2022). Ancak yumurta, önemli bir gıda güvenliği riski olan *Salmonella* ile horizontal (dışkı, su, altlık, kan, toprak, toz vb.) ve/veya vertikal (üreme organları) olmak üzere iki farklı yolla kontamine olmaktadır. *Salmonella*'nın insanlara geçişinde yumurta ve ürünleri önemli bir aracı gıda olarak kabul edilmektedir (Sırıken ve Haldun 2013). Avrupa Birliği (AB)'nde 2018 yılında rapor edilen salmonelloz salgınlarının %45,6'sının, 2020 yılında ise %21.9'unun "yumurta ve yumurta ürünleri" tüketimi ile ilişkili olduğu rapor edilmiştir (EFSA 2021). Avrupa Birliği üyesi 24 ülkede yapılan bir araştırmada, yumurta tavuklarının insanlarda görülen salmonellozdan sorumlu suşların ana rezervuarı olduğu ve tüm insan enfeksiyon vakalarının %42,4'üne neden olduğu bildirilmiştir (De Knegt ve ark 2015). Amerika Birleşik Devletleri'ndeki salmonelloz salgınları ile ilişkili hastalıkların çoğunun yumurta ve/veya yumurta ürünleri kaynaklı *Salmonella* kontaminasyonu sonucunda oluştuğu belirtilmektedir (Callejón ve ark. 2015).

Sofralık yumurtalarda (A sınıfı) *Salmonella* kontaminasyonunu azaltmak için fiziksel (ışınlama, mikrodalga, ultraviyole ışık teknolojisi, ultrason vb.), biyolojik (bitki ekstraktlarının kullanımı, probiyotikler vb.) ve kimyasal (hidrojen peroksit, elektrolize su, ozon, organik asitler vb.) dekontaminasyon yöntemleri kullanılmaktadır (Juven ve Pierson 1996, Al-Haq ve ark. 2005, Galiş ve ark. 2013, Keerthirathne ve ark. 2017). Organik asitler ortamın pH'sını düşürerek ve/veya mikroorganizmaların yaşaması için gerekli olan metallerle şelat oluşturarak ya da hücre membranını değiştirip subsrat taşınımını bozarak antimikrobiyal etki gösterirler (Cemeroğlu ve ark. 2004). Ülkelere göre sofralık yumurtaların yıkanması ile ilgili yasal mevzuatlar farklılık göstermektedir. Türk Gıda Kodeksi Yumurta Tebliğinin madde 5.2. (b) bendinde "A sınıfı yumurta (sofralık) yıkanarak veya başka bir yöntemle temizlenemez ve yağlama işlemine tabi tutulamaz" ibaresinden dolayı böyle yumurtaların yıkanması yasaktır (Anonim 2014). Benzer şekilde Avrupa Birliği'nde A sınıfı yumurtaların yıkanmasına izin verilmemektedir (Galiş ve ark. 2013). ABD, Kanada ve Avustralya gibi bazı ülkelerde sofralık yumurtaların yıkandıktan sonra satışında ise herhangi bir yasal engel bulunmamaktadır. Ancak yumurtaların yıkanması yumurta kabuğunda hasarlara neden olabilmektedir (Galiş ve ark. 2013). Yumurta kabuğunun taramalı elektron mikroskobu (scanning electron microscope, SEM) görüntüleri daldırma (immersiyon) sonrası özellikle kütikül tabakasının hasar gördüğü veya tamamen ortadan kalktığını göstermektedir. Bu da *Salmonella* gibi patojenlerin yumurtaya penetrasyonunu kolaylaştırmakta ve patojenlerin kolayca yumurtanın içine doğru hareket etmesine katkı sağlamaktadır (Galiş ve ark. 2013, Keerthirathne ve ark. 2017, Juven ve Pierson 1996, Al-Haq ve ark. 2005, Cemeroğlu ve ark. 2004, Ofongo ve ark. 2022, Grudlewska-Buda ve ark. 2022).

Sirke ve limon suyunun düşük pH değerlerine sahip olması ve içeriğindeki organik asitler başta *Salmonella* olmak üzere gıda kaynaklı patojenlere karşı değişen oranlarda antimikrobiyal etki göstermesine neden olur (Elhan 2014, Gökırmaklı ve ark. 2019). Bazı tüketiciler içeriğindeki organik asitlerden dolayı asidik yıkama solüsyonu olarak sirke ve limon suyunu gıda maddelerinde kullanabilmektedir (Şengün ve Karapınar 2006, Henley 2013, Öncül ve Karabıyıklı 2019, Vatral ve Quinlan 2021). Tüketici düzeyinde yapılan araştırmalar tüketicilerin bir kısmının asidik yıkama solüsyonlarının kanatlı hayvan ürünlerini daha güvenli hale getirdiğine dair çok yanlış bir izlenime sahip olabileceğini ortaya koymaktadır (Prior ve ark. 2010, Koppel ve ark. 2014, Henley ve ark. 2016, Henley ve ark. 2018). Ancak asidik yıkama sıvılarının çiğ kanatlı hayvan ürünlerinde *Salmonella* gibi patojenlerin riskini tamamen ortadan kaldırması pek mümkün olmadığı gibi hem ürünün raf ömrünü olumsuz etkileyebilmekte hem de çapraz kontaminasyonlar yoluyla halk sağlığı problemlerine neden olabilmektedir. Türkiye, AB, İngiltere, ABD ve Kanada gibi birçok ülke yumurta gibi çiğ kanatlı hayvan ürünlerinin tüketiciler tarafından su dahil herhangi bir yıkama solüsyonu ile yıkanmasını, çapraz kontaminasyonlara neden olabileceğinden önermemektedir [Anonim 2014, Anonim 2022(a), Anonim 2022(b)].

Bu çalışmada asidik (sirke, limon suyu ve bunların karışımları) ve asidik olmayan (su) yıkama sıvılarının: i) yumurta kabuğu dış yüzeyinde *Salmonella*'nın yaşamı üzerine etkisi ve ii) yumurta kabuğunun fiziksel yapısında meydana getirdiği değişiklerin belirlenmesi amaçlanmıştır.

MATERYAL ve METOT

Bu çalışmada Lohmann Sandy ırkı tavuklardan elde edilen ortalama 53-62 g ağırlığındaki yumurtalar materyal olarak kullanıldı. Yumurtalar Dicle Üniversitesi Ziraat Fakültesi Yumurta Tavukçuluğu Araştırma Uygulama Ünitesinden temin edildi. Kirli (dışkı, kan vb.), çatlak ve/veya şekil bozukluğu (kalın kabuklu, aşırı derecede kalsifiye, buruşuk kabuklu vb.) olan yumurtalar çalışmaya dahil edilmedi. Bu çalışma

üç kez tekrar edildi ve her tekrarda 75 adet yumurta olmak üzere toplam 225 adet yumurta kullanıldı.

Kontaminasyon sıvısının hazırlanması

Salmonella typhimurium (ATCC 14028) ve *Salmonella enteritidis* (RSKK 91) suşları yumurtaların kontaminasyonu amacıyla kullanıldı. Stoktan (-20 °C'de bekletilen) alınan her bir *Salmonella* suşu 10 ml'lik Tryptic Soy Broth (TSB) (LABM, Lancashire, UK)'da 37 °C'de 18-24 saat inkübe edildi. Sonra santrifüjle (Nüve NF 800 R, Ankara, Türkiye) 4200 rpm'de 4 °C' de 15 dakika süre ile santrifüj edildi. Santrifüj sonunda üstte kalan süpernatant uzaklaştırıldı. Geriye kalan pelete 9 ml %0,1'lik peptonlu su (LABM, Lancashire, UK) ilave edildi ve 4200 rpm'de 4 °C de 15 dakika süre ile santrifüj edildi ve bu işlem 2 kez tekrar edildi. Santrifüj sonunda üstte kalan süpernatant uzaklaştırıldı ve peletler eşit miktarda karıştırılarak %0,1 peptonlu suda süspansiyon haline getirilerek 300 ml steril %0,1' lik peptonlu su ile tamamlandı. Hazırlanan kontaminasyon sıvısındaki *Salmonella* sayısının belirlenmesi için 10^8'e kadar seri dilüsyonlar hazırlandı ve her bir dilüsyondan 0,1 ml alınarak Xylose Lysine Tergitol 4 (XLT4) (Merck, Germany) agar içeren petrilere transfer edildi. 37 °C'de 18-24 saat inkübasyonu takiben XLT4 agarda üreyen tipik kolonilerin sayımı sonrası kontaminasyon sıvısındaki *Salmonella* miktarının 10^6 kob/ml düzeyinde olduğu tespit edildi. Hazırlanan kontaminasyon sıvısı 30 dakika içinde kullanıldı. Her bir grup için 3 litre olacak şekilde her tekrarda 15 litre kontaminasyon sıvısı hazırlandı.

Yumurtaların *Salmonella* ile deneysel kontaminasyonu

Deneysel aşamada kullanılacak her bir yumurta örneği aseptik koşullarda %70'lik alkol içinde 1 dakika bekletildi ve böylece yumurta kabuğunun yüzeyinde bulunan mikroorganizmaların elimine edilmesi sağlandı. Alkol uygulamasını takiben yumurtalar yaklaşık 60 dakika oda sıcaklığında kurumaya bırakıldı. Bu işlem sonrası yumurtaların *Salmonella* ile kontaminasyonu gerçekleştirildi (Wang ve Slavik 1998). Yumurtalar 10^6 kob/ml düzeyinde *Salmonella typhimurium* (ATCC 14028) ve *Salmonella enteritidis* (RSKK 91) suşlarını içeren kontaminasyon solüsyonu içinde tüm yüzeyi temas edecek şekilde 3 dakika bekletildi. Daha sonra *Salmonella* suşlarının yumurta yüzeyine tutunması için yumurtalar oda sıcaklığında 60 dakika bekletildikten sonra yumurtaların yıkanması aşamasına geçildi (Wang ve Slavik 1998).

Yumurtaların yıkama sıvılarına daldırılması ve deneysel grupların oluşturulması

Kontrol, distile su, limon suyu, sirke, limon suyu+sirke olmak üzere Tablo 1'de gösterildiği gibi gruplar oluşturuldu. *Salmonella* ile kontamine edilen her bir yumurta daha önceden hazırlanan yıkama sıvılarına daldırılarak (immersiyon) 3 dakika bekletildi. Süre sonunda aseptik koşullarda yumurtalar çıkarılarak laminar kabinde oda sıcaklığında 60 dakika bekletildi (Wang ve Slavik 1998). Bunu takiben yumurtalar steril spatüller yardımı ile plastik viyollere birbirlerine temas etmeyecek şekilde yerleştirildi ve üç farklı koşulda [4°C'de 28 gün, 25 °C'de 28 gün, 25 °C'de 18 gün ve sonrasında 4°C'de 10 gün] muhafaza edildi.

Tablo 1. Deneysel gruplar
Table 1. Treatment groups

Kontrol (K)	Herhangi bir uygulama yapılmamış grup
Distile su (DS)	Yalnız suya daldırılan grup
Limon suyu (L)	Yalnız limon suyuna daldırılan grup
*Sirke (S)	Yalnız sirkeye daldırılan grup
Limon suyu+sirke (LS)	Limon suyu+sirke(1:1)'ye daldırılan grup

* %4-5 asetik asit, pH: 2,38

Yumurta kabuğunda mikrobiyolojik analizlerin yapılması

Muhafazanın 0., 6., 18., 21. ve 28. günlerinde *Salmonella* sayısında meydana gelen değişimleri belirlemek amacıyla her örneklem gününde aseptik koşullarda yumurtalar steril numune alma poşetlerine konuldu ve üzerine 50 ml steril tamponlanmış petonlu su (BPW) (LABM, Lancashire, UK) ilave edildi. *Salmonella* patojeninin yumurta kabuğundan tamponlanmış peptonlu suya geçişini kolaylaştırmak amacıyla her bir yumurtaya 10 dakika boyunca dikkatli bir şekilde dışarıdan elle masaj işlemi uygulandı. Daha sonra 1/10'luk düzende seri dilüsyonlar hazırlandı ve 0,1 ml her bir dilüsyondan alınarak Xylose Lysine Tergitol 4 (XLT4) (Merck, Germany) agar içeren petrilere yayma yöntemine göre ekim yapıldı ve 37 °C'de 18-24 saat bekletildi. Üreyen tipik *Salmonella* kolonileri sayılarak not edildi. Ayrıca, *Salmonella* sayısının tespit seviyesinin altına düşmesi durumuna karşı örneklere zenginleştirme işlemi uygulandı. Bu amaçla her bir örneklem gününde *Salmonella* sayımı için ekimler gerçekleştirildikten sonra numune alma poşetlerinde geriye kalan homojenizatlar (BPW+yumurta) 37 °C'de 18-24 saat bekletildi (ön zenginleştirme). Bunu takiben ön zenginleştirilmiş sıvıdan 0,1 ml alınarak içerisinde 10 ml Rappaport

Vassiliadis Soy broth (LABM, Lancashire, UK) bulunan polipropilen konik tüplere aktarıldı ve tüpler 41,5 °C'de 18-24 saat inkübe edildi (selektif zenginleştirme). Daha sonra zenginleştirilmiş sıvıdan bir öze dolusu alınarak XLT4 agar besiyerine geçildi ve 37 °C'de 18-24 saat bekletilerek tipik *Salmonella* kolonilerinin varlığı yönünden incelendi.

pH analizi

Distile su, limon suyu, sirke ve limon suyu+sirkenin (1:1) pH analizleri pH metre (EcoMet P25, Kore) cihazı kullanılarak her tekrar öncesi gerçekleştirildi. Bu amaçla pH metrenin kalibrasyonu pH'sı bilinen tampon çözeltiler ile yapıldıktan sonra pH ölçümü yapılacak sıvılar bir behere aktarıldı. pH metrenin probu distile su ile yıkanıp kurutulduktan sonra ölçümü yapılacak sıvıya daldırıldı. Bir süre bekledikten sonra ekranda sabitlenen pH değerleri kaydedildi.

Yumurta kabuğunun mikro yapısal özelliklerinde meydana gelen değişimlerin belirlenmesi

Yumurtaların su ve asidik sıvılara daldırılması sonrası yumurta kabuğunda meydana gelen değişiklikler taramalı elektron mikroskobu (SEM) kullanılarak incelendi. Bu amaçla kontrol ve daldırma sonrası her bir gruptan (DS, S, L, LS) 1 cm²'lik yumurta kabuğu aseptik koşullarda alındı. Kurutma sonrası yumurta kabukları standart SEM modu Quanta FEG 250 (FEI, Netherland) kullanılarak X500 büyütmede incelendi (II, Hitachi, Tokyo, Japonya).

İstatistiksel Analiz

Salmonella tespit seviyesi (the level of detection, LOD), kullanılan BPW miktarı (50 ml) ile BPW+yumurta homojenizatından alınan miktarın (0,1 ml) ml başına saptanabilir en düşük koloni sayısıyla (10 kob) çarpılmasıyla elde edildi. Bu çalışmada *Salmonella* sayısı için tespit seviyesi (the level of detection, LOD) 500 kob/yumurta kabuğu (2,69 log kob/yumurta kabuğu) olarak hesaplandı. Tüm gruplarda *Salmonella* sayıları log kob/yumurta kabuğu'na dönüştürülerek 5x5x3 (test grubu x örneklem zamanı x muhafaza sıcaklığı) olacak şekilde varyans analizi (ANOVA) gerçekleştirildi ve gruplar arası, günler arası ve sıcaklıklar arası farklılıklar karşılaştırıldı. Ortalamalar, General Linear Models (GLM) prosedürlerine göre Fisher'in en küçük kareler farkı (Fisher's LSD) kullanılarak ayrıldı ve istatistiksel önem seviyesi P≤0,05 olarak kabul edildi. İstatiksel analizlerin gerçekleştirilmesinde Statistical Analysis System (SAS) paket programı kullanıldı (SAS 1999).

BULGULAR

Bu çalışmada farklı muhafaza sıcaklıkları ve yıkama sıvılarının, yumurtanın 28 günlük muhafazası sırasında *Salmonella* varlığı üzerine etkileri incelendi. Muhafazanın 0. gününde K ve DS gruplarında *Salmonella* sayısı sırasıyla 3,2±1,0 log kob/yumurta kabuğu ve 3,6±0,4 log kob/yumurta kabuğu olarak tespit edilirken L, S ve LS gruplarında ise sırasıyla 1,7±0,5 log kob/yumurta kabuğu, 1,9±0,8 log kob/yumurta kabuğu ve 1,8±0,8 log kob/yumurta kabuğu düzeyinde olduğu saptandı. Bu da özellikle L, S ve LS'nin uygulandığı anda (0. gün) DS grubuna göre *Salmonella* üzerine inhibisyon etkisinin fazla olduğunu göstermektedir. Farklı muhafaza sıcaklıklarına göre *Salmonella* sayısında meydana gelen değişimler Tablo 2'de verilmiştir. DS, L, S ve LS'nin pH'sı sırasıyla ortalama 6,10±0,02, 2,02±0,02, 2,38±0,02 ve 2,19±0,03 olarak belirlendi.

Limon suyu, sirke ve karışımlarının farklı sıcaklıklardaki muhafaza sırasında *Salmonella* üzerine etkisi

Yumurta örneklerinin 4 °C'de muhafazası sırasında K grubu dâhil tüm gruplarda *Salmonella* sayısında azalma saptandı. K ve DS gruplarında *Salmonella* sayısının muhafazanın 0. gününden 28. gününe kadar sırasıyla toplam 1,4 log kob/yumurta kabuğu ve 2,6 log kob/yumurta kabuğu azaldığı ve muhafaza süresince tespit seviyesinin içinde kaldığı belirlendi. *Salmonella* sayısındaki azalmanın DS grubu (3,1±0,5 log kob/yumurta kabuğu) ile S (1,3±0,2 log kob/yumurta kabuğu) ve LS (1,2±0,1 log kob/yumurta kabuğu) grupları arasında muhafazanın 6. gününde istatistiksel açıdan önemli olduğu bulundu (P<0,05). Her ne kadar istatistiksel açıdan gruplar arasında farklılık, muhafazanın sadece 6. gününde tespit edilmiş olsa da muhafazanın diğer günlerinde de S, L ve LS gruplarının K ve DS gruplarına göre *Salmonella*'nın inhibisyonu üzerine daha etkili olduğu görüldü. Her 3 tekrarda muhafazanın 18. gününde LS grubunda, 21. gününde L grubunda ve 28. gününde S grubunda *Salmonella*'nın tespit seviyesinin altında ve ancak zenginleştirme işlemi sonrası pozitif olduğu saptandı.

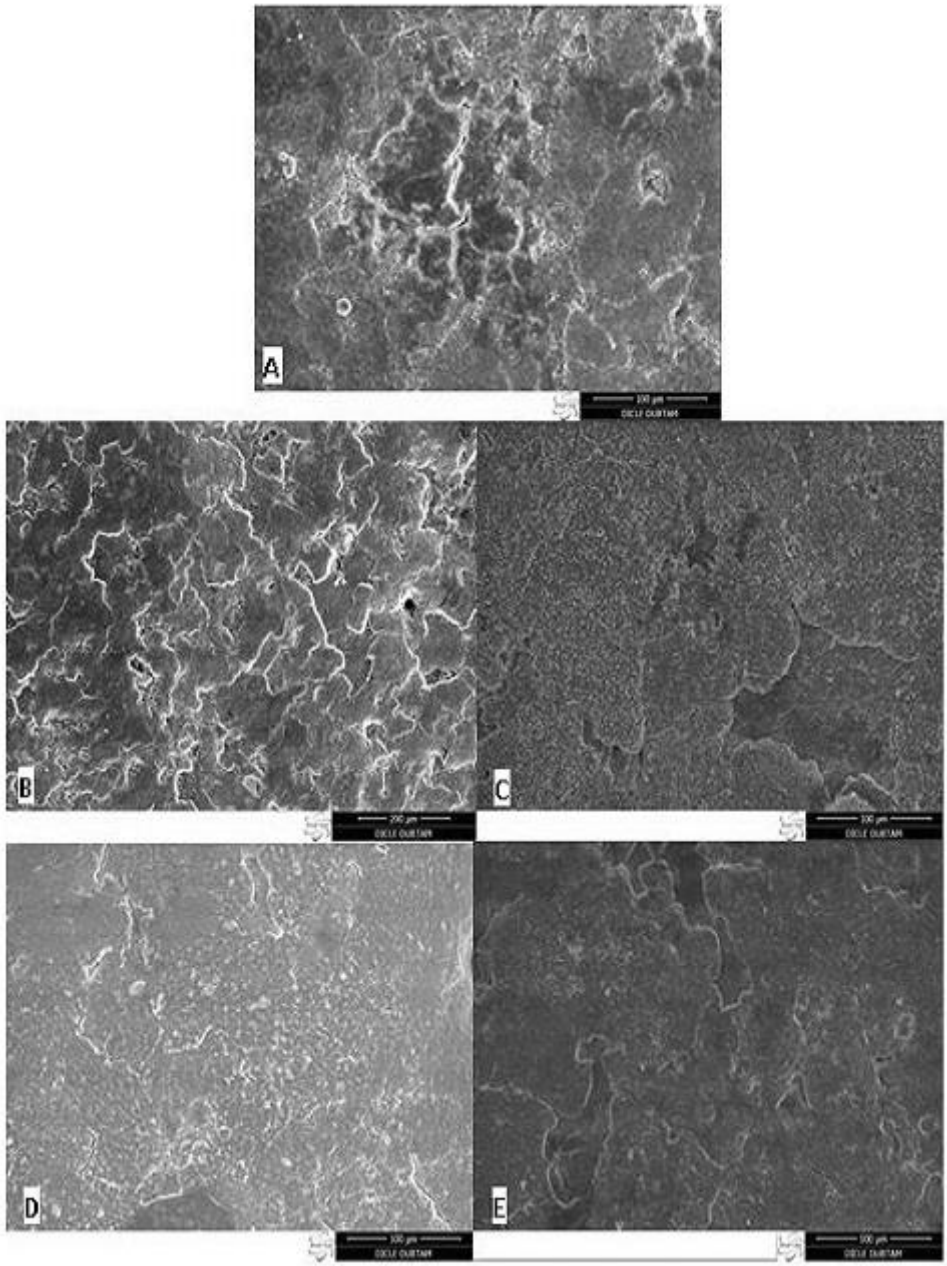
Yumurtaların 25 °C'deki muhafazası sırasında tüm gruplarda *Salmonella* sayısında kademeli bir düşüşün meydana geldiği belirlendi. 0. günün dışında gruplar arasında *Salmonella* sayılarındaki değişimin istatistiksel açıdan önemli olmadığı tespit edildi (P>0,05). Her ne kadar gruplar arasında istatistiksel açıdan *Salmonella* sayısında bir farklılık tespit edilmemiş olsa da özellikle 18. gün ve sonrasındaki muhafaza günlerinde L, S ve LS gruplarında *Salmonella* inhibisyonunun diğer gruplara göre daha fazla olduğu saptandı. K ve DS gruplarında muhafazanın sonunda (28 gün) *Salmonella* sayısında sırasıyla toplam 2,1 log kob/yumurta kabuğu ve 2,3 log kob/yumurta kabuğu düzeyinde azalma saptandı. L, LS ve S gruplarında muhafazanın 6. gününde sırasıyla 1,7±0,8 log kob/yumurta kabuğu, 1,7±0,5 log kob/yumurta kabuğu ve 1,2±0,2 log kob/yumurta kabuğu olan *Salmonella* sayısının L ve LS gruplarında muhafazanın 18. gününde, S grubunda ise 21. gününde tespit seviyesinin altında ve ancak zenginleştirme işlemi sonrası pozitif olduğu bulundu.

Yumurtaların 25 °C'de 18 günlük muhafazası sırasında *Salmonella* sayısı L ve LS gruplarında tespit seviyesinin altında, K, DS ve S gruplarında ise sırasıyla 1,6±0,2 log kob/yumurta kabuğu, 1,8±0,4 log

kob/yumurta kabuğu ve 1,3±0,1 log kob/yumurta kabuğu düzeyinde olduğu tespit edildi. Muhafazanın 0. günü dışında gruplar arasında *Salmonella* sayısındaki değişiklikler istatistiksel açıdan önemsiz bulundu (P>0,05). Diğer muhafaza sıcaklıklarından farklı olarak bu sıcaklık kombinasyonunda muhafazanın sonunda DS grubundaki *Salmonella* sayısındaki azalmanın daha sınırlı olduğu görüldü. Muhafazanın 21. gününde *Salmonella* sayısı S grubunda tespit seviyesinin altında ancak zenginleştirme işlemi sonrası pozitif olduğu bulundu, K ve DS gruplarında ise 21. günde sırasıyla 1,2±0,2 log kob/yumurta kabuğu ve 2,1±0,4 log kob/yumurta kabuğu, 28. günde ise 1,2±0,4 log kob/yumurta kabuğu ve 1,7±0,7 log kob/yumurta kabuğu olduğu saptandı.

Daldırmanın yumurta kabuğunun mikro yapısı üzerine etkisi

Daldırma işlemi sonrası yumurta kabuklarında meydana gelen değişikliklere ait görüntüler Şekil 1'de verilmiştir. Kontrol grubu ve distile suya daldırma işleminin uygulandığı grup arasında önemli farklılıklar görülmemekle beraber distile su ile yıkanan grupta çatlakların arttığı ve hafif düzeyde kütikül hasarının meydana geldiği gözlendi. Kontrol grubuna göre S, L ve LS'ye daldırmanın yumurta kabuğunun kütikül tabakasında önemli oranlarda kayıplar ve/veya hasarlara neden olduğu belirlendi.



Şekil 1: Yumurta kabuğunun SEM görüntüsü (x500) **A.** Kontrol (K); **B.** Distile Su (DS); **C.** Limon Suyu (L); **D.** Sirke (S); **E.** Limon Suyu + Sirke (LS)

Figure 1: SEM images of eggshell (x500) **A.** Control (K); **B.** Distilled Water (DW); **C.** Lemon Juice (L); **D.** Vinegar (S); **E.** Lemon Juice + Vinegar (LS)

Tablo 2. Farklı koşullarda 28 günlük muhafaza süresince yumurta kabuğu dış yüzeyinde *Salmonella* sayısında meydana gelen değişimler
Table 2. Changes in the number of *Salmonella* in outer surface of chicken eggshell during 28 days of storage at different conditions

Muhafaza Sıcaklığı	Muhafaza Süresi(gün)	Gruplar ve pH Değerleri				
		K[ı]	**DS**[ı] (pH: 6,10)	**L**[ı] (pH: 2,02)	**S**[ı] (pH: 2,38)	**LS**[ı] (pH: 2,19)
4 °C	**0**	3,2±1,0^{xA}	3,6±0,4^{xA}	1,7±0,5^{yA}	1,9±0,8^{xyA}	1,8±0,8^{yA}
	6	2,4±0,2^{xyA}	3,1±0,5^{xAB}	1,5±0,3^{xyA}	1,3±0,2^{yA}	1,2±0,1^{yA}
	18	2,3±0,7^{xA}	1,5±0,9^{xyA}	1,2±0,8^{yA}	1,2±0,2^{yA}	<
	21	2,2±0,6^{xA}	1,3±0,8^{xB}	<	1,2±0,3^{xA}	<
	28	1,8±0,2^{xA}	1,1±0,3^{xB}	<	<	<
25 °C	**0**	3,2±1,0^{xA}	3,6±0,4^{xA}	1,7±0,5^{yA}	1,9±0,8^{xyA}	1,8±0,8^{yA}
	6	1,7±0,4^{xAB}	1,6±0,5^{xB}	1,7±0,8^{xA}	1,2±0,2^{xA}	1,7±0,5^{xA}
	18	1,2±0,4^{xB}	1,4±0,5^{xB}	<	1,1±0,04^{xA}	<
	21	1,3±0,1^{xB}	1,4±0,4^{xB}	<	<	<
	28	1,2±0,2^{xB}	1,3±0,2^{xB}	<	<	<
25°C + 4 °C ¥	**0**	3,2±1,0^{xA}	3,6±0,4^{xA}	1,7±0,5^{yA}	1,9±0,8^{xyA}	1,8±0,8^{yA}
	6	1,8±0,4^{xAB}	2,3±0,3^{xAB}	1,6±0,2^{x}	1,5±0,2^{xA}	1,9±0,1^{xA}
	18	1,6±0,2^{xAB}	1,8±0,4^{xAB}	<	1,3±0,1^{x}	<
	21	1,2±0,2^{xB}	2,0±0,4^{xAB}	<	<	<
	28	1,2±0,4^{xB}	1,7±0,7^{xB}	<	<	<

* Aynı satırda farklı harfler (x, y) ile gösterilen sonuçlar istatistiksel olarak önemlidir (P≤0,05)
* Aynı sütunda farklı harfler (A,B) ile gösterilen sonuçlar istatistiksel olarak önemlidir (P≤0,05)
* <:*Salmonella* sayısının tespit seviyesinin altına düştüğü durumları ifade eder.
* K: kontrol, DS: distile su, L: limon suyu, S: sirke, LS: limon suyu+sirke
¥ İlk 18 gün 25 °C'de sonraki 10 gün 4 °C'de muhafaza
ı log kob/yumurta kabuğu

TARTIŞMA

Bu çalışma, farklı sıcaklık ve dekontaminantların muhafaza süresi boyunca tavuk yumurtalarında *Salmonella*'nın varlığı üzerine etkilerinin incelenmesi amacıyla gerçekleştirildi. Çalışma bulgularımız daldırma işlemi uygulanmayan kontrol (K) ve distile su (DS) ile yıkanan gruplarda *Salmonella* patojeninin muhafaza süresince yumurta kabuğunda 1 log kob/yumurta kabuğu'nun üstündeki sayılarda yaşayabileceğini ve 4 °C'ye göre 25 °C'deki muhafaza sırasında *Salmonella*'daki azalmanın daha hızlı olduğu bulundu. Mikroorganizmalar, oda sıcaklığı gibi ortam sıcaklıklarına göre düşük sıcaklıklarda veya buzdolabı sıcaklıklarında azalan metabolik aktivitelerinden dolayı daha uzun süre hayatta kalabilmektedirler. Enzim katalizli reaksiyonlar sıcaklığa bağlı olduğundan, bu aktiviteler düşük sıcaklıkta büyük ölçüde azalır (Jay ve ark. 2005). Radkowski (2002) tarafından yapılan bir araştırmada, *Salmonella enteritidis* ile kontamine edilen 1440 yumurtanın 0, 7, 14 ve 21 gün boyunca 2 °C, 20 °C ve 30 °C olmak üzere üç farklı muhafazası sırasında *Salmonella enteritidis* 20 °C ve 30 °C'ye göre 2 °C'de daha sık saptanmıştır (Radkowski 2002). Başka bir çalışmada *Salmonella enteritidis* ile kontamine edilen yumurta kabuklarının oda sıcaklığına göre 7 °C'de daha uzun süre canlı kaldığı bildirilmiştir (Baker 1990). Muhafaza süresince yumurta kabuk yüzeyinde *Salmonella*'nın yaşamı üzerine muhafaza sıcaklığının dışında relatif rutubetin de etkili olduğu ve rutubet oranı arttıkça *Salmonella*'nın yaşam kabiliyetinin arttığı farklı araştırmacılar tarafından bildirilmiştir (Park ve ark. 2015). Çalışmamızda distile su (DS) ile yıkanan yumurtaların 4 °C'lik muhafaza sırasında *Salmonella* sayısındaki azalma hızının 25 °C'dekine göre çok daha yavaş olduğu görüldü.

Gıda maddelerinde kullanımı genel olarak güvenli kabul edilen (GRAS) sitrik asit limonda, asetik asit ise sirkede doğal olarak bulunur ve bu asitler limon suyu ve sirkenin patojenlere karşı antimikrobiyel etki göstermesinde önemli rol oynarlar (Lytou ve ark 2019). Ancak kullanılan limon suyu veya sirke konsantrasyonu, uygulama süresi, muhafaza sıcaklığı ve gıda tipi gibi değişkenler antimikrobiyel etkinin ortaya çıkmasında farklılıklara neden olabilmektedir. Şengün ve Karapınar (2004), 10^8 düzeyinde *Salmonella typhimurium* ile kontamine ettikleri havuçların 15, 30 ve 60 dakika limon suyuna daldırılması sonrasında *Salmonella typhimurium* sayısında sırasıyla 2,68, 2,68 ve 3,95 log kob/g'lık bir azalmanın meydana geldiğini bildirmiştir (Şengün ve Karapınar 2004). Aynı çalışmada 15., 30. ve 60. dakikalar arasında *Salmonella typhimurium* sayısındaki azalma istatistiksel açıdan önemsiz iken ($P>0,05$), limon suyunun *Salmonella typhimurium*'a karşı ani antimikrobiyal etkisi 0. dakikada anlamlı bulunmuştur ($P<0,05$). Kışla ve Üzgün (2008), 0. dakikada limon suyunun midye dolmalarında *Salmonella typhimurium*'a karşı ani antimikrobiyal etkisinin ihmal edilebilir olduğunu bulmuştur ($P>0,05$). Ancak antimikrobiyal etkinin 5. dakikadan itibaren başladığını ve istatistiksel açıdan önemli olduğunu bildirmişlerdir ($P<0,05$) (Kışla ve Üzgün 2008). Başka bir araştırma da Bingöl ve ark. (2011), limon suyunun çiğköftede *Salmonella enteritidis*'e karşı 10 ve 30 saniyedeki ani antimikrobiyal etkisinin ihmal edilebilir düzeyde olduğunu ($P>0,05$), ancak antimikrobiyal etkinin 5. dakikadan itibaren görüldüğünü ($P<0.05$), 5. ve 15. dakikalar arasında anlamlı bir fark bulunmazken 30. ve 60. dakikalar arasında farklılığın önemli olduğu tespit edilmiştir ($P<0,05$). Çalışma bulgularımıza göre *Salmonella* ile kontamine edilen yumurta kabuk dış yüzeylerinin limon suyu ile üç dakika maruz bırakılması sonrası ilk uygulama anında (0.gün) *Salmonella* sayısında yaklaşık 2 log kob/yumurta kabuğu bir azalmaya neden olduğu ve bu azalmanın K ve DS grubuna göre istatistiksel açıdan önemli olduğu bulundu ($P<0,05$).

Çalışmamızda limon suyunun aksine sirke ile yıkanan yumurta örneklerinde *Salmonella* sayısının muhafazanın 21. (25 °C ve 25 °C+4 °C sıcaklıkta) ve 28. (4 °C sıcaklıkta) gününde tespit seviyesinin altına düştüğü belirlendi. Bu da muhafaza sıcaklığına bağlı *Salmonella* sayısında 1,92 log kob/yumurta kabuğu bir azalma olduğunu göstermektedir. Çalışma bulgularımıza benzer şekilde Lytou ve ark. (2019) %2 asetik asit içeren sirke ile yapılan marinasyonun tavuk göğüs filetolarında, 4 °C'nin aksine daha yüksek muhafaza sıcaklıklarında (8, 12 ve 16 °C'de) ve daha uzun muhafaza sürelerinde (7. ve 9. günler) tespit seviyesinin altına düşmesine neden olduğunu saptamıştır (Lytou ve ark 2019). Hawkins ve ark. (2016), *Salmonella* ile kontamine ettikleri tavuk etlerine sprey kabinleri aracılığıyla 15, 30, 45 ve 60 saniye %0,8 sirke uygulanmasının *Salmonella* sayısında sırasıyla 0,19, 0,32, 0,51, 0,63 log kob/g azalmaya neden olduğunu bildirmiştir. Aynı araştırmada 4 °C'de 3 gün muhafaza sonunda sirke uygulanan grupta *Salmonella* sayısında 0,18 log kob/g gibi çok düşük düzeyde azalma görüldüğü bildirilmiştir (Hawkins ve ark. 2016).

Bu çalışmada limon suyu ve sirkenin tek başına kullanıldığı gruplara göre limon suyu+sirke karışımında muhafazanın 6. gününden itibaren her üç farklı koşulda *Salmonella*'nın tespit seviyesinin altına düştüğü saptandı. Bu da limon suyu+sirke karışımının kullanılmasının *Salmonella* inhibisyonunu arttırdığını göstermektedir. Çalışma bulgularımıza benzer şekilde Şengün ve Karapınar (2004), havuçların farklı sürelerde tek başına limon suyu veya sirke ile muamele edilmesinin *Salmonella typhimurium* üzerinde sırasıyla 0,79-3,95 ve 1,57-3,58 log kob/g arasında önemli azalmalara neden olduğunu, limon suyu-sirke karışımının 30 dakika uygulanmasından sonra patojen

sayısının ise tespit seviyesinin altına düştüğünü bildirmiştir (Şengun ve Karapınar 2004).

Tavuk filetolarının marinasyonu için sitrik asit ve asetik asidin *Salmonella*'nın varlığı ve gelişimi üzerine etkisinin karşılaştırıldığı bir çalışmada, asetik asidin belirli bir pH ve konsantrasyonunun sitrik asite göre daha etkili olduğu, bunun da asetik asidin daha yüksek miktarlarda ayrışmamış formda olması ile ilişkili olduğu bildirilmiştir (Lytou ve ark 2019). Aynı çalışmada asit miktarının eşit olduğu durumda (örneğin, limon suyu içinde sitrik asit %2 [v/v] ve elma sirkesi içinde asetik asit %1 [v/v]) asetik asidin patojen üzerine etkisinin daha fazla olmasında toplam asit konsantrasyonu ile ayrışmamış asit konsantrasyonu dışında asit tipinin de antimikrobiyal etkiye katkıda bulunabileceği ifade edilmiştir. Ancak bu çalışmanın aksine çalışmamızda sirke (%4-5 asetik asit, pH: 2,38) grubunda limon suyu ve limon suyu+sirke karışımı gruplarına göre *Salmonella* sayısının tespit seviyesinin altına ancak muhafazanın 21. gününden itibaren düştüğü belirlenmiştir. Antimikrobiyal etkinin ortaya çıkmasında sirke tipi, uygulanan gıda modeli ve *Salmonella* tespitinde kullanılan mikrobiyolojik yöntemler gibi değişkenler sonuçlar arasında farklılıklara neden olabilmektedir.

Sofralık yumurtalarda (A sınıfı) *Salmonella* kontaminasyonunu azaltmak için kullanılan başta organik asitler olmak üzere termal veya termal olmayan dekontaminasyon uygulamalarının yumurta kabuğunun fiziksel yapısında hasarlara neden olabileceğini göstermektedir (Galiş ve ark. 2013, De Souza 2019, Lakins ve ark. 2008). Kütikül, iç proteinleri (sarısı ve beyaz) dehidrasyondan ve bakteriyel kontaminasyondan korur. Yapılan bir çalışmada dezenfektanların yumurta kütikülüne zarar verebileceğini ve muhafaza süresine bağlı olarak bakteri hasarının fazla olduğu bildirilmiştir (Wang ve Slavik 1998). Li ve ark. 2019, %2 laktik asidin spreyleme yoluyla yumurta kabuklarına uygulamasının *Salmonella enteritidis* sayısında 5,63 logaritmalık bir azalmaya neden olduğunu ancak asit veya su ile yıkama işlemi yapılmayan kontrol grubuna göre laktik asit uygulanan yumurta kabuğunun SEM görüntülerinde kütikül kayıplarının ve hasarlarının meydana geldiğini bildirmiştir (Li ve ark. 2019). Benzer şekilde çalışmamızda asidik sıvılara daldırılan yumurtalara ait kabuklarda kütikül bütünlüğünün bozulduğu ve kütikül kayıplarının oluştuğu belirlendi.

SONUÇ

Çalışma sonuçlarımıza göre limon suyu ve sirkenin yumurta kabuğunda *Salmonella* sayısında azalmalara neden olduğu ancak yumurtadaki *Salmonella* riskini tamamen ortadan kaldırmadığını göstermektedir. Yumurtaların sirke ve limon suyuna daldırılması ile tüketiciler tarafından böyle gıdaların "güvenli gıda" olarak algılanarak tüketilmesi sonrası oluşabilecek halk sağlığı riskleri de göz önünde bulundurulmalıdır. Bu kapsamda başta tüketiciler olmak üzere gıda hazırlayıcıların ve ilişkili alanlarda istihdam edilen gıda çalışanlarının bu konuda bilinçlendirilmesinin gerekli olacağı düşünülmektedir. Tavuk yumurtalarında *Salmonella* kontaminasyonlarının önlenmesi ve kontrol altına alınmasında, çiftlikten sofraya gıda güvenliği konsepti yaklaşımının sıkı bir şekilde uygulanması önem arz etmektedir. Özellikle ulusal düzeyde *Salmonella* kontrol programlarının etkin olarak devam ettirilmesi yumurta kaynaklı salmonelloz vakalarının azaltılmasında ciddi katkılar sağlayacaktır.

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RESEARCH ARTICLE

Some Egg Quality Characteristics and Hatching Performances of Leghorn Hybrids

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ABSTRACT

This study examined the hatchability performance of the offspring and some egg quality characteristics, which will be obtained from crossing Leghorn breed chickens and five different genotypes. The study's experiment was carried out in the Prof. Dr. Hümeyra Özgen Research and Application Farm in Selcuk University. In the present study, which was designed to have one male and twelve females belonging to each genotype, a total of 186 eggs from each flock were examined. No adverse results were found in the incubation results of the crosses made with our local breed Denizli and Araucana, Brahma, and Cornish breeds, whose breeders increased locally. When egg quality characteristics were examined, the difference in egg weight between F_1 genotypes was insignificant and ranged between 46.91-51.54 g on average. When the F2 generation was investigated, egg weight differed between genotype groups, and the average weights ranged between 57.6-67.14 g. In addition, the effect of genotype on egg shell strength values were found to be significant. In addition, the effect of genotype on egg shell strength and shell weight values were found to be significant. However, the effect on the Haugh Unit and yellow height were insignificant in the same generation. As a result, hybridizing genotypes with low yield performance with commercially important genotypes could provide a genotype for alternative production systems for future generations.

Keywords: Egg quality, genotype, hatching efficiency, hybridization, Leghorn.

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Leghorn Melezlerine Ait Bazı Yumurta Kalite Özellikleri Ve Kuluçka Performansları

ÖZ

Bu çalışma Leghorn ırkı tavuklar ile beş farklı genotipin melezlemesi sonucunda elde edilecek yavruların kuluçka performansı ve bazı yumurta kalite özelliklerini incelemek için yapılmıştır. Çalışmanın deneyi Selçuk Üniversitesi Prof. Dr. Hümeyra Özgen Çiftliğinde gerçekleştirildi. Her bir genotipe ait bir erkek yirmi dişi hayvan olacak şekilde planlanan çalışmada toplamda her sürüden 186 yumurta incelendi. Yerli ırkımız olan Denizli ve lokal olarak yetiştiriciği artan Aracuana, Brahma, Cornish ırkları ile yapılan birleştirmelerde kuluçka sonuçlarında olumsuzluk bulunmamıştır. Yumurta kalite özellikleri incelendiğinde ise F_1 genotipler arasında yumurta ağırlığı bakımından farkın önemsiz olduğu ve ortalama 46.91-51.54 g arasında değiştiği tespit edildi. F_2 kuşağı incelendiğinde ise yumurta ağırlığının genotip grupları arasında farklılık gösterdiği ve ağırlık ortalamalarının 57.6-67.14 g arasında değiştiği tespit edildi. Ayrıca aynı kuşakta genotipin kabuk mukavemeti ve kabuk ağırlığı değerlerine etkisi önemli, Haugh Unit ve sarı yüksekliğine etkisi ise önemsiz bulundu. Sonuç olarak, düşük verim performansına sahip genotiplerin ticari önemi olan genotiplerle melezlemeleri gelecek nesiller için alternatif üretim sistemlerine bir genotip kazandırılabileceği sonucuna varıldı.

Anahtar Kelimeler: Genotip, kuluçka randımanı, Leghorn, melezleme, yumurta kalitesi.

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INTRODUCTION

Rapid population growth in the world has increased the need for animal products, and intensive system breeding has become widespread to meet this demand. Cracked, broken, defective, and unshelled eggs in poultry farms cause severe damage to the national economy. The rate of broken shells in the eggs produced varies between 6-20% (Çetin and Gürcan 2006). In addition to the shell defects, this situation may impair the quality characteristics of the egg in general. Egg quality, which is examined in two areas as internal and external quality characteristics, significantly affects hatchability. For the chickens we obtain from hatching eggs to have a high life force and achieve high hatching efficiency, the necessary hatching conditions must be provided, and the quality of hatching eggs must be high. External quality of the egg; egg weight, shell thickness, breaking strength, and shape index parameters determine the internal quality, white and yellow appearance, flesh-blood stain, Haugh Unit, and nutrient composition parameters. The optimum level of all these features is a good start for chick hatching. Any abnormality in the quality characteristics of the egg leads to the deterioration of the primary functions that will provide the best conditions for embryo development. Egg production at a level to meet the hatching requirements is not at the desired level despite the efforts of breeding establishments. The lack of chick hatching between 20-40% in chicken eggs indicates this situation. Studies have reported that some parameters in eggs cause embryonic mortality (Narushin and Ramanov 2002). It has been stated that the parameters that make up the egg quality characteristics have a more positive effect on the hatching yield rather than having very high or low values (Wolc and Olori 2009).

The hatching yield is usually 82-85%. It is impossible to interpret hatching results without knowing the problems that cause low hatchability. Moreover, there is a loss of 15-18% may be caused by egg storage conditions, bacterial and fungal contamination, eggshell quality, mechanical errors, feed used, diseases and genetic structure of animals, breeding flock, egg processing, and hatchery errors (Wilson 1991).

Hatching efficiency is an essential parameter for the continuity of production of generations in poultry. It can vary with the effect of factors such as the age of the breeder flock, health status, maintenance feeding procedure, transportation of hatching eggs, disinfection, storage conditions and duration, hatchery and incubation conditions (Burton and Tullett 1983, Peebles and Brake 1985, Peebles et al. 2001, Reis et al. 1997). incubation conditions in the hatchery practice and the shell characteristics of hatching eggs cause weight loss in eggs, which can affect hatching results. Burton and Tullet (1983) reported that the eggs' weight loss (as water vapour) during hatching affects the hatching results. Reis et al. (1997) reported an of 11.22-11.64% weight loss in eggs until the end of hatching in chickens. Peebles et al. (2001) reported that there would be a lower weight loss in infertile eggs and eggs with early embryo mortality compared to other egg types. Peebles and Marks (1991) reported that gas exchange was affected by an increase in shell thickness, and thus, early embryonic mortality were formed. In addition, Padhi et al. (2013) reported that the average shell thickness was between 0.34 mm and 0.38 mm.

MATERIAL AND METHODS

This study was carried out at Prof. Dr. Hümeyra Özgen Research and Application Farm in Selcuk University. Three roosters from each breed and 30 chickens from each breed were used for five breeds (Brahma, Denizli, Araucana, Leghorn, Cornish) to form the breeder flock to be grown as material. Leghorn parent breeder chickens were obtained from Poultry Research Institute, Ankara. Ankara Poultry Research Institute. After adaptation feeding, 20 females will be placed in the mating chamber made with fencing wire for mating so that one male has 20 females (F_0). In order to determine which animal the fertile eggs belonged to, the breeder chickens were given wing numbers, and their wing and family numbers were given on the cages. After all the females were kept in the mating chamber with the roosters for ten days, they were placed in the egg-laying cages and placed in individual cages of 30x45x50 cm (width × length × height). Fertile eggs were obtained from cage cells, and hen and rooster numbers were recorded at regular intervals. 40 randomly selected eggs from each flock were examined in terms of quality characteristics. During the examination, cracks etc. eggs were not evaluated. Before the eggs were transferred to the incubator, the eggs stored in the holding rooms at room temperature 22 ^{o}C and 75% relative humidity for one week were transferred to the incubator at 37.5 ^{o}C and 65% humidity conditions after fumigation. At the end of the 18th day of incubation, the egg records were written in gauze bags and on small papers, and the transfer process was carried out. The egg were weighted at the end of storage, and recorded before ıncubation. The eggs were transferred to the brooder machine at 37.2 °C and 70% relative humidity and kept for 3 days. As a result, crossbred F_1 chickenof Brahma x Leghorn (BxL), Denizli x Leghorn (DxL), Araucana x Leghorn (AxL), Leghorn x Leghorn (LxL), and Cornish x Leghorn (CxL) were obtained in female line with Leghorn breed, respectively. The wing numbers of the hatched chickens were given by writing the parent number. The healthy hatched chickens were raised in the brooder machine, where they will be kept for three weeks. Initially, 2.5%

sugary water was given, and after 3-4 hours of fasting, feeding was started. The vaccination program for poultry (Marek, Gumboro, Newcastle) was implemented in the The vaccination program for chicken (Marek, Gumboro, Newcastle) was implemented in the Prof. Dr. Hümeyra Özgen Research and Application Farm Alternative Poultry Unit in Selcuk University. 300 (F_1) animals were obtained from five families, and members of five families with sufficient male females from the herd were separated as breeders to obtain the F_2 herd. In order to obtain F_2, the same procedure was applied in the F_0 generation for each breed (15 males and 300 females F_1 individuals). As a result, the quality characteristics and hatchability of the eggs of F_1 and F_2 genotypes were evaluated.

Early embryonic mortality rate, storage period and temperatures of eggs below or above average, errors due to fumigation, temperature shock in eggs during transport, high-temperature values in the first week of embryo development, very young or very old breeders, the health status of the flock, chromosomal abnormalities affected by many factors. The middle embryonic mortality rate (7-17st days) is shaped by improper temperature, humidity, turning and ventilation in the machine, contamination of eggs, nutritional deficiencies in breeder houses, and lethal genes. Late embryonic mortality rate (18-21st days); It may occur in situations such as unsuitable temperature, humidity and ventilation, fumigation, excessive cooling of the eggs during transfer, contamination, nutritional deficiencies, and the inability of the embryo to take the normal hatching position in the hatching machines (Wilson 1991).

In this study were used to below formulas;

Hatching efficiency rate (%): (Number of live chicks hatched/Number of eggs laid in hatching)*100

Hatching rate (%): (Number of live chicks hatching/Number of fertile eggs hatched)*100

Specific Gravity (g/cm3)=Egg weight in air (g)/(Egg weight in air(g)-weight in pure water (g))

Early embryonic mortality rate (%): (Number of embryos mortality 0-6st days incubation/Number of fertile eggs)*100

Middle embryonic mortality rate (%): (number of embryos mortality 7-17st days of incubation/Number of fertile eggs)*100

Late embryonic mortality rate (%): (Number of embryos mortality 18-21st days of incubation/Number of fertile eggs)*100

Table 1. The rations contents used in the experiment

Week **Nutritional Contents**	**1-3** **Starter ration**	**4-10** **Growth raiton**	**11-16** **Growth raiton-II**	**17-40** **Breeder hen ration I**	**40-64** **Breeder hen ration II**
Dry matter %	88	88	88	88	88
Crude Ash %	8	8	8	8	8
Crude Protein %	19	18	16	18	17
Energy kkal/kg	2900	2800	2700	2800	2700
Crude cellulose %	4-5	5-6	6	6	6
Ca %	1.0-1.2	1.0-1.1	0.9-11	4.0-4.2	3.5.0-4.5
P %	0.45	0.42	0.40	0.40	0.35
Metionin %	0.55	0.45	0.35	0.50	0.40
Lysine %	1.15	1.0	0.75	0.75	0.75
Salt %	0.50	0.50	0.40	0.40	0.35
Vit A IU	13 000	13 000	13 000	13 000	13 000

The data of the study were evaluated using the IBM SPSS 21 package program licensed by XX. The normal distribution of the data was analyzed using the Shapiro-Wilk test. Multiple analysis of variance (ANOVA) was used for parametric tests and Duncan test was used for comparisons between groups. In addition, Kruskal Wallis-H test was used from non parametric tests. Intergroup significance was evaluated on the basis of $P<0.05$.

RESULTS

Eggs obtained from the F_0 flock were placed in the incubator as 300 from each genotype group. At the end of the incubation, as a result of the embryonic mortality evaluation made at the end of the 22nd day, the incubation machine efficiency and embryonic mortality percentages were calculated and presented in Table 2.

In the study carried out with an equal number of eggs, hatchability characteristics and embryonic mortality are given in Table 2. The group with the highest hatchability of set/total eggs was the LL_1 cross (93.67). When infertility rates are examined, while the highest rate was found in the CL group, the lowest rate was 3.3% in the LL group. It was calculated that embryonic mortality, which were examined in as early, late middle, and sub-crustacean periods, ranged from 0.3% to 4% in the groups.

Table 2. Fertility, hatchability and embriyonic mortality rate of F1 (%)

Genotype	Hatchability	HSE	Fertility	EEM	MEM	LEM
(Brahma x Leghorn) BL	88.33	92.98	95.0	1.7	1.0	4
(Denizli x Leghorn) DL	86.00	92.81	92.7	2.0	2.3	2.3
(Aracuana x Leghorn) AL	88.67	93.99	94.3	2.7	0.3	2.6
(Leghorn x Leghorn) LL	93.67	96.90	96.7	1.0	0.3	1.7
(Cornish x Leghorn) CL	82.67	89.86	92.0	3.3	2.7	3.3

HSE: Hatchability of set/total eggs, EEM: Early Embryonic Mortality, MEM: Mid Embryonic Mortality, LEM: Late Embryonic Mortality

Cornish x Leghorn F_2 (CL_2) was the lowest hatchability with 79.0%, while the highest hatchability was obtained from Leghorn x Leghorn F_2 (LL_2) crosses with 90.5%, belonging to the F_2 genotype groups.

Table 3. Fertility, hatchability and embriyonic mortality rate of F1 (%)

Genotype	Fertility	HSE	İnfertile	EEM	MEM	LEM
(Brahma x Leghorn) BL	87.5	92.1	5.0	2.5	1.5	3.5
(Denizli x Leghorn) DL	83.5	88.4	5.5	3.0	3.5	4.5
(Aracuana x Leghorn) AL	86.0	90.5	5.0	3.0	2.5	3.5
(Leghorn x Leghorn) LL	90.5	95.3	5.0	1.5	0.5	2.5
(Cornish x Leghorn) CL	79.0	84.0	6.0	5.5	4.0	5.5

HSE: Hatchability of set/total eggs, EEM: Early Embryonic Mortality, MEM: Mid Embryonic Mortality, LEM: Late Embryonic Mortality

Egg weights of F_1 genotype groups were found to be 46.91-51.54 g, and the difference between the groups was insignificant ($P>0.05$).

Table 4. Egg weights and specific gravity values of the F1 generation

Genotypes	DL	AL	LL	BL	CL	Mean±SE	P
Egg weight (g)	48.61±0.63	46.91±0.63	47.97±1.08	49.66±1.17	51.54±2.28	48.94±0.59	**0.128**
Specific gravity (gr/cm³)	1.077±0.01	1.098±0.02	1.076±0.01	1.074±0.01	1.075±0.01	1.080±0.01	**0.297**
n	37	37	37	38	37	186	

In the F_2 generation, which was obtained by combining the F_1 genotype groups among themselves, the egg weights were found to be the highest in BL_2 and were determined as 67.14 g. The lowest egg weight was found in CL_2 with 57.6 g. In addition, the mean egg weight of the study groups was determined as 62.0 g, and it was determined that there were statistically significant differences between the genotypes in terms of egg weight ($P<0.05$).

Table 5. Egg weights and some egg quality characteristics of the F2 generation

	LL	CL	DL	BL	AL	P	Total
Egg weight (g)	63.24±0.71^{b}	57.6±0.86^{d}	61.95±0.97^{bc}	67.14±0.66^{a}	60.21±0.81^{c}	0.001	62.00±0.44
Haught Unıt*	73.52±2.86	62.73±2.29	65.52±3.03	70.69±2.65	69.1±2.57	0.073	68.39±1.23
Egg height (mm)	5.44±0.43	6.79±2.36	5.06±0.37	5.82±0.3	5.41±0.26	0.796	5.68±0.43
Egg shell strength (mm/kg)	33.79±1.76^{c}	47.52±1.53^{a}	37.69±1.92^{bc}	40.86±1.7^{b}	40.79±1.82^{b}	0.001	40.24±0.86
Shell weight (g)	6.2±0.11^{b}	10.97±3.22^{a}	6.12±0.13^{b}	6.6±0.09^{b}	6.11±0.09^{b}	0.042	7.08±0.58

The difference between groups carrying different letters on the same line is statistically significant.

DISCUSSION

Economic sustainability in commercial breeding hatcheries depends on success in hatching. One of the factors affecting hatching performance is genetic factors. Hatching performance and machine yield values of F_1 genotypes are presented in Table 2. The hatchability values obtained in the study ranged from 82.67 to 93.67. When this difference was examined, ıt was higher especially in the Leghorn breed than other breeds. It could be because this breed was more bred. Likewise, the lowest yield in Cornish Leghorn (CL) hybrids can be expressed in this situation. Due to the higher embryonic mortality in CL crosses, the hatchery efficiency is lower than in other crosses. Ledur et al. (2000) obtained 94-97% fertility and 86.65-90.60% hatchability in their crosses in White Leghorn lines of different ages. The fact that it was found to be lower than the findings of this study may be due to the age factor as well as the difference in care and feeding conditions.

Alewi and Melesse (2013) and Bamidele et al. (2020) reported that the hatching performance of eggs obtained from hybrids is between 67.9-89%. Alabi et al. (2012) and Wondmeneh et al. (2011) stated that this value varies between 52.4-87% in domestic chicken breeds and crosses. In this study, as indicated in Table 3, the hatchability of F_2 genotypes was found to vary between 79-90.5%. Detection of a higher value than the values were reported in the literature; may have resulted from genotype, male-female ratio, or differences in care and feeding.

The difference between the F_1 genotype groups was insignificant in terms of egg weight. However, significant differences were found between the F_2 genotype groups regarding egg weight. The main reason for this difference may be due to age, feeding, and seasonal changes between generations. It was concluded that due to the decrease in the variation in egg weight in the F_2 generation.

Putra et al. (2021) calculated the egg weights in three Turkish layer genotypes as 59.7, 53.7, and 55.86 g in White Leghorn, Lohman Brown, and Atak-S chickens, respectively. These values were higher than the egg weights of the F_1 genotype groups in this study and lower than those obtained from the F_2s. Ewa et al. (2005), on the other hand, reported the egg weights of four inbred domestic chicken genotypes as 56.30, 56.72, 39.45, and 39.21 g in Black Olimpia (ESA), Brown Nick (ESB), LTA, and LTB genotypes, respectively. When the egg weights stated in Table 4 were compared with the values of Ewa et al. (2005), it was determined that the egg weights were similar to the commercial chicken lines and higher than the local lines. In addition, egg weights obtained from F_2s were higher than the values of the four genotypes reported by Ewa et al. (2005). Sirri et al. (2018) calculated the egg weight as 64.5 g in commercial chickens and 52.9 g in domestic genotypes, stating that the statistical difference is very significant (p=0.01). Regarding the similarity of egg weight, it was found that the commercial line was closer to the F_2s specified in our study, and the egg weight obtained from the local line was closer to the F_1 generation. Drabik et al. (2021), on the other hand, found 47.24, 60.93, and 66.32 g in their study on Araucana, Marans, and Leghorn breed chickens, and they found the effect of genotype on egg weight to be very important (P<0.001). The study evaluated that the findings of 46.91 g in AL_1 and 63.24 g in LL_2 were close to the values of Drabik et al. (2021).

The egg-specific gravity values of the F_1 generation are given in Table 4, and the average specific gravity value was found to be 1.080 g/ml (1.074-1.098) (p=0.297). Putra et al. (2021), on the other hand, reported 1.017, 1.07, and 1.11 and found the difference statistically significant (P<0.05). Drabik et al (2021) found 1.078 g/ml in Aracuana, 1.078 in Marans, and 1.081 g/ml in Leghorn (p=0.299).

The Haugh Unit value of the F_2 generation presented in Table 5, and the difference between genotypes found no significant (p=0.073). The highest Haugh Unit value was found in LL_2 with 73.52, and the lowest Haugh Unit value with 62.73 in CL_2. Drabik et al. (2021), on the other hand, reported that the Haugh Unit value was 80.53 in Araucana, 84.01 in Marans, and 84.82 in Leghorn (p=0.215). Sirri et al. (2018) found the Haugh Unit value to be 81.8 in commercial

chickens and 76.4 in local chickens ($p>0.05$). On the other hand, Yaman et al. (2020) examined egg Haugh Unit values in 6 different genotypes and found a statistical difference in subtypes ($p<0.05$). The bark weight value of the F_2 generation presented in Table 5, and the difference between genotypes found significant ($p=0.042$). Although the CL_2 genotype had the lowest egg weight, the highest eggshell weight was determined. In addition, it determined that this value, 10.97 g in CL_2, was different from other genotype groups. It was concluded that this situation might be a genotype-specific situation. Putra et al. (2021), on the other hand, determined the eggshell weight value obtained from the White Leghorn as 8.76 g and stated that this value was higher than the other study groups. This study determined that eggshell weight values of LL_2 were higher than all other genotype groups lower than CL_2.

CONCLUSION

It was concluded that obtaining offspring without significant brood losses in the offspring generation was obtained using Leghorn in the main line. No adverse results founded in the incubation results of the crosses made with our local breed Denizli and the green layer Araucana, Brahma, and Cornish breeds, whose breeders increased locally.

It is necessary to develop local chicken breeds with low production performance, to increase their production performance, create disease-resistant local breeds, and search for alternative feeding opportunities, to introduce and protect traditional flavors. Moreover, further studies are needed in this context to bring new types from local breeds to the next generations and contribute to the protection of the gene resources of the countries.

ETHICS STATEMENT

Approval was obtained from the Selcuk University Experimental Research and Application Center, Animal Experiments Ethics Committee with the decision number 2014/61 dated 29.09.2014.

Conflict of interest: The authors have no conflicts of interest to report.

Ethical approval: This study was carried out at Selçuk University Faculty of Veterinary Medicine Hümeyra Özgen Research and Application Center Farm Poultry Unit. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, University of Selçuk (report no: 2014/61).

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CASE REPORT

Primary Hypothyroidism in An Adult Cat

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ABSTRACT

A 10-year-old cross breed female cat was brought with the complaint of weight gain while only the same amount of food was given. As a result of the evaluation of clinical examination, anamnesis, laboratory results and thyroid panel parameters, a rare hypothyroid disease in cats was determined. Levotroxine was used in the treatment and after 1.5 months, the parameters were within normal ranges at the control. As a result, the opinion that primary hypothyroidism in cats has gained more importance in veterinary endocrinology in recent years compared to previous years.

Keywords: T4, FT4, Cat, Hypothyroidism, Feline Endocrinology

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Yetişkin Bir Kedide Primer Hipotroidizm

ÖZ

Bu olgunun materyalini aynı miktarda günlük mama verilmesine rağmen kilo artışı meydana gelen 10 yaşındaki melez ırkı dişi kedi oluşturdu. Klinik muayene, anamnez, laboratuar sonuçları ve troid paneli parametrelerinin sonuçlarının değerlendirilmesi sonucunda kedilerde nadir görülen hipotroid hastalığı belirlendi. Tedavisinde levotroksin kullanıldı ve 1,5 ay sonra kontrol değerlerinde parametreler normal aralıklar arasında elde edildi. Sonuç olarak son yıllarda önceki yıllara göre kedilerde hipotroid olabileceği görüşü veteriner endokrinolojisinde daha çok önem kazanmıştır.

Anahtar kelimeler: Hipotroidizm, Kedi, Kedi Endokrinolojisi, T4, FT4

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INTRODUCTION

A disorder that may occur at any point in the hypothalamus-pituitary-thyroid axis causes thyroid hormone deficiency, but in 95% of clinically observed hypothyroidism events, the problem is the disorders occurring in the thyroid gland itself (primary hypothyroidism). The two most common causes of hypothyroidism in adult are lymphocytic thyroiditis and atrophy of the thyroid gland, and these have been reported to play an equal role in hypothyroidism. Less common causes of hypothyroidism are iatrogenic conditions, tumors developing in the thyroid gland, and congenital (or developing at a young age) hypothyroidism (Reusch et al 2015, Sevinç and Koral 2017).

It is a clinical condition resulting from insufficient secretion of tetraiodothyronine (T4, levotroxine, thyroxine), triiodothyronine (T3, liothyronine) by the thyroid gland (Chastain and Panciera 1995). 80-90% of the secreted hormone is T4 and 10-20% is T3. Biologically, T3 is 10 times more active than T4 on a cellular basis (Sevinç and Koral 2017). Thyroid hormone synthesis and secretion are regulated by extrathyroidal (thyrotropin, TSH) and intratrodial (autoregulatory) mechanisms. Thyrotropin (TSH) is the major modulator of thyroid activity and the result of this activity is the secretion of thyroid hormones. TSH secreted from the pituitary gland; It is regulated by the negative feedback mechanism from the thyroid hormone. The regulation of the thyroid hormone, namely the TSH feedback loop, is regulated by the thyrotropin-releasing hormone (TRH) secreted from the hypothalamus (Reusch et al 2015).

Clinical findings of hypothyroidism are weight gain, mental dullness, bilateral trunkal non-pruritic alopecia, exercise intolerance. Most dogs become obese due to the reduced metabolic rate and increased appetite. Interestingly, most pet owners even say that their animals start to gain weight despite not being hungry and having the same appetite. Most clinical signs in cat were similar to those in dogs (Rand et al 1993). Due to decreased metabolic rate, heat production is reduced. Therefore, some of the patients are hypothermic (Aytuğ 2011). Skin-related problems often occur in hypothyroidism. The skin may be dry or greasy, with a malodorous characterized by scale. One of the most important findings is bilateral, symmetrical, non-pruritic truncal alopecia. As a result of hair loss in tail, a condition called mouse tail occurs. (Kemppainen 2001, Schoeman 2011, Sevinç and Koral 2017). Weight gain, hypothermia and bradycardia can be observed in hypothyroidism in cats. (Scott-Moncrieff 2007).

As a laboratory finding in hypothyroidism, as well as decrease in thyroid hormones occur with hypercholesterolemia, normocytic normochromic anemia, elevated in liver enzymes such as ALT, AST, and ALP (Sevinç and Koral 2017). The diagnosis of the hypothyroidism is made by measuring serum total T4 and total T3 concentrations. It causes; a decrease in total T4 due to the disease and drug use that causes the euthyroid sick syndrome mentioned Table 1 and an increase in the total T3 concentration. Therefore, the specificity of measuring total T4 alone to diagnosis of hypotyhroidism is 70%. Free T4 (FT4); It is the form that is not bound to proteins in the T4 fraction and is free at the tissues. Free T4 concentration is measured by equilibrium dialysis method. The determination of free T4 and free T3 is used in human and veterinary medicine to differentiate the diagnosis of euthyroid sick syndrome from thyroid diseases. FT4 is used to determine the definitive diagnosis hypothyroidism, as it is not be affected by drugs and the diseases mentioned Table 1. While TT4 and FT4 levels decrease in hypothyroidism, TSH level becomes normal or high (Greco 2006, Sevinç and Koral 2017). Table 1 shows the change in total T4, free T4 and TSH to differentiate hypothyroidism and euthyroid sick syndrome. Thyroid hormones are suppressed by the use of drugs such as glucocorticoid, phenobarbutal, primidone, diazepam, sulfonamide, carprofen, aspirin, meloxicam and unbalanced nutrition. In addition, diabetes mellitus, liver diseases, chronic renal failure, hyperadrenocorticism, renal and heart failures often reduce the concentration of thyroid hormones. These cases cause decreasing in the thyroid hormones and are called euthyroid sick syndrome (Daminet 2010, Sevinç and Koral 2017).

Sodium levothyroxine as synthetic T4 is the first treatment option. The recommended initial dose of treatment is 20-44 μg/kg twice a day (Greco 2016).

CASE HISTORY

A 10-year-old cross breed female cat was brought with the complaint of severe weight gain despite eating the same amount of food. The clinical examination performed was normal and as a result of biochemical parameter, an increase in ALT, ALP, glucose and cholesterol was determined. Biochemical parameters were measured in the fuji nx-600 (Japan) instrument. Red blood cells (RBC), haematocrit (Hct), haemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were measured in Mindray BC-5000 vet (China). FT4, TT4, TSH were measured in animal laboratory. In addition, microcytic hypochromic anemia was determined in the complete blood count. No glycosuria was observed in the urine examination.

Table 1. Hypothyroidism-euthyroid sick syndrome table of thyroid hormones.

	FT4	TT4	TSH
Hypothyroidism	**Low**	**Low or Low-Normal**	**High**
Euthyroid sick syndrome	**Normal**	**Low or Normal**	**Normal or Slightly increase in recovery**

After confirming that the cat did not use any other medication drug or did not have any disease in the anamnesis, the thyroid panel (TT4, FT4, TSH) was measured. Thyroid panel parameters was measured in special animal laboratory. A decrease in TT4 and FT4 levels and an increase in TSH levels were determined (Table 2). According to the thyroid panel, a rare diagnosis of hypothyroidism in cats has been made. Twice a day levotroxine treatment at a dose of 20 μg/kg was started immediately. At the 1.5 months later, it was observed that TT4, FT4 and other parameters returned to normal reference value (Table 2). The complaint of weight gain, which is a clinical symptom, disappeared immediately and weight control was achieved.

Table 2. The cat's laboratory results on the first day and control laboratory results of the cat after 1.5 months later

PARAMETRES	RESULTS of FIRST DAY	RESULTS of AFTER 1.5 MONTHS	REFERENCE VALUES (Turgut 2000)
FT4 (ng/dL)	0.68	1.4	1.0-2.5
TT4 (ug/dL)	0.50	1.6	1.5-5.0
TSH (ng/dL)	0.45	0.26	0-0.38
ALT (U/L)	117	88	10-80
ALP (U/L)	58	43	10-80
Glucose (mg/dL)	268	173	70-150
GGT (U/L)	10	10	1-10
Cholesterol (mg/dL)	187	154	90-205
RBC (10^{12}/L)	3.70	4.70	5.0-10.0
HCT (%)	24.20	29	24-45
Hb (g/dL)	8.20	9.10	8.0-15.0
MCV (fL)	30	41	37-49
MCHC (g/l)	260	300	290-360

DISCUSSION

Naturally occurring hypothyroidism in cats is very rare. When TT4 is decreased in the cats, it is generally thought that non-thyroid diseases cause this decrease. Sometimes, a decrease in TT4 level can be observed due to the overdose of drugs used in the treatment of hyperthyroidism. The symptoms of hypothyroidism in cats are the same as in dogs. As reported by researchers
(Tanase et al. 1991, Greco 2006, Scott-Moncrieff 2007), in cats with hypothyroidism, weight gain was also detected in this case despite decreased or normal appetite.

Total T4 contains both bound and free T4. More than 99% of the T4 hormone is "protein-bound". This means it is bound to binding proteins in the blood and can never reach the tissues. Therefore, the TT4 result alone can often lead to wrong interpretations. With using medication or any other disease, it can change the amount of T4 bound to proteins in the blood. Therefore, determination of TT4 concentration in

thyroid disorders is not a definite indicator alone, it must be evaluated together with FT4 (Scott-Moncrieff 2007, Dodds and Laverdure 2011). It has been reported that total T4, total T3, free T4, and free T3 can be evaluated in determining thyroid disorder in animals, but it is more reliable to evaluate TT4 and FT4 together in the diagnosis of the hypothyroidism (Scott-Moncrieff 2007, Daminet 2010, Dodds and Laverdure 2011). However, since the concentration of free T4 is not affected by the protein level in the blood, it is thought to be more reliable than total T4 in evaluating thyroid activity (Ferguson 1994, Scott-Moncrieff 2007, Daminet 2010, Dodds and Laverdure 2011). In this case report; as a result of the thyroid panel examinations, a decrease in TT4 and FT4 levels and an increase in TSH levels were determined. With these results, the cat in our case was diagnosed with primary hypothyroidism. The diagnosis of primary hypothyroidism in the cat in this case is compatible with Greco (2006), Scott-Moncrieff (2007), Dodds and Laverdure (2011). Clinicopathologic findings such as normocytic normochromic anemia resulting from erythropoeitin deficiency, decreased bone marrow activity, and decreased serum iron and iron-binding capacity would be expected to be observed in approximately 30% of hypothyroid cats (Greco 2016). In this case report, the cat's laboratory results on the first day showed us microcytic hypochromic anemia. This type of anemia is related to decreased serum iron and iron-binding capacity.

In the treatment of hypothyroidism, thyroxine (thyroxine; that is T4) is applied. For this purpose, synthetic thyroid hormones; it is more preferred because it stays as a standard better and longer. Sodium levothyroxine as synthetic T4 is the first treatment option. The half-life of levotroxine is 12-16 hours and peak concentration is reached within 4-12 hours after administration. The recommended initial dose of treatment is 20-44 μg/kg twice a day (Greco 2016). In this case, levotroxine treatment was started at a dose of 20 μg/kg twice a day. The complaint of weight gain, which is a clinical symptom, disappeared immediately and weight control was achieved. At the follow-up 1.5 months later, it was observed that TT4, FT4 and other parameters returned to normal values (Table 2). As reported by the researchers, levotroxine administration was also seen in this case, to be effective in cat hypothyroidism

CONCLUSION

As a result, the opinion that primary hypothyroidism in cats has gained more importance in veterinary endocrinology in recent years compared to previous years. We think that the evaluation of TT4, FT4 and TSH levels is of diagnostic importance in the diagnosis of feline hypothyroidism. Although hypothyroidism is rarely seen in cats, it should not be forgotten in the endocrinology of cats.

Conflict of interest: The authors declare that there is no actual, potential or perceived conflict of interest for this article

Project Support Information: During this study, any pharmaceutical company that has a direct connection with the subject of the research, a company that provides and/or produces pharmaceutical instruments, equipment and materials, or any commercial company, the evaluation process, the evaluation process, the material and / or morale that will negatively affect the result of the study. No support has been received.

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

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